machen. Die geringfügige Vermehrung der Lipide in der Speicherphase von Stamm H 16 betrifft also alle Lipidklassen annähernd gleichmässig, zumal auch bei der dünnschichtchromatographischen Auftrennung der Neutrallipide und der polaren Lipide keine wesentlich verschiedenen Lipidmuster in den verschiedenen Wachstumsphasen zu finden waren. Wir möchten daher annehmen, dass die Lipidvermehrung bei Stamm H 16 nicht auf einer echten Speicherung beruht, sondern auf einer Neubildung von Membranlipiden. Eine solche Neubildung wird notwendig, da die bei Speicherung angehäufte PHBS in intrazellulären, wahrscheinlich mit Membranen umgebenen Grana deponiert wird. Bei Stamm H 16 sind solche Granamembranen bisher zwar noch nicht gesichert worden, aber in anderen Bakterienarten (*B. cereus* und *B. megaterium*) wurden sie elektronenmikroskopisch nachgewiesen[6]. Aus elektronenmikroskopischen Aufnahmen an Stamm H 16[7] geht hervor, dass sich bei beginnender Speicherung die einzelnen PHBS-Grana vergrössern und erst bei fortschreitender Speicherung miteinander verschmelzen. Bei beginnender Speicherung dürften sich daher die Granamembranen vergrössern, während bei der späteren Verschmelzung der Grana keine weitere Vergrösserung der Granamembranfläche stattfindet. Diese Vorstellung steht im Einklang mit unseren Befunden; denn wir fanden zu Beginn der Speicherphase die grösste Lipidzunahme, die um so geringer wird, je stärker die PHBS-Speicherung zunimmt.

Die beträchtliche Lipidvermehrung bei Stamm 11/x bei gleichzeitiger Speicherung von Kohlenhydraten sehen wir als echte Speicherung von Depot-Lipiden an. Denn die in der Speicherphase angehäuften Lipide bestehen überwiegend aus Neutrallipiden und diese zu über 80% aus Triglyceriden. Die Triglyceridnatur dieser Hauptkomponente wurde durch ihre Verseifbarkeit, ihr dünnschichtchromatographisches und säulenchromatographisches Verhalten sowie durch die enzymatische Glycerinbestimmung gesichert. Triglyceride sind im Bakterienreich durchaus selten; mit Sicherheit wurden sie bisher nur in Mycobakterien gefunden.

Summary. The ability to accumulate lipids was investigated in two strains of hydrogen oxidizing bacteria (Hydrogenomonas H 16 and strain 11/x). Along with the deposition of poly-β-hydroxybutyrate the amount of other lipids is shown to increase 1.8 times in strain H 16. It is suggested that the increase of the latter lipids is due to the formation of membrane lipids that are needed for the formation of membranes around the intracellular globules of poly-β-hydroxybutyrate. In strain 11/x the amount of lipids increases 7 times along with the storage of carbohydrates. In this case, the majority of lipids consists of triglycerides. It is suggested that there is a true storage of neutral fat in strain 11/x.

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Effect of α-Tocopherol and Tocopheronolactone on Ethanol Induced Fatty Liver and Triglyceridemia

The ethanol-induced fatty liver is inhibited by α-tocopherol and it is assumed that this inhibition mechanism is probably developed by the antioxidant activity inherent in α-tocopherol[1,2]. Tocopheronolactone, a water-soluble active metabolite of α-tocopherol, was found to increase the tissue ubiquinone level and to prevent the lipid peroxidation[3]. Thus, tocopheronolactone may also modify the ethanol-induced fatty liver. The present paper reports an attempt to elucidate the effects of α-tocopherol and tocopheronolactone on the fatty liver as well as the triglyceridemia after ethanol administration.

Female Wistar strain rats with an average weight of 200 g, kept on Clea MF rat diet, were used for the study. The animals were fasted for 8 h prior to receiving a single oral dose of ethanol (0.6 g/100 g of body weight as a 50% solution). Control rats were given an equivalent amount of either saline or isocaloric glucose. 10 mg/100 g body weight of α-tocopherol acetate and 6 mg/100 g body weight of equimolar tocopheronolactone (a synthetic product from the Eizai Co., Ltd., Tokyo, Japan) were dissolved in 0.1 ml of propyrene glycol and administered i.p. at 26, 12 and 2 h prior to the administration of ethanol. In this case, control rats received propylenglycol i.p. The animals were sacrificed 10 h after the ethanol administration. In the Triton experiments, the animals were treated in the same manner as described above, except that α-tocopherol acetate dissolved in saline was administered i.m. 60 min before being sacrificed, the animals were given i.v. 50 mg/100 g body weight of Triton (Triton WR 1339, Winthrop Lab., New York). Triglycerides in liver and plasma were determined by the method of SARDESAI and MANNING[4]. Serum lipoproteins were separated using agarose gel electrophoresis according to the method of NOBLE[5]. Hepatic contents of NAD and $NADH_2$ were determined by the method of CIOTTI and KAPLAN[6].

