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.

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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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Table I. Liver lipids and phospholipids of rat on different experimental treatments

Experimental treatments	Liver lipids[a] 16th day[b]	30th day	Liver phospholipids[a] 16th day	30th day
Standard diet + water	4.12 ± 0.83[c]	4.31 ± 0.49	2.55 ± 0.12	2.71 ± 0.21
Standard diet + ethanol 10%	8.47 ± 1.93	5.10 ± 1.31	3.39 ± 0.53	2.45 ± 0.39
Standard diet + ethanol 20%	9.45 ± 2.36	6.11 ± 2.03	3.66 ± 0.62	3.05 ± 0.94
Standard diet + sucrose isocaloric with ethanol 10%	4.25 ± 0.51	4.17 ± 0.53	2.63 ± 0.28	2.58 ± 0.29
Standard diet + sucrose isocaloric with ethanol 20%	4.30 ± 0.43	4.28 ± 0.42	2.70 ± 0.31	2.65 ± 0.28

[a] g/100 g of wet liver. [b] During the first 15 days the rats received alcohol-water or sucrose-water in isocaloric quantities, and in the following 15 days all the rats received only water to drink. [c] Each value represents the mean ± S.D. of separate determinations in 6 rats.

Table II. Percentage of liver phospholipids fractions of rat on different experimental treatments

Experimental treatments		Start	Lyso-lecithin	Sphingo-myelin	Phosphatidyl-choline	Phosphatidyl-inositol and serine	Phosphatidyl-ethanol amine	Cardio-lipin
Standard diet	16th day[b]	1.25 ± 0.48[a]	1.29 ± 0.29	6.22 ± 2.13	54.62 ± 6.85	11.75 ± 3.39	19.30 ± 1.58	5.56 ± 0.85
+ water	30th day	1.15 ± 0.79	1.11 ± 0.14	6.46 ± 1.51	55.32 ± 0.02	11.89 ± 2.26	18.75 ± 1.19	5.31 ± 0.61
Standard diet	16th day	0.87 ± 0.34	2.19 ± 0.14	5.95 ± 2.33	50.86 ± 3.68	10.55 ± 0.84	24.22 ± 1.29	5.36 ± 1.01
+ ethanol 10%	30th day	1.75 ± 0.29	1.31 ± 0.25	5.75 ± 2.09	51.40 ± 5.32	12.57 ± 1.97	21.35 ± 1.17	5.87 ± 1.02
Standard diet	16th day	0.73 ± 0.29	2.38 ± 0.31	5.69 ± 2.35	50.76 ± 4.10	10.50 ± 0.55	25.02 ± 1.46	4.91 ± 0.63
+ ethanol 20%	30th day	1.17 ± 0.46	2.02 ± 0.46	6.39 ± 2.41	52.56 ± 3.85	11.71 ± 0.42	20.22 ± 0.92	5.93 ± 0.42
Standard diet	16th day	1.44 ± 0.30	1.37 ± 0.30	6.60 ± 1.63	52.71 ± 8.00	12.44 ± 1.40	20.02 ± 1.29	5.42 ± 0.76
+ sucrose isocaloric with ethanol 10%	30th day	1.07 ± 0.22	1.15 ± 0.33	6.78 ± 1.44	52.98 ± 5.22	12.49 ± 2.48	19.67 ± 0.81	5.85 ± 0.74
Standard diet	16th day	1.35 ± 0.49	1.46 ± 0.56	6.58 ± 1.42	53.55 ± 9.72	12.12 ± 2.17	19.41 ± 0.64	5.53 ± 0.67
+ sucrose isocaloric with ethanol 20%	30th day	1.49 ± 0.45	1.34 ± 0.63	6.18 ± 1.52	52.79 ± 4.25	12.37 ± 2.01	19.90 ± 1.15	5.92 ± 0.78

[a] Each value represents the mean ± S.D. of separate determinations in 6 rats. [b] During the first 15 days the rats received alcohol-water or sucrose-water in isocaloric quantities, and in the following 15 days all the rats have received only water to drink.

On the 30th day the amounts of total lipids and phospholipids are back to normal in the group receiving 10% alcohol in water, whereas in the rats receiving 20% alcohol the decrease of liver lipids is clear but not near the normal values; phospholipids approach normal amounts to a very limited extent. Table II shows the results of the separation of liver phospholipids after the 2 experimental periods. Phospholipid fractions lysolecithin, sphyngomyelin, phosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl inositol and serine, cardiolipin are greatly increased by the 16th day in both groups receiving alcohol.

The increase of each phospholipid fraction is rather uniform in the various individuals. On the 30th day there is a clear normalization of the values for the rats receiving 10% ethanol, but high values remain in those receiving 20% ethanol. We must briefly consider that food and drink consumptions were analogous in the various groups, also weight gains were similar. We have not found important differences between values of rats receiving control diet and water and the 2 groups receiving sucrose and water.

On the basis of these results, it appears possible that the biochemical damage induced by ethanol in liver phospholipids may be more severe than the accumulation of neutral fats.

We might think that among the factors inducing the transformation of steatosis into cyrrhosis a greater importance can be ascribed to phospholipid changes than to neutral fat accumulation.

Riassunto. È stato studiato l'effetto dell'etanolo assunto come bevanda a concentrazioni diverse (10 e 20%) sul ratto in fase di accrescimento. Sono stati studiati i danni determinati dall'etanolo sui lipidi epatici, in particolare a livello dei fosfolipidi delle membrane cellulari e l'entità della «restitutio ad integrum», ottenibili in queste condizioni con la sospensione dell'assunzione dell'etanolo.

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