NADPH and Ascorbate Catalyzed Peroxidation of Microsomal Lipids During Ageing

Lipid peroxides and malonyldialdehyde (MDA) are formed in microsomes during NADPH and ascorbate oxidation[1]. These reactions are somewhat different in that NADPH is linked to the microsomal drug hydroxylating enzyme system[2] whereas ascorbate appears to utilize only minor portions of this chain[1,3]. Ascorbate causes disarrangement of membranes with diminution of phospholipid content[4].

Increased formation of lipid peroxides is considered one of the main cell-deranging events during ageing[5,6], resulting in lipofuscin accumulation[7]. Since the activity of the microsomal drug metabolizing enzyme system and the NADPH oxidation decrease in rats from 30 up to 250 days of age[8] and microsomes are the chief peroxidizing site in the cell[9], lipid peroxide formation by rat liver microsomal preparations in the presence of NADPH and ascorbate has been investigated at 20, 120, 400 and 600 days of age by measuring MDA.

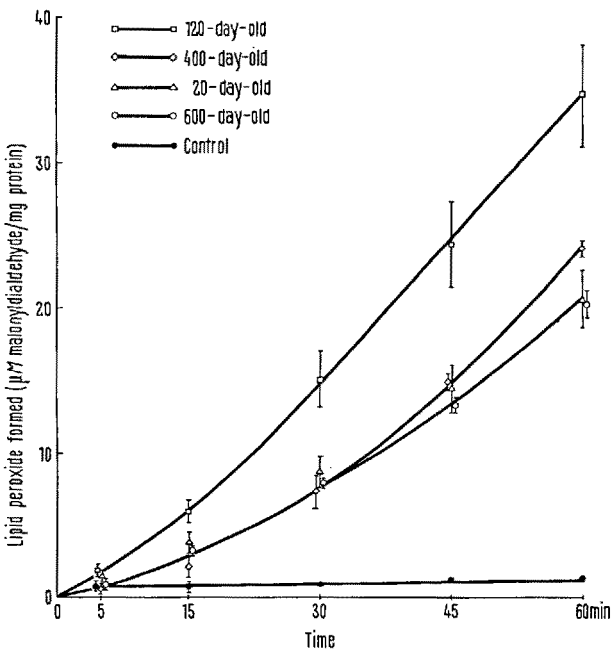

Fig. 1. Lipid peroxide formation in the presence of NADPH and ADP-Fe. Each point represents an average with S.E. (vertical bars) of 3 experiments.

Male Wistar rats fed on a commercial pelleted diet and water ad lib. were used. Animals were employed without previous starvation and microsomes obtained according to WILLS[1]. Livers were homogenized in 0.25 *M* sucrose, centrifuged at 750 *g* and 5000 *g* for 10 min and then at 105,000 *g* for 60 min in a Spinco preparative ultracentrifuge. Microsomal pellets were suspended in 125 m*M* KCl and diluted to a protein concentration of 0.5–0.7 mg (estimated colorimetrically by the method of LOWRY et al.[10]) per 0.3 ml sample employed. Incubations were carried out at 37 °C for 60 min with NADPH 40 μ*M*, ADP-Fe 3 m*M*[11] (Boehringer, Mannheim), Na-phosphate buffer 20 m*M* pH 7.0 and KCl 90 m*M*. When employed, ascorbate 0.5 m*M* replaced NADPH and ADP-Fe and final pH was 6.0. Samples of the incubation mixture were taken at intervals and the reactions stopped with 5% TCA. MDA was determined on the supernatants with 0.67% 2-thiobarbituric acid at 80 °C for 15 min[12]. MDA was calculated using 1,1,3,3-tetraethoxypropane standards[13].

As is shown in the Figures, little lipid peroxide is formed by control microsomes. When NADPH and ADP-Fe (Figure 1) or ascorbate (Figure 2) are added formation of lipid peroxide is greatly increased[1]. Lipid peroxide is greater in the presence of NADPH than ascorbate[1,11] except in 600-day-old rats. From Figure 1

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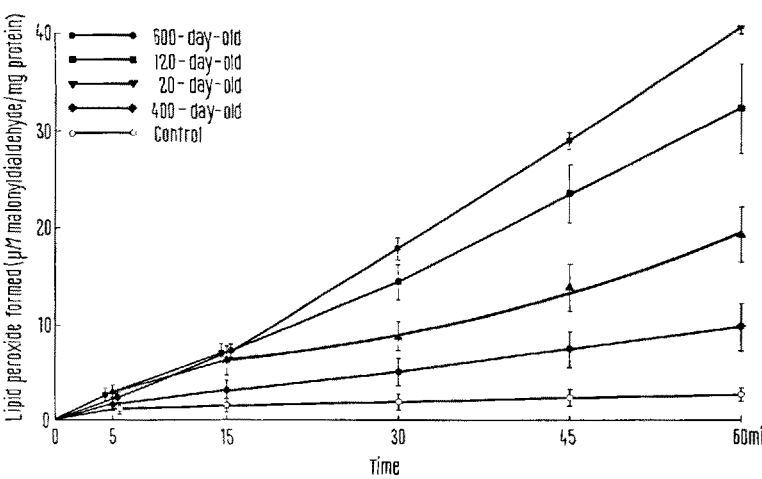

