Table III. Effect of α-tocopherol and tocopheronolactone on the hepatic NAD and $NADH_2$ level following ethanol administration[a]

Group	No. of rats	NAD (μg/g)	$NADH_2$ (μg/g)	NAD + $NADH_2$ (μg/g)	NAD/ $NADH_2$
Ethanol plus propylene glycol	6	502.0 ± 45.5	318.5 ± 90.7	821.6 ± 112.5	1.66 ± 0.44
Ethanol plus α-tocopherol	5	479.0 ± 76.2	301.7 ± 79.6	780.8 ± 104.3	1.68 ± 0.52
Ethanol plus tocopheronolactone	5	471.1 ± 55.1	202.7 ± 19.2[b]	674.2 ± 69.2	2.33 ± 0.21
No treatment	7	594.9 ± 76.2	247.4 ± 70.0	843.0 ± 138.3	2.47 ± 0.42

[a] Values are expressed as mean ± S.D. [b] The difference to ethanol-propylene group is statistically significant ($p < 0.05$).

the increase in $NADH_2$/NAD ratio in hepatic cell stimulates triglyceride synthesis[9]. From this point of view, α-tocopherol as well as tocopheronolactone may modify the ethanol-induced fatty liver through the enzyme system which couples the reduction of tocopheronolactone with the oxidation of $NADH_2$[10]. To clarify their participation on the reoxidation of $NADH_2$, levels of NAD and $NADH_2$ were examined in the ethanol-induced fatty liver with and without the treatment with α-tocopherol and tocopheronolactone. As shown in Table III, hepatic $NADH_2$ levels, which were elevated by the administration of ethanol, could be modified by the administration of ethanol, and by the treatment with tocopheronolactone, but not by the treatment with α-tocopherol. The results suggest that it is tocopheronolactone but probably not α-tocopherol that maintains NAD/$NADH_2$ ratio, but the former does not inhibit triglyceride accumulation in the liver by ethanol feeding.

Thus, the present investigation discloses that it is α-tocopherol but not tocopheronolactone that exerts an inhibitory effect on the ethanol-induced fatty liver. It seems that the major effect of α-tocopherol is not to modify the intrahepatic triglyceride metabolism but to accelerate triglyceride transport from liver to plasma.

Zusammenfassung. Es wird nachgewiesen, dass α-Tocopherol, nicht aber Tocopheronolacton, die Entstehung der Fettleber durch einmalige Alkoholbelastung bei der Ratte hemmt.

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Chloroplast Aging in vitro and Relationships to Fatty Acids and Polyphenoloxidase Activity

Since the discovery that isolated chloroplasts can undergo dark- and-light-induced swelling[1], several reports have appeared describing the conditions and the factors which affect these phenomena. One of the most interesting features of chloroplast swelling is that it occurs slowly in the dark, while it is activated in the light[1,2], and that both of these processes are irreversible in darkness[1]. These properties have suggested that swelling is the result of a deterioration process of the chloroplast membrane system[1-3]. The validity of this hypothesis has been confirmed by electron microscopic studies which have shown that light-activated and the slower dark-induced swelling cause drastic morphological changes in the architecture of the chloroplast lamellar system[4-6]. New evidences that chloroplast swelling was in essence a deteriorative ('and/or aging') process arose recently from studies on photochemical reactions in chloroplasts[2,7]. Indeed, simultaneous with the swelling phenomenon, the capacity of chloroplasts to carry out light-dependent shrinkage diminished. Also, aging of chloroplasts in the dark was accompanied by decreases in activities for photohydrolysis, cyclic and non-cyclic synthesis of ATP, photoreduction of $NADP^+$, and O_2 evolution. There was a striking parallelism between swelling rate and loss of these chloroplast activities. Moreover, chloroplast incubation in the presence of light was found to act synergetically towards the aging process observed in darkness. In many respects, these results suggested[7] that the effects of chloroplast aging were comparable to the action of fatty acids on the structure and photochemical activities of these organelles[8-11]. The purpose of this note is to bring into focus such a correlation. Also, in the same connection, a relationship between polyphenoloxidase activity (*O*-diphenol-O_2-oxydoreductase, EC. 1.10.3.1.) and loss of O_2 evolution capacity during aging was established.