Following the administration of ethanol, as seen from Table I, liver triglyceride levels were significantly elevated ($p < 0.01$). It was α-tocopherol that caused an inhibition of liver triglyceride accumulation as well as a hypertriglyceridemia. Tocopheronolactone did not show such activities. To investigate the cause for the elevated levels of serum triglycerides, serum lipoprotein patters were observed by agarose gel electrophoresis. As shown in Figure 1, pre-β-lipoprotein and in a less degree chylomicron became larger in the ethanol plus α-tocopherol group. The results indicate that the most part of elevated

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serum triglycerides is endogenous and synthesized in the liver. The relationship between liver and plasma triglyceride levels under these conditions is illustrated in Figure 2. As is apparent from Figure 2, a significant inverse correlation between both levels was observed with a correlation coefficient of -0.77 ($p < 0.01$).

Furthermore, to assess the rate of triglyceride secretion from liver to plasma compartment, Triton experiment, which inhibits peripheral utilization of lipoprotins, was performed (Table II). In comparison with the control ethanol group, a higher degree of hypertriglyceridemia was observed in the α-tocopherol treated group. The results demonstrate that α-tocopherol is regarded as a useful vehicle for triglyceride transport from liver to plasma. Since hepatic lipoprotein synthesis was not altered by the administration of ethanol[7,8], α-tocopherol seems to be effective in accerelating hepatic release of lipoproteins, at least of lipid moiety.

Apart from the problem concerning peroxide formation, studies on ethanol-induced fatty liver also indicate that

Table I. Effect of α-tocopherol and tocopheronolactone on ethano-induced fatty liver[a]

Group	No. of rats	Triglyceride Serum (mg/100 ml)	Liver (mg/g)
Ethanol plus propylene glycol	7	36.7 ± 9.1	45.3 ± 7.4
Ethanol plus α-tocopherol	5	72.0 ± 18.6[c]	30.5 ± 8.9[b]
Ethanol plus tocopheronolactone	5	41.4 ± 10.1	46.3 ± 5.6
Glucose	5	37.5 ± 16.0	9.0 ± 1.0

[a] Values are expressed as mean ± S.D. [b,c]. The difference to ethanol-propylene glycol group is statistically significant ($p < 0.05$, 0.01).

Table II. Effect of ethanol and α-tocopherol on rates of hepatic triglyceride secretion[a]

Condition	Serum triglyceride (mg/100 ml) Pretriton	Posttriton	Triglyceride secretion (mg/min/ 100 ml plasma)
Ethanol	72 ± 21 ($p < 0.05$)	568 ± 42 ($p < 0.05$)	8.3
Ethanol plus α-tocopherol	123 ± 36	923 ± 278	13.3

[a] Each group consists of 5 animals. Values are expressed as mean ± S.D.

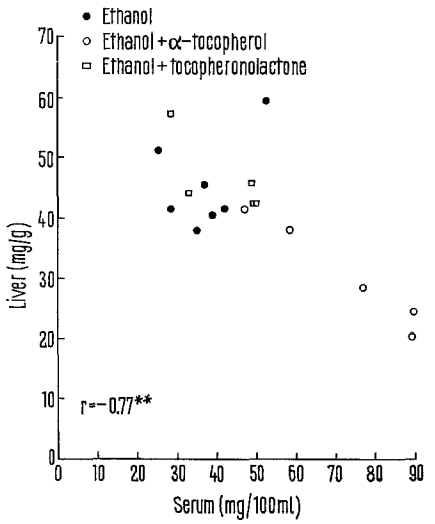

Fig. 2. Relationship between liver and serum triglycerides in rats under the conditions of ethanol (●), ethanol plus α-tocopherol (○) and ethanol plus tocopheronolactone (□) administration.

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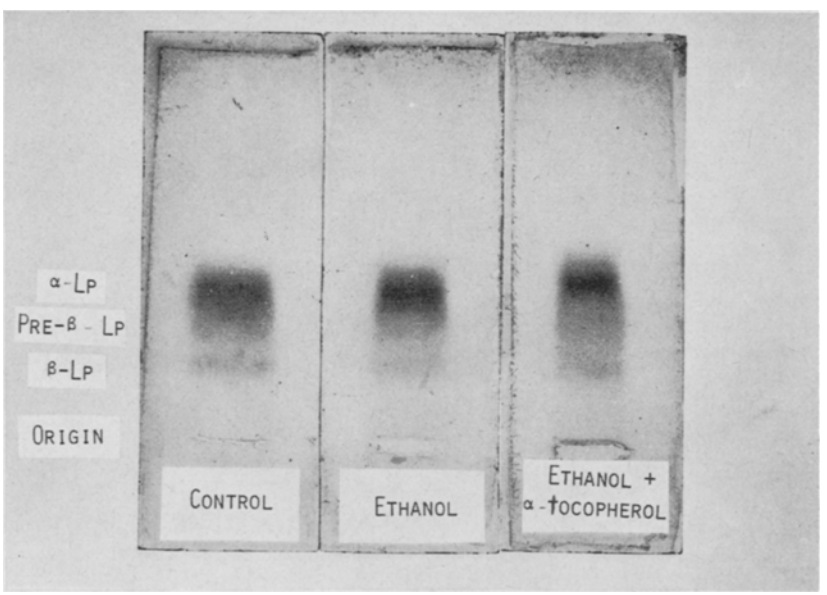

Fig. 1. Lipoprotein patterns of rat serum by agarose gel electrophoresis; control group, ethanol group and ethanol plus α-tocopherol group. α-Lp, α-Lipoprotein; pre-β-Lp, pre-β-Lipoprotein; β-Lp, β-Lipoprotein.

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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