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Studies on the hyperplasia ('regeneration') of the rat liver following partial hepatectomy

Changes in lipid peroxidation and general biochemical aspects

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Using the experimental model of partial hepatectomy in the rat, we have examined the relationship between cell division and lipid peroxidation activity. In rats entrained to a regime of 12 h light/12 h dark and with a fixed 8 h feeding period in the dark phase, partial hepatectomy is followed by a rapid regeneration of liver mass with cycles of synchronized cell division at 24 h intervals. The latter phenomenon is indicated in this study by pulses of thymidine kinase activity having maxima at 24 h, 48 h and 72 h after partial hepatectomy. Microsomes prepared from regenerating livers show changes in lipid peroxidation activity (induced by NADPH/ADP/iron or by ascorbate/iron), which is significantly decreased relative to that in microsomes from sham-operated controls, again at 24 h, 48 h and 72 h after the operation. This phenomenon has been investigated with regard to possible underlying changes in the content of microsomal fatty acids, the microsomal enzymes NADPH:cytochrome *c* reductase and cytochrome *P*-450, and the physiological microsomal antioxidant α-tocopherol. The cycles of decreased lipid peroxidation activity are apparently due, at least in part, to changes in microsomal α-tocopherol content that are closely associated in time with thymidine kinase activity.

INTRODUCTION

Previous studies have shown that lipid peroxidation, a free-radical-mediated process, is generally greatly decreased in liver tumour cells and tumour cell fractions compared with corresponding samples of normal liver (Thiele & Huff, 1960; Utsumi *et al.*, 1965; Fonnesu *et al.*, 1966; Lash, 1966; Burlakova & Pal'mina, 1967; Ugazio *et al.*, 1969; Burlakova, 1975; Bartoli & Galeotti, 1979; Player *et al.*, 1979; Ahmed & Slater, 1981; Dianzani *et al.*, 1984; Cheeseman *et al.*, 1986*a*, 1988*a*; Masotti *et al.*, 1988). Detailed studies on the Novikoff (Cheeseman *et al.*, 1986*a*) and Yoshida (Cheeseman *et al.*, 1988*a*) tumours have identified an increased tumour cell content of α-tocopherol as a major contributory factor in the decreased rate of lipid peroxidation.

The question arises naturally: is a decreased rate of lipid peroxidation, and a corresponding increase of α-tocopherol, a special feature of liver tumour cells, or is it more a reflection of cell division? To answer this question we have turned to the rat liver model following partial hepatectomy. It is known that a regenerative hyperplasia of the liver (hereafter abbreviated as 'regeneration') occurs in this model system following operation so that the original mass of the liver is quickly regained (see Bucher & Malt, 1971, for a review). When the rats are entrained to a 12 h/12 h light/dark regime, with access to food during the first 8 h of darkness, liver regeneration is marked by cycles of DNA synthesis at approx. 24 h intervals (Hopkins *et al.*, 1973). We have used this model of liver cell division to study variations in lipid peroxidation at times of high and low DNA synthesis.

METHODS

Entrainment of animals, partial hepatectomy and preparation of microsomes

Male Wistar rats were obtained from Charles River Ltd. (Margate, Kent, U.K.) and were entrained to an inverted lighting and feeding schedule for a period of 3 weeks before partial hepatectomy. The lighting and feeding regimen was that of Hopkins *et al.* (1973): the rats were housed in a windowless room lit from 20:00 h to 08:00 h and they were allowed access to a standard laboratory diet (Expanded Breeder Diet No. 3; Special Diet Services, Witham, Kent, U.K.) from 08:00 h to 16:00 h, corresponding to the first 8 h of the dark period. At the time of operation the mean body weight was 270 g. The rats were subjected to 65% partial hepatectomy (Higgins & Anderson, 1931) under ether anaesthesia; sham-operated animals were subjected to laparotomy.

The rats were killed by cervical dislocation at various intervals after operation and the liver was quickly removed. The very small necrotic stubs were removed from

Abbreviations used: TBA, thiobarbituric acid; MDA, malondialdehyde.

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the livers of the partially hepatectomized rats before homogenization. Only the minor liver lobes (corresponding to the remaining liver lobes in the hepatectomized animals) were taken from the sham-operated animals. These liver lobes were homogenized and microsomes were prepared as described previously (Slater & Sawyer, 1969). Microsomal pellets were rinsed with 0.15 M-KCl and stored at −70 °C or −20 °C for up to 60 h before resuspension.

Microsomal enzyme and lipid peroxidation assays

Microsomal pellets were resuspended for use in cold 0.15 M-KCl, such that 1 ml of suspension contained the equivalent of 1 g wet wt. of liver. Cytochrome *P*-450 and NADPH:cytochrome *c* reductase were assayed as described previously (Slater & Sawyer, 1969). CCl_4-dependent lipid peroxidation and ascorbate/iron-dependent lipid peroxidation were assayed using the thiobarbituric acid (TBA) test for malondialdehyde (MDA) production as previously described (Slater & Sawyer, 1971; Beswick *et al.*, 1981). NADPH/ADP/iron-induced lipid peroxidation was measured by oxygen uptake (Slater, 1968) using a Clark-type oxygen electrode. The time (min) between addition of ADP/iron to the microsomal suspension mixed with NADPH and the start of the maximal rate of oxygen uptake was derived from the recorded traces and is termed here the 'induction period' (for example, see Fig. 3).