Isolation of spinach chloroplasts was carried out in a solution containing 175 m*M* NaCl and 100 m*M* *Tris*-HCl (pH 8)[2]. Chloroplast ageing in vitro was obtained by incubating these organelles at 20 °C in test tubes containing the same medium either in darkness or in light (3.45×10^5 ergs $cm^{-2} \times sec^{-1}$)[2].

The first comparison was made between the effect of aging in vitro and the action of linolenic and palmitic acids on chloroplast photophosphorylation. Table I A

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shows that a 2 h aging period in darkness causes the same inhibition of this activity as a 50 μM linolenic acid treatment of fresh chloroplasts. Increasing concentration of linolenic acid up to 200 μM diminishes photophosphorylation activity down to the same level as a 2 h aging period of chloroplasts in the light. At both of these concentrations, palmitic acid has practically no inhibitory effect. The second comparison concerned light-induced shrinkage capacity. Table I B shows, as in the case of photophosphorylation activity, that a 2 h aging period in darkness inhibits by half light-induced shrinkage as does a 50 μM linolenic acid treatment. A 2-h aging period in the light and a 200 μM linolenic acid treatment accentuate the inhibitory effect down to a comparable level. Again, palmitic acid does not greatly affect this activity. The third comparison was established for O_2 evolution with aged or fatty acid treated chloroplasts. The experiments reported in Table II A lead to the same conclusions as with photophosphorylation and light-induced shrinkage.

Thus, these results reveal that there is a striking parallelism between the consequences of chloroplast aging in vitro and the action of linolenic acid on the photochemical activities tested, i.e. photophosphorylation, light-induced structural changes and O_2 evolution. These findings strengthen the view[7] that inhibition of photochemical activities during aging is caused, at least in part, by an accumulation of free fatty acids in the plastids, probably due to increased enzymatic hydrolysis of endogenous lipids during this phenomenon[8–11]. In this connection, the greater inhibition observed with chloroplasts aged in the light should be interpreted as the result of a greater accumulation of these compounds. Also, it is interesting to mention that linolenic acid, which is the more abundant fatty acid in the chloroplast lipids[13] and therefore the main degradation product of lipids during aging[10], is much more effective than palmitic acid.

Table I. Comparison of chloroplast aging in vitro and the effect of 2 fatty acids on photophosphorylation and light-induced shrinkage capacity by chloroplasts

Experimental conditions		Control	% of control	
A) Cyclic photophosphorylation				
Aging in vitro:	Dark-0 min (control)	171 [a]	100	100
	Dark-120 min		52 (5)	
	Light-120 min			8 (5)
Linolenic acid	0 μM	126 [a]	100	100
	50 μM		55 (7)	
	200 μM			7 (7)
Palmitic acid	50 μM		94 (7)	
	200 μM			90 (7)
B) Light-induced shrinkage capacity				
Aging in vitro:	Dark-0 min (control)	181 [b]	100	100
	Dark-120 min		42 (6)	
	Light-120 min			4 (6)
Linolenic acid	0 μM (control)	181 [b]	100	100
	50 μM		50 (6)	
	200 μM			26 (6)
Palmitic acid	50 μM		89 (6)	
	200 μM			88 (6)

ATP synthesis was determined by measuring the uptake of Pi (HORWITT's technique[12]) in the following basic reaction mixture: NaCl (35 mM), *Tris*-HCl (20 mM, pH 8), $MgCl_2$ (5 mM), KH_2PO_4 (0.5 mM, pH 8), ADP (0.5 mM), phenazine methosulfate (PMS, 20 μM) and chloroplasts (20 μg chlorophyll/ml) over a 10 min period during which photophosphorylation was linear. Light-induced shrinkage was estimated by measuring light-scattering increase at 90° (546 nm) with a modified[2] Photovolt fluorimeter (model 540) in the following reaction mixture: KH_2PO_4 (50 mM, pH 6), NaCl (35 mM), $MgCl_2$ (5 mM), PMS (20 μM) and chloroplasts (20 μg chlorophyll/ml). In the experiments involving fatty acids, all the reaction mixtures contained 0.5% ethanol. The number of experiments are shown in parentheses. [a] μmoles phosphate esterified/mg chl/h. [b] Increases in scattering intensity following illumination with red light are expressed as a percentage change of the initial scattering level (100).