Fig. 2. Lipid peroxide formation in the presence of ascorbate. Each point represents an average with S.E. (vertical bars) of 3 experiments.

it is evident that the peroxide formed in the presence of NADPH is less in rats aged 20 and 600 than 120 and 400 days. These results are in keeping with those indicating a decreased activity of the microsomal drug metabolizing enzyme system in infancy and during ageing of rats[8] and the increased lag period of the NADPH:cytochrome c reductase induction with increasing age[14].

In the presence of ascorbate (Figure 2) results are somewhat different than with NADPH and a tendency toward an increased peroxidation of microsomal lipids by this substance with increasing age is presented. Sole exceptions are the 400-day-old rats, showing minor lipid peroxide values. This rather paradoxical result is difficult to explain. It might be due to better preservation and function of the structural subunits of microsomal membranes at this age period, as shown for the tryptophan pyrrolase activity[15], or to higher protecting effects by thiol groups[16].

Lipid peroxidation in the presence of ascorbate has been shown to release the bound hydrolases from lysosomal granules[12]. A similar increased release occurs also during ageing[17]. Present results suggest that an increased susceptibility of subcellular membranes to the disrupting action of ascorbate occurs in old rat tissues. This might be due to a lower concentration of GSH[16] and other thiol compounds during ageing[18]. An increased activity of liver glutathione peroxidase has been demonstrated in this condition[19]. On the contrary, Vitamin E deficiencies do not occur in the liver of senescent rats[20].

Riassunto. La formazione di lipoperossidi nei microsomi di fegato di ratto in presenza di NADPH diminuisce con la senescenza. Il contrario sembra invece avvenire in presenza di ascorbato. Ciò potrebbe indicare una maggiore suscettibilità delle membrane microsomiali a fenomeni lipoperossidativi aspecifici in tale condizione.

R. Comolli

Istituto di Patologia generale dell'Università di Milano, e Centro di Patalogia cellulare del C.N.R., Via Mangiagalli 31, I-20133 Milano (Italy), 10 March 1971.

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Liver Damage by Protracted Ethanol Uptake and its Reversibility

The observation that a fatty liver is often associated with alcoholism is old. Nevertheless, the mechanisms involved in the accumulation of lipids in the liver and its relation to liver cirrhosis remain the subject of much debate[1–3].

We believe that the problem involves both neutral fats and phospholipids, and that the changes of the two lipid classes are probably disjuncted for certain aspects.

After all, our theory agrees with the observations of French[4] that phospholipid modifications are concomitant with glyceride accumulation.

We believe it is interesting to study the damage to the liver of alcohol given in different quantities, 10 and 20% in water as a drink for 2 weeks; enough time to cause changes in total lipids and phospholipids, and we intended to see if this damage disappears during the two following weeks after stopping of the alcohol administration.

Materials and methods. We have used 30-day-old female rats of the Wistar strain divided into groups of 6 individuals each, and carefully randomized. 2 groups received as drink 10% and 20% alcohol in water respectively for a period of 15 days. 2 additional groups received water containing sucrose in isocaloric amounts of the cited amounts of alcohol. A final group received only water.

The diet was identical under all experimental conditions and administered ad libitum, and was in agreement with the nutritional requirement for rats[5]. Temperature in the room was kept at 19 ± 2°C; air humidity was kept at 70 ± 2%.

After 15 days, alcohol administration ceased, and all groups received water as the only drink. Food and drink consumptions were calculated, and also the weight changes were combined.

On the 16th and 30th day of experiment, 6 individuals of each group were sacrificed by decapitation. Immediately after withdrawal, the livers were assayed for total lipids according to Folch et al.[6]; phospholipids were separated according to Marks et al.[7]; the P content was determined according to Hoogwinkel et al.[8].

Phospholipids were isolated and quantitatively determined by bidimensional TLC according to Abramson and Blecher[9]. The separations were controlled by standards and by P determination of the individual fractions and calculating the recovery of the amounts put on the plates.

Results and discussion. Table I shows the pattern of liver lipids, phospholipids and neutral fats of the different groups sacrified on the 16th and 30th day of experiment; the values found at the first sacrifice agree with those in the literature both for the controls and for the rats receiving alcohol. At the 30th day, at the 2nd sacrifice, we observe an appreciable reversal of the biochemical pattern, especially evident in the group receiving alcohol in lower amounts.

Total phospholipids are significantly increased at the first sacrifice both in rats receiving 10% and 20% alcohol, in respect to the controls and to the groups receiving sucrose in isocaloric amounts.

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