Lipid and lipid-soluble antioxidant assays

Samples of liver homogenate were taken for analysis of liver triacylglycerols and fatty acid composition. The homogenate was extracted using at least 20 vol. of

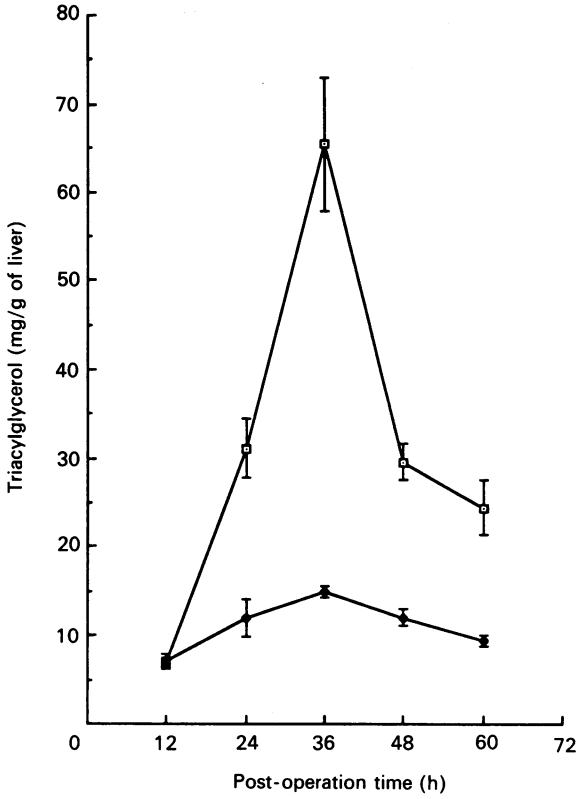

Fig. 1. Transient accumulation of triacylglycerol in the regenerating rat liver (□) compared with sham-operated control rat liver (◆) at up to 60 h post-operation

Points are means ±S.E.M.

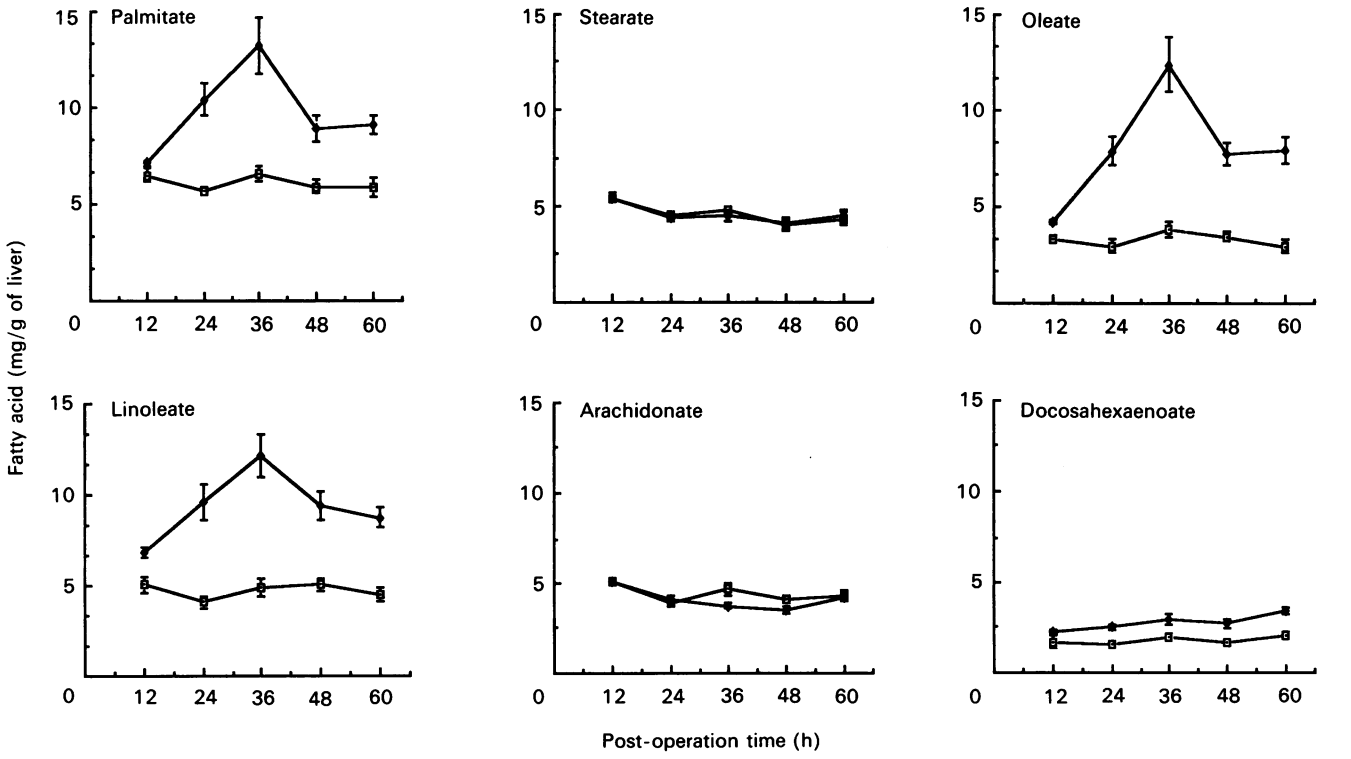

Fig. 2. Changes in the total fatty acid profile of the whole liver up to 60 h after partial hepatectomy

Lipids were extracted from regenerating livers (◆) or from the livers of sham-operated control rats (□) and the fatty acids were analysed as described in the Methods section. Values are means ± S.E.M.