Table II. Comparison of chloroplast aging in vitro and the effect of 2 fatty acids on O_2 evolution and polyphenoloxidase activity by chloroplasts

Experimental conditions		Control	% of control	
A) O_2 evolution				
Aging in vitro:	Dark-0 min (control)	72 [a]	100	100
	Dark-120 min		60 (5)	
	Light-120 min			7 (5)
Linolenic acid	0 μM (control)	51 [a]	100	100
	50 μM		48 (6)	
	200 μM			0 (6)
Palmitic acid	50 μM		89 (7)	
	200 μM			94 (6)
B) Polyphenoloxidase activity (O_2 fixation) [c]				
Aging in vitro	Dark-0 min (control)	17 [a]	100	100
	Dark-120 min		206 (13)	
	Light-120 min			200 (13)
Latent period	Dark-0 min (control)	7 [b]	100	100
	Dark-120 min		66 (10)	
	Light-120 min			5 (10)
Linolenic acid	0 μM (control)	17 [a,d]	100	100
	50 μM		126 (8)	
	200 μM			249 (5)
Latent period	Linolenic acid 0 μM	3 [b,d]	100	100
	Linolenic acid 50 μM		63 (8)	
	Linolenic acid 200 μM			35 (5)

O_2 evolution in the light and polyphenoloxidase activity (uptake of O_2) in the dark were determined polarographically with a Clark electrode at 20 °C. The reaction mixtures contained NaCl (35 mM), *Tris*-HCl (20 mM, pH 8), $MgCl_2$ (5 mM), K ferricyanide (1 mM), 0.5% ethanol for the fatty acids experiments and chloroplasts (20 μg chlorophyll/ml) for O_2 evolution and Na-phosphate buffer (0.1 M, pH 6.5), 4-methylcatechol as substrate (2.0 mM, pH 6.5) and chloroplasts (55 μg chlorophyll/ml) for the polyphenoloxidase activities determination. [a] Oxygen evolved or fixed in μmoles/mg Chl/h. [b] Min. [c] These experiments were performed by Mrs. P. VAUCHER. [d] For 20 μg Chl/ml in the reaction mixture.

Since polyphenoloxidase activity was found to be localized in chloroplasts[14], it was tested in aging chloroplasts in order to detect whether this activity could also be responsible for the inhibition of O_2 evolution during aging. Indeed, Table II B shows that, in the presence of 4-methylcatechol as substrate, polyphenoloxidase activity is stimulated to the same extent (100%) in 2-h dark-and light-aged chloroplasts. In view of the synergetic effect of light towards aging[3,7], it is rather surprising to find the same rate of stimulation for the chloroplasts aged

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in light and darkness. Preliminary observations indicate however that light causes first a stimulation and then an inhibition of O_2 fixation. The most striking difference between dark-and-light-aging occurs at the level of the latent period of the enzyme activity. In fresh chloroplasts, a 7 min average latent period was observed. After a 2 h chloroplast incubation in vitro, this period decreases to 66 and 5% in darkness and light, respectively. It appears that the impairment of the latent period in the light (and also to a lesser extent in darkness) reflects an advanced state of chloroplast aging which was already observed for morphological[2] and photochemical[7] parameters. In this connection, the effect of linolenic acid shows an interesting resemblance to aging. Indeed, increasing concentrations of linolenic acid accelerate the rate of polyphenoloxidase activity and diminish the latent period. Also, these results indicate a close interrelationship between inhibition of O_2 evolution and activation of polyphenoloxidase activity by a linolenic acid or aging treatment.