chloroform/methanol (2:1, v/v) by the Folch procedure (Folch *et al.*, 1957). The chloroform phase was separated and concentrated by rotary evaporation. For analysis of whole liver fatty acids a sample was taken for saponification, and trans-esterification and g.l.c. analysis of the fatty acid methyl esters were done as described previously (Esterbauer *et al.*, 1982). For analysis of the phospholipid-derived fatty acids, a sample of the chloroform extract was separated on a silica gel G t.l.c. plate (Merck, cat. no. 5187) that had been previously sprayed with Rhodamine B and activated at 100 °C; development was in hexane/diethyl ether/acetic acid (40:10:1, by vol.). The zone corresponding to phospholipids was scraped off, eluted and subjected to the same trans-esterification and g.l.c. analysis procedure already referred to. In the above procedures, diheptadecanoyl-L-α-phosphatidylcholine and heptadecanoic acid were used as internal standards. For analysis of liver triglycerides, a sample of chloroform extract was dried down, redissolved in ethanol and assayed using a test kit from Boehringer–Mannheim (cat. no. 124 966).

Liver microsomal fatty acids were extracted and analysed as for the homogenates. Microsomal α-tocopherol was extracted and assayed using a method based on that of Burton *et al.* (1983). Microsomal suspension (1 ml) was mixed with 2 ml of SDS (25 mM), 3 ml of ethanol and 1 ml of n-heptane. After rotary mixing for 1–2 min, the mixture was centrifuged briefly and the heptane phase was taken for h.p.l.c. analysis of α-tocopherol (Burton *et al.*, 1985*a*) and for measurement of total microsomal lipid-soluble antioxidant activity by the inhibited styrene oxidation method (Burton *et al.*, 1983).

Other procedures

Thymidine kinase activity was measured in the post-microsomal supernatant (cytosol) fractions essentially as described by Ives *et al.* (1969) with the following slight modification: non-phosphorylated [^{3}H]thymidine was removed from the ion-exchange filter paper discs (Whatman DE81) by mounting them in a Millipore filtration apparatus and washing each of them with 45 ml of 1 mM-ammonium formate and 30 ml of distilled water, drawn through by suction. Protein was determined in the various liver fractions by the method of Lowry *et al.* (1951).

RESULTS

Regeneration of liver mass and lipid changes

Following partial hepatectomy, the remaining minor lobes of the liver grow rapidly in order to restore the original liver mass. In the sham-operated rats the weight of the minor lobes was on average about 4 g during the period of study, whereas in the partially hepatectomized rats these lobes weighed about 8 g at 48 h post-operation and about 10.5 g at 96 h post-operation. As reported by Cheeseman *et al.* (1988*b*), the rate of regeneration of liver mass follows a somewhat step-wise time course indicative of circadian influences. In the period 24–48 h post-operation, the regenerating livers appear pale in colour due to triacylglycerol accumulation, which is maximal at 36 h at some 430% of the control value (Fig. 1). This triacylglycerol accumulation is paralleled by changes in the fatty acid composition of the total lipid extracted from the whole liver: transient elevations in palmitic,

Table 1. Fatty acid composition of liver microsomal lipids at various times after partial hepatectomy

Lipids were extracted from liver microsomes of sham-operated or partially hepatectomized (Hep) rats and the fatty acid composition was determined; for experimental details see the Methods section. All values are means ± S.E.M. for groups of 6–9 animals. Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Hep versus Sham).

Post-operation time (h)	Total fatty acid content (μg/mg of protein)		Fatty acid composition (% of total fatty acids)											
			$C_{16:0}$		$C_{18:0}$		$C_{18:1}$		$C_{18:2}$		$C_{20:4}$		$C_{22:6}$	
	Sham	Hep	Sham	Hep	Sham	Hep	Sham	Hep	Sham	Hep	Sham	Hep	Sham	Hep
12	283.6±35.5	254.9±20.9	22.7±1.3	22.4±0.3	21.9±0.7	19.1±1.1	9.5±0.7	10.2±0.1	15.5±0.9	18.6±0.7*	20.7±1.1	19.2±0.9	7.4±0.3	6.5±0.2*
24	249.5±15.0	246.2±21.1	23.4±0.5	22.5±0.8	23.6±1.3	21.1±0.6	8.5±0.7	12.0±1.6	14.2±0.4	17.6±0.5***	20.7±0.9	18.4±0.7	6.4±0.4	5.9±0.4*
36	256.1±12.1	249.6±11.3	21.2±0.6	22.8±1.4	25.5±1.0	23.2±0.9	8.8±0.5	10.0±0.4	13.5±0.7	17.5±0.3***	24.4±1.0	20.0±0.7**	6.6±0.4	6.6±0.5
48	313.7±50.0	372.2±45.8	23.7±0.9	22.8±0.5	22.3±0.6	17.9±1.7*	10.7±0.4	14.3±1.4*	17.7±0.8	21.2±1.8	19.1±1.5	16.4±2.0	6.6±0.4	7.3±0.4
60	331.5±22.1	260.9±16.2*	22.2±0.3	23.4±0.5*	23.2±0.2	21.8±0.4*	8.4±0.3	9.4±0.5	14.9±0.2	15.2±0.3	22.9±0.2	20.5±0.4***	8.4±0.1	9.7±0.4

Table 2. Effect of partial hepatectomy on the ratio of stearic acid/oleic acid in various liver lipid fractions

The fatty acid composition of liver homogenates, whole liver phospholipid fractions and liver microsomes from sham-operated or partially hepatectomized (Hep) rats were determined and the stearate/oleate ratio was calculated. All values are means ± S.E.M. with number of animals in parentheses. Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Hep versus Sham groups).

Post-operation time (h)	Stearic acid/oleic acid ratio					
	Total liver lipid		Phospholipid		Microsomal lipid	
	Sham	Hep	Sham	Hep	Sham	Hep
12	1.64±0.10 (6)	1.29±0.05 (6)**	3.03±0.14 (6)	2.38±0.13 (6)**	2.07±0.37 (4)	1.96±0.10 (5)
24	1.67±0.14 (9)	0.60±0.04 (9)***	3.21±0.22 (6)	2.77±0.16 (6)	2.85±0.18 (9)	2.36±0.29 (9)
36	1.35±0.19 (6)	0.38±0.03 (6)***	3.12±0.21 (6)	2.72±0.44 (6)	2.96±0.21 (6)	2.38±0.19 (6)
48	1.18±0.07 (9)	0.53±0.04 (9)***	2.68±0.35 (9)	2.29±0.23 (9)	2.14±0.12 (8)	1.29±0.19 (9)**
60	1.48±0.05 (6)	0.61±0.10 (6)***	3.20±0.13 (6)	2.78±0.13 (6)*	2.76±0.11 (5)	2.34±0.13 (6)

oleic and linoleic acids are evident with maxima again at 36 h post-operation (Fig. 2). No significant changes in the fatty acid composition of the whole liver phospholipids were seen (results not shown), and only minor changes in the fatty acid composition of the microsomal lipids of the regenerating liver were apparent (Table 1). For reasons mentioned in the Discussion section, the stearic acid/oleic acid ratio was calculated for the aforementioned lipid fractions: in the whole liver lipids this ratio was greatly decreased following partial hepatectomy reaching a minimum at 36 h post-operation (Table 2). No major changes were apparent in this ratio in the whole liver phospholipids or in the microsomal lipids (Table 2).

Lipid peroxidation and antioxidants

NADPH/ADP/iron-dependent lipid peroxidation was measured by oxygen uptake in liver microsomes from partially hepatectomized or sham-operated rats. In all

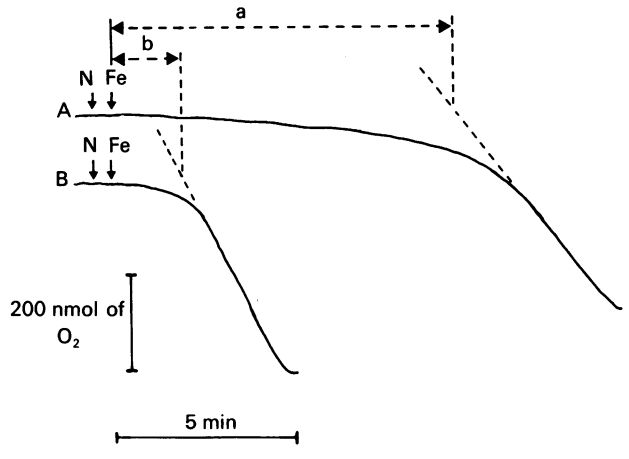

Fig. 3. Examples of recordings of oxygen uptake during NADPH/ADP/iron-induced liver microsomal lipid peroxidation

A, microsomes from regenerating liver 48 h after partial hepatectomy; B, liver microsomes from sham-operated rats at the same time point. Broken lines indicate the measurement of the respective induction periods in 'a' and 'b' for samples A and B. N, NADPH addition; Fe, ADP/iron addition.

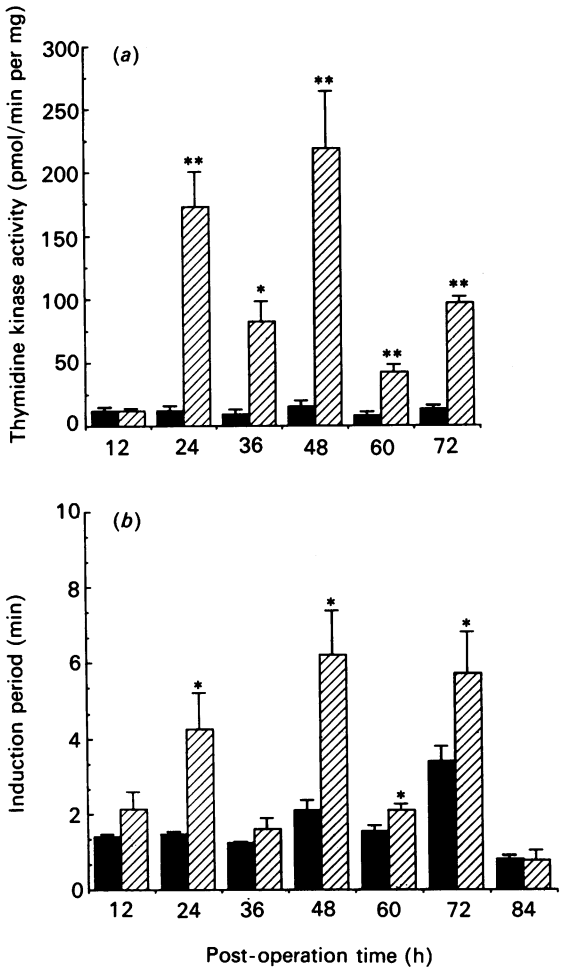

Fig. 4. Cyclical changes in (*a*) thymidine kinase activity and (*b*) the induction period of NADPH/ADP/iron-induced microsomal lipid peroxidation in liver samples from sham-operated (solid bars) and partially hepatectomized (hatched bars) rats

Mean values are given ± S.E.M. The number of rats used for thymidine kinase assays was three at 12 h, nine at 48 h and six at other time points; for induction period assays the number of rats was 17 at 24 h, nine at 48 h and six for other time points. * $P < 0.01$ and ** $P < 0.001$ (Student's *t* test) for differences between hepatectomized and sham operated groups.