Thus, it appears that the increase of polyphenoloxidase activity during chloroplast aging and the discrepancy of the latent period behaviour, towards dark and light incubation of chloroplast, and, towards various linolenic acid concentrations, represent new parameters which must be taken into consideration in our study of aging of the photosynthetic apparatus in vitro[15].

Résumé. Un vieillissement in vitro de chloroplastes isolés d'épinard et un traitement par l'acide linolénique, à des concentrations croissantes, provoquent des inhibitions, comparables, de la photophosphorylation, de la capacité des plastides à se contracter et à dégager de l'O_2. De plus, ces deux traitements stimulent dans la même proportion l'activité des polyphénoloxydases. Ainsi, l'acide linolénique semble être l'un des facteurs responsables du vieillissement in vitro des chloroplastes.

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The Effects of Stimulation of the Olfactory Bulbs on the Serum Proteins of the Rat

In a previous paper[1] we found that bilateral excision or sectioning of the olfactory bulbs in the rat produced a decrease in the total serum proteins, albumin, and α- and β-globulins, while no change was observed in γ-globulin. These results led us to believe that stimulation of the olfactory bulbs could produce opposite effects. The present study has been effected to verify this hypothesis.

Materials and methods. 85 white rats of both sexes weighing from 140–220 g each and chosen from stock bred in our Institute, were used.

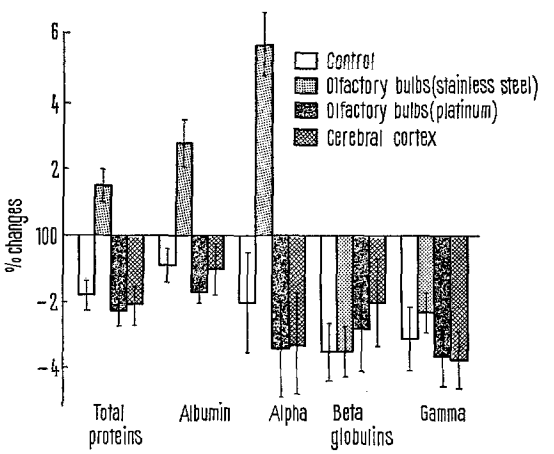
The animals were divided into 4 lots, as follows: a) bilateral insertion of stainless steel electrodes in both bulbs, no current being applied (control group – 14 subjects); b) stainless steel electrodes in both bulbs (electro-chemical stimulation – 22 subjects); c) platinum electrodes in both bulbs (6 subjects); d) bilateral insertion of stainless steel electrodes in the parietal cortex (electro-chemical stimulation – 7 subjects).

The electro-chemical stimulation was effected by means of stainless steel electrodes[2] of 0.3 mm in diameter, using a Nuclear Chicago Stimulator, model 7153, to provide monophase, square wave, direct current with an intensity of 1 mA for 10 sec.

In order to discard any possibility of the effects found being due to the lesions caused by the stainless steel electrodes instead of to the stimulation created by the iron ion deposit, platinum electrodes were also used, since the latter element does not produce a metallic ion deposit.

A stereotaxic apparatus, under visual control, was used to insert the electrodes to a depth of 1 mm in the parietal cortex and in the posterior part of the olfactory bulbs. The neutral electrode was placed in the stereotaxic apparatus close to the subject. Trepanation of the skull was performed under ether anesthesia in the area of the olfactory bulbs or in that corresponding to the parietal cortex. Total serum proteins and their subfractions were determined prior to operating and also 1, 3 and 5 h after stimulation.

The concentration of total serum proteins was determined by the biuret method, paper electrophoresis being used for that of the different subfractions. Tail sectioning



Changes in serum proteins 3 h after stimulation of the olfactory bulbs and cerebral cortex. Values are expressed in percentages of their initial value which is taken as 100%. Bars represent the mean ± S.E.

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