Table 3. Ascorbate/iron-induced microsomal lipid peroxidation at various times after partial hepatectomy

Liver microsomes prepared from sham-operated or partially hepatectomized (Hep) rats were incubated with ascorbate (500 μM) and iron (5 μM) at 37 °C for either 5 (*a*) or 15 min (*b*). Lipid peroxidation was measured by the TBA test (see the Methods section). All values are means ± S.E.M. with numbers of animals in parentheses. Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Post-operation time (h)	Lipid peroxidation			
	a (nmol of MDA/5 min per mg)		*b* (nmol of MDA/15 min per mg)	
	Sham	Hep	Sham	Hep
12	—	—	22.94 ± 1.97 (6)	15.60 ± 2.15 (6)*
24	11.34 ± 4.53 (14)	4.86 ± 5.27 (21)**	22.40 ± 1.73 (12)	14.30 ± 3.17 (14)**
36	15.51 ± 1.55 (6)	12.38 ± 1.86 (6)	30.00 ± 1.10 (6)	20.43 ± 2.43 (6)**
48	4.78 ± 1.99 (6)	0.22 ± 0.13 (6)**	19.45 ± 2.73 (9)	3.75 ± 1.91 (9)***
60	15.45 ± 0.62 (3)	9.59 ± 4.61 (3)	36.30 ± 2.30 (6)	22.50 ± 2.22 (6)**
72	2.29 ± 0.99 (7)	0.17 ± 0.04 (8)*	15.74 ± 2.97 (8)	7.83 ± 2.82 (8)
84	9.72 ± 0.72 (4)	9.15 ± 1.90 (4)	16.84 ± 0.21 (4)	17.70 ± 0.74 (4)

cases an induction period was apparent between adding ADP/iron to the microsomal suspension plus NADPH and the maximum rate of lipid peroxidation; examples are shown in Fig. 3. It was obvious that at certain post-operation times (24 h, 48 h and 72 h), there was marked and significant extension of the induction period in the microsomes from regenerating livers relative to the control microsomes from sham-operated animals. The length of the induction period was measured (min) and found to exhibit a marked periodicity in microsomes from regenerating livers with maxima at 24 h, 48 h and 72 h post-operation and minima at the intervening 12 h, 36 h, 60 h and 84 h time points (Fig. 4). This cyclical change in the induction periods was apparent to a relatively minor degree (with the exception of the 72 h time point) in the liver microsomes from sham-operated controls. The maximum rate of NADPH/ADP/iron-induced lipid peroxidation had a mean value of around 70 nmol of O_2/min per mg of protein, and there was no significant difference between the two groups, though there was a tendency for lower maximum rates in samples with extended induction periods (results not shown).

Ascorbate/iron-induced microsomal lipid peroxidation was measured by MDA production after 5 min and 15 min of incubation. MDA production after 5 min of incubation exhibited marked periodicity with minima at 24 h, 48 h and 72 h post-operation in liver microsomes from both partially hepatectomized and sham-operated rats (Table 3). MDA production was significantly lower in microsomes from regenerating livers compared with liver microsomes from sham-operated controls. Using

Table 4. CCl_4-induced lipid peroxidation in liver microsomes prepared from partially hepatectomized (Hep) or sham-operated rats

Lipid peroxidation was measured as MDA production during a 15 min incubation as described in the text. Values are means ± S.E.M. for numbers of animals in parentheses.

Post-operation time (h)	CCl_4-induced lipid peroxidation (nmol of MDA/min per mg of protein)	
	Sham	Hep
12	0.18 ± 0.03 (6)	0.23 ± 0.03 (6)
24	0.11 ± 0.03 (9)	0.13 ± 0.02 (9)
36	0.12 ± 0.01 (6)	0.13 ± 0.01 (6)
48	0.17 ± 0.02 (9)	0.14 ± 0.01 (9)
60	0.21 ± 0.02 (6)	0.13 ± 0.02 (6)

Table 5. Total lipid-soluble antioxidant and α-tocopherol in liver microsomes from partially hepatectomized (Hep) or sham-operated rats

Total lipid-soluble chain-breaking antioxidant and α-tocopherol were extracted into heptane and determined as described in the Methods section. All values are means ± S.E.M. with numbers of animals in parentheses. Student's *t* test for Sham versus Hep: * $P < 0.05$; ** $P < 0.01$. Within the Hep group: † $P < 0.01$ (36 h versus 24 h); ‡ $P < 0.001$ (36 h versus 48 h); § $P < 0.002$ (48 h versus 60 h).

Post-operation time (h)	Total antioxidant (nmol/mg of lipid)		α-Tocopherol (nmol/mg of lipid)	
	Sham	Hep	Sham	Hep
24	1.49 ± 0.18 (7)	2.35 ± 0.36 (7)*	1.43 ± 0.16 (7)	2.44 ± 0.32 (7)**
36	1.59 ± 0.11 (7)	1.49 ± 0.07 (7)†	1.47 ± 0.07 (7)	1.36 ± 0.08 (4)†
48	2.07 ± 0.16 (7)	2.15 ± 0.11 (7)‡	1.93 ± 0.13 (6)	2.23 ± 0.13 (7)‡
60	1.10 ± 0.17 (4)	1.17 ± 0.09 (4)§	1.05 ± 0.22 (4)	1.30 ± 0.14 (4)§

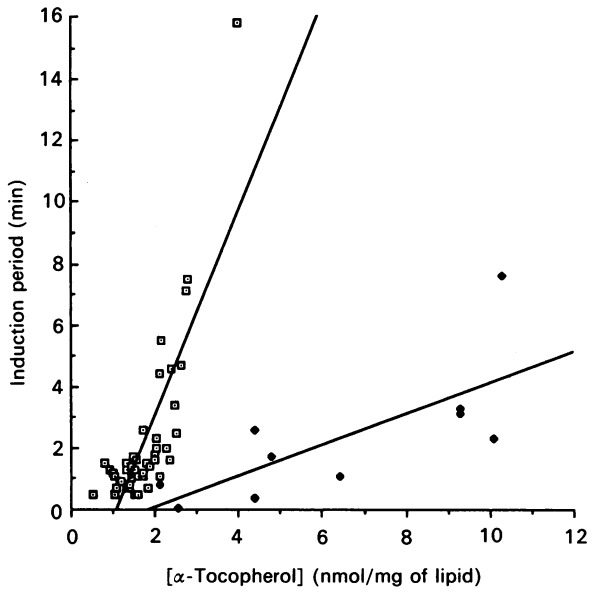

Fig. 5. Correlation of the induction period in the NADPH/ADP/iron-induced microsomal lipid peroxidation system with microsomal α-tocopherol content

Rats from the regenerating liver experiments (□; $r = 0.81$) and control rats dosed with varying amounts of α-tocopherol (◆; $r = 0.73$) in order to artificially increase the liver microsomal α-tocopherol concentration were investigated.

the 15 min incubation time, a similar but less pronounced pattern of change was apparent.

In contrast, CCl_4-induced lipid peroxidation was not significantly different in the regenerating liver microsomes compared with the corresponding sham groups at any of the time points studied (Table 4).

The lipid-soluble antioxidant content of the liver microsomes over the post-operation period of 24–60 h was determined by the inhibited styrene oxidation method (Burton *et al.*, 1983) and by measuring the α-tocopherol content (Table 5). Virtually all of the lipid-soluble antioxidant in each sample could be accounted for by α-tocopherol. There was clear indication of cycling in the α-tocopherol content of the regenerating liver microsomes, with maxima at 24 h and 48 h; this cycling was not evident in the liver microsomes from sham-operated rats. However, the microsomal α-tocopherol content in the regenerating livers was significantly elevated above the control value only at 24 h post-operation. The induction periods measured in the NADPH/ADP/iron lipid peroxidation system were found to correlate strongly with the α-tocopherol content of the liver microsomes (Fig. 5). In a completely separate group of unoperated control rats a range of microsomal α-tocopherol levels was obtained by injection of various doses of the vitamin 16 h before killing the rats. Whereas a correlation between induction period and α-tocopherol concentration was again strongly apparent, the linear relationship between the two parameters was not the same as in the regenerating liver microsomes (Fig. 5). Thus a given level of α-tocopherol in the regenerating liver microsomes was associated with a longer induction period than the same level of α-tocopherol in the liver microsomal suspensions prepared from unoperated 'dosed' rats.

Microsomal enzymes

Liver microsomal NADPH:cytochrome *c* reductase activity was not significantly different in the two groups at any time point and no periodic cycling was apparent (Table 6). The cytochrome *P*-450 contents of the liver microsomes were significantly depressed 48 h and 60 h after partial hepatectomy compared with sham-operated controls (Table 6). Again, no cycling in *P*-450 content was apparent. There was no significant difference between the two groups at any time point with regard to microsomal protein contents per g wet wt. of liver (Table 7).

Thymidine kinase

Thymidine kinase activity measured in the post-microsomal supernatant was greatly elevated in the regenerating livers compared with sham-operated controls. The thymidine kinase activity showed a very marked periodicity with maxima at 24 h, 48 h and 72 h post-operation and minima at 12 h, 36 h and 60 h post-operation. Fig. 4 shows the close temporal association between this thymidine kinase activity and the induction periods in the NADPH/ADP/iron-dependent

Table 6. Cytochrome *P*-450 content and NADPH:cytochrome *c* reductase activity in liver microsomes prepared from partially hepatectomized (Hep) or sham-operated rats

For experimental details see the Methods section. All values are means ± S.E.M. with numbers of animals in parentheses. Student's *t* test: * $P < 0.05$; ** $P < 0.01$ versus corresponding sham group; † $P < 0.01$ for 48 h sham group versus 36 h and 84 h sham groups.

Post-operation time (h)	NADPH:cytochrome *c* reductase (nmol/min per mg of protein)		Cytochrome *P*-450 (nmol/mg of protein)	
	Sham	Hep	Sham	Hep
12	86.4 ± 12.4 (6)	82.9 ± 6.6 (6)	0.55 ± 0.04 (6)	0.56 ± 0.02 (6)
24	91.4 ± 6.2 (12)	91.1 ± 6.0 (14)	0.50 ± 0.03 (12)	0.48 ± 0.02 (14)
36	85.3 ± 6.1 (6)	86.1 ± 4.5 (6)	0.47 ± 0.02 (6)†	0.41 ± 0.02 (6)
48	135.4 ± 20.6 (9)	118.1 ± 15.3 (9)	0.72 ± 0.07 (9)†	0.49 ± 0.04 (9)*
60	101.3 ± 10.4 (6)	92.4 ± 9.2 (6)	0.62 ± 0.03 (6)	0.41 ± 0.01 (6)**
72	140.5 ± 22.3 (8)	132.6 ± 25.1 (8)	0.54 ± 0.04 (8)	0.33 ± 0.02 (8)**
84	78.5 ± 5.2 (3)	53.8 ± 1.2 (4)	0.40 ± 0.03 (3)†	0.22 ± 0.02 (4)

Table 7. Protein content of liver microsomal fractions at various times after operation for partial hepatectomy (Hep) or a sham procedure

All values are means ± S.E.M. for numbers of animals in parentheses.

Post-operation time (h)	Microsomal protein (mg of protein/g of liver	
	Sham	Hep
12	19.3 ± 1.3 (6)	22.0 ± 2.7 (6)
24	21.7 ± 0.6 (14)	20.5 ± 0.5 (14)
36	20.7 ± 1.9 (6)	21.9 ± 1.7 (6)
48	17.2 ± 1.6 (9)	16.7 ± 1.3 (9)
60	18.0 ± 1.0 (6)	19.0 ± 1.2 (6)
72	20.8 ± 2.1 (8)	17.6 ± 1.5 (8)
84	23.6 ± 0.6 (4)	21.1 ± 0.8 (4)

microsomal lipid peroxidation system. The values given in Fig. 4(*a*) are expressed as pmol/min per mg of protein. No change occurs in the pattern of results when the values are expressed as pmol/min per g of liver, since there is no significant difference in cytosolic protein/g of liver between the sham-operated and partially hepatectomized groups of rats at equivalent time points (results not shown).

DISCUSSION

Following removal of the two major lobes of the rat liver, the remaining minor lobes rapidly undergo a hyperplastic hypertrophy; many studies have been carried out in attempts to elucidate the controlling mechanisms for this liver regeneration (Bucher & Malt, 1971; Allison, 1986).

A complex and interactive set of biochemical reactions is set into operation in the residual minor lobes of the liver following partial hepatectomy. A number of growth-stimulating factors have been identified (Rixon *et al.*, 1979; Nakamura *et al.*, 1984; Tei *et al.*, 1984; Diaz-Gil *et al.*, 1986; Francavilla *et al.*, 1986; Jones, 1987), and changes in the levels of *c*-myc and *c*-ras proteins are observed (Goyette *et al.*, 1983; Makino *et al.*, 1984), Moreover, studies of gene transcription and translation have indicated quantitative changes in some genes normally expressed only at very low levels (Huber *et al.*, 1986). From this highly complicated variety of important events we have presented data here on a limited but important aspect: possible interactions between DNA synthesis and antioxidant content.

Following entrainment of rats to a strict light/dark cycle, with restricted access to food, there is a marked periodicity in DNA synthesis and in cell division (Hopkins *et al.*, 1973). Using this model to study processes associated with liver cell division, we have found similar marked periodicities in the response of liver microsomal suspensions to pro-oxidant conditions. For example, as shown in Fig. 4, microsomal lipic peroxidation stimulated by NADPH/ADP/Fe^{2+} shows cyclical variations in induction periods that correlate very well in time with changes in thymidine kinase activity. Since the induction period is a measure of antioxidant activity (Burton *et al.*, 1985*b*), this means that high thymidine kinase activity in liver cytosol is associated in time with higher antioxidant levels in liver microsomes. A similar cyclical variation also occurred when microsomal lipid peroxidation was stimulated by ascorbate/iron (Table 3). In this case, the experimental measurement was of TBA-reacting material and was directly related to the extent of lipid peroxidation, as opposed to the induction period, which relates to antioxidant content. The relatively short incubation time of 5 min was deliberately chosen so as to allow the effect of an increased microsomal antioxidant concentration to be manifest; incubation times significantly longer than the induction period would tend to obscure the presence of increased antioxidant. With the ascorbate/iron system, and with microsomes prepared from regenerating livers, there was very little MDA production at 24, 48 and 72 h: With this method, however, microsomal suspensions prepared from livers following 'sham' operations also exhibited circadian cycling, but the values at times corresponding to peaks of thymidine kinase activity were never so low as found after partial hepatectomy (Table 3).

Of interest is the finding (Table 4) that CCl_4-stimulated lipid peroxidation does not change during the period of liver regeneration studied here. This implies that fluctuations of α-tocopherol (Table 5) are in regions of the microsomal membrane spatially distinct from the locus of CCl_4 activation.

Several previous studies have reported changes in lipid peroxidation during liver regeneration (Wolfson *et al.*, 1956; Pal'mina & Burlakova, 1970; Hino *et al.*, 1974; Cockerill *et al.*, 1983; Ueda *et al.*, 1983; Gavino *et al.*, 1985; Cheeseman *et al.*, 1986*b*). With the exception of our own preliminary report (Cheeseman *et al.*, 1986*b*), and Wolfson *et al.* (1956), who studied ascorbate-stimulated peroxidation in liver homogenates at 10, 48 and 144 h post-operation, none of these studies produced evidence of periodic changes in peroxidation correlated in time with indicators of DNA synthesis such as thymidine kinase activity as found here; Pal'mina & Burlakova (1970) and Burlakova *et al.* (1971), however, have made a careful study of changes in antioxidant activity during liver regeneration. We believe that the use of the entrained rat schedule of Hopkins *et al.* (1973) has been crucial in allowing the close relationship in time between stimulated peroxidation and thymidine kinase activity to be uncovered.

During the early period of liver regeneration (0–48 h) there is a major accumulation of lipid in the liver (Fig. 1); this is known to be mainly triacylglycerol (Glende & Morgan, 1968). The microsomal membrane fraction, however, undergoes only minor changes in fatty acid content and composition (Table 2). Thus, the marked cyclical changes in lipid peroxidation referred to above are not the result of cyclical changes in the polyunsaturated fatty acid substrates of lipid peroxidation. The ratio of stearic/oleic acids in whole liver samples and in microsomal suspensions was calculated (Table 2) since it has been reported that this ratio is significantly decreased in rapidly growing rat liver tumours (Cheeseman *et al.*, 1986*a*, 1988*a*) and in erythrocytes of cancer patients (Wood *et al.*, 1985) compared with the corresponding normal samples. Although there is a substantial change in the ratio in the whole liver value obtained for regenerating liver compared with the corresponding sham (Table 2), there are no changes in the ratio in total liver phospholipid or in microsomal total lipid. Presumably,

the change in the whole liver value is due to alterations in the composition of triacylglycerol that is known (Glende & Morgan, 1968, see Fig. 1) to increase markedly in regenerating liver. The microsomal membrane fraction does not show marked differences between the sham and regenerating groups in terms of protein content (Table 7) or the NADPH/flavoprotein activity measured by cytochrome *c* reduction (Table 6). In addition, although cytochrome *P*-450 (Table 6) does decrease in the regenerating group in later stages of regeneration (48 h onwards), there is no indication of a cyclical pattern of change that might relate to the periodic fluctuations in lipid peroxidation. Cytochrome *P*-450 is known to be capable of initiating new free radical events, for example by metabolism of lipid hydroperoxides (Svingen *et al.*, 1979).

The relatively small decrease observed in cytochrome *P*-450 content is consistent with a number of previous studies that have reported decreases in approx. 30% during the first days of regeneration (Barker *et al.*, 1969; Cockerill *et al.*, 1983; Babany *et al.*, 1985). Some considerable fluctuations were found here (Table 6) in the sham group means, unlike the constancy reported by Cockerill *et al.* (1983). Cytochrome *P*-450 is known to undergo circadian variations in normal rat liver (Slater, 1978).

Measurements of total lipid-soluble chain-breaking antioxidants in the microsomal suspensions (Table 5), and in whole liver and in isolated hepatocytes (results not shown) of sham and regenerating groups, have shown that this is almost entirely due to the content of α-tocopherol. In this sense, the present results are identical with our previous studies on normal liver and liver tumours (Cheeseman *et al.*, 1986*a*, 1988*a*). Although there is evidence of periodic changes in the α-tocopherol content of microsomal suspensions during regeneration (Table 5) the fluctuations are not large in comparison with the sham group values. Nonetheless, the variations found in the regenerating group point very clearly to a net transport of α-tocopherol in and out of the regenerating liver microsomal membranes during regeneration. This is an unexpected finding and warrants further study. In relation to transport of α-tocopherol, it is interesting to note that an α-tocopherol binding protein has been reported in liver cytosol (Rajaram *et al.*, 1973; Catignani, 1975) and that the kinetics of secretion of α-tocopherol into the extracellular fluid and into bile have been studied (Bjorneboe *et al.*, 1987).

There are not many results in the literature concerning changes in lipid-soluble antioxidant content in liver during regeneration, although several measurements have been made of catalase, superoxide dismutase and glutathione transferase and peroxidase activities; these are generally decreased (Lamy *et al.*, 1973; Peskin *et al.*, 1977; Dreosti & Record, 1978; Oberley *et al.*, 1978; Bartkowiak & Bartkowiak, 1981; Ueda *et al.*, 1983; Liott *et al.*, 1987; Rossi *et al.*, 1987). Glutathione itself has been reported to be little changed during regeneration (Rosi *et al.*, 1981; Roberts *et al.*, 1983), although we have found a significant increase in the GSH content of isolated hepatocytes prepared from regenerating liver compared with the sham-operated controls (results not shown). Two studies have been concerned with α-tocopherol but neither has been with the entrained rat model used here (Ueda *et al.*, 1983; Gavino *et al.*, 1985), and neither showed data consistent with cyclical variations in content.

Although the fluctuations in α-tocopherol content of regenerating microsome suspensions are not substantial (Table 5) the α-tocopherol content correlates very strongly with the induction period for ADP/iron-stimulated lipid peroxidation (Fig. 5); it is this induction period that shows marked periodic variations during regeneration (Fig. 4). A critical point, however, is that supplementation of normal rats with α-tocopherol yields a correlation with induction periods that has a completely different slope (Fig. 5). The implication of this is that some other feature of the microsomal membrane in the regenerating liver is also cycling periodically, and that this additional feature works together with α-tocopherol to modify greatly the induction period. This additional feature cannot be a conventional lipid-soluble chain-breaking antioxidant, as already discussed above in relation to the results in Table 5. One possibility is that it is concerned with recycling the α-tocopherol free radical in a way previously discussed for ascorbate (Tappel, 1968; Packer *et al.*, 1979), or perhaps it may be a glutathione-related phenomenon (McCay *et al.*, 1976; Burk *et al.*, 1980; Channa Reddy *et al.*, 1982; Ursini *et al.*, 1982; Burk, 1983; Hill & Burk, 1984; Tan *et al.*, 1984).

In summary, the results of this study have shown that in the regenerating liver there are marked cyclical variations in the ability of the microsomal suspensions to undergo lipid peroxidation. Maximal rates of peroxidation are observed at times of low thymidine kinase activity, and vice versa. Thus, as previously observed with liver tumours (Cheeseman *et al.*, 1986*a*, 1988*a*), the onset of DNA synthesis and the preparation for mitosis are linked to an increased level of antioxidant and a down-regulation of lipid peroxidation. This is consistent with the hypothesis (Slater, 1978; Slater *et al.*, 1984) that these phenomena are interdependent.

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