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FREE RADICALS AND LIPID PEROXIDATION IN LIVER OF RATS KEPT ON A DIET DEVOID OF CHOLINE

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Rodents kept on a choline devoid (CD) diet up to 14 months develop hepatic lesions progressing through two broad stages. The first is chracterized by severe steatosis and increase in cell turnover, the second by a gradual clearance of the deposited fat and fibrosis. Hepatocellular carcinomas eventually arise in rats fed for over 12 months, even though the animals aer not exposed to chemical carcinogens. It has been suggested that the diet may trigger generated thereby may be responsible for intiation of liver cancer and promotion. The radicals would lead to DNA damage, and the altered DNA in a proliferating liver would result in initiation of the carcinogenic process. In this communication we present evidence that the diet used in the above studies contained stable fatty acid isomers with conjugated dienes, which are absorbed and deposited in rat liver. This finding cast doubts on whether a CD diet does indeed cause a peroxidation of cellular membrane lipids. Electron spin resonance (ESR) spectroscopy was also used to investigate whether any abnormal pattern of free radicals exists in the liver of rats fed a CD diet. No significant differences were noted in ESR spectra of either transition metal-centered signals, or organic free radicals.

KEY WORDS: Free radicals, electron spin resonance, conjugated dienes, choline devoid diet, cancer.

INTRODUCTION

Tumors in the liver, and at other organ sites, develop in rats fed choline-devoid (CD) diets as the sole treatment of the animals.[1,2] It has been proposed that these diets lead to generation of free radicals that trigger a peroxidation of cellular membranes lipids, with formation of peroxyl radicals that damage DNA and thus initiate carcinogenic processes.[3] The proposal is based on detection of conjugated dienes in total-lipid extracts of liver nuclei and other subcellular organelles, isolated from rats fed a CD diet for a few days or weeks;[4-6]detection of as yet undefined alterations in liver DNA;[7] and absence of conjugated dienes in liver lipids of rats fed a CD diet and, at the same time, treated with N-tert-butylphenylitrone, a free radical trapping agent.[3] We have recently re-examined the question of the origins of the conjugated dienes found in liver total-lipids of rats fed a CD diet, and investigated by means of electron spin resonance (ESR) spectroscopy whether any abnormal pattern of free radicals is present in the liver of these animals. The results obtained are the object of this communication.

MATERIALS AND METHODS:

Male Fisher-344 rats weighing 90–100 g were placed for up to 2 weeks on purified choline-supplemented (CS) or choline-devoid (CD) diet (DYETS, Bethlehem, PA).

Samples of liver, perirenal adipose tissue and of small intestine were taken. Intestinal mucosa cells and liver nuclei and microsomes were prepared as indicated.[8,9] Total lipids were extracted from tissue preparation and samples of the diets, and some of the former were separated into neutral and phospholipids, also as indicated.[9] Conjugated dienes were detected by means of 2nd derivative U.V. absorption spectra of the lipids.[10]

ESR investigations were carried out on groups of 8 rats each fed the CS or CD diet for 3 weeks or 3 months. The livers were taken and immediately frozen in liquid nitrogen. At the moment of recording spectra, the livers were coarsly chopped and inserted in a large ESR silica tube. Spectra were recorded at liquid nitrogen temperature, using two different instrumental settings: 4000 G scan width, 5 G modulation amplitude, 50 mW microwave power; and, 500 G scan width, 5 G modulation amplitude, 2 mW microwave power. The first setting was deemed to show, in particular, oxidized iron-centered cytochromes, and the second free radical species.

RESULTS

In 2nd derivative U.V. spectra of lipids, conjugated dienes are revealed by signals with minima at 233 and at 242 nm, due to absorption by trans-trans and cis-trans conjugated-diene isomers, respectively.[10] Analyses were performed on the tissue lipids of 3 to 5 rats fed the CS or CD diet for 1, 3, 7 and 14 days. Typical results are shown in Figures 1–3. conjugated dienes were consistently detected in total and neutral lipids of liver microsomes intestinal mucosa cells and adipose tissue, but not in pho-

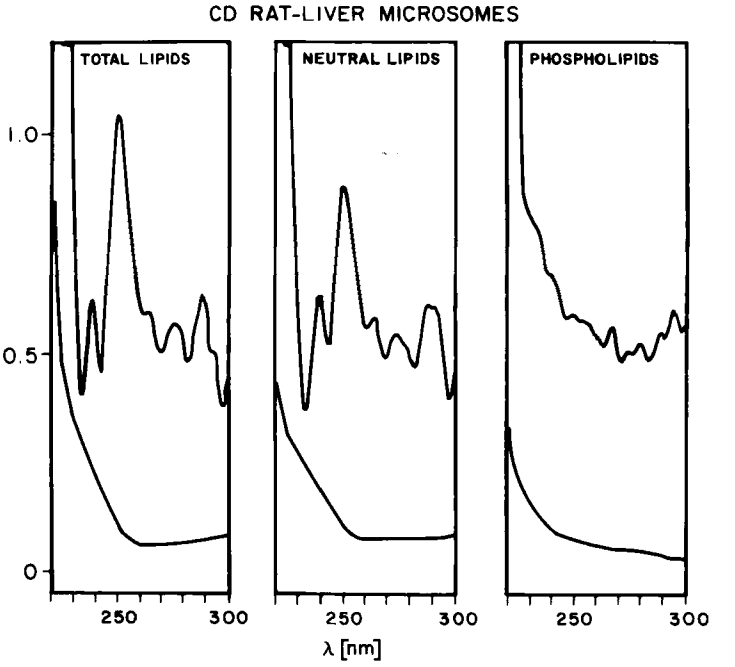


FIGURE 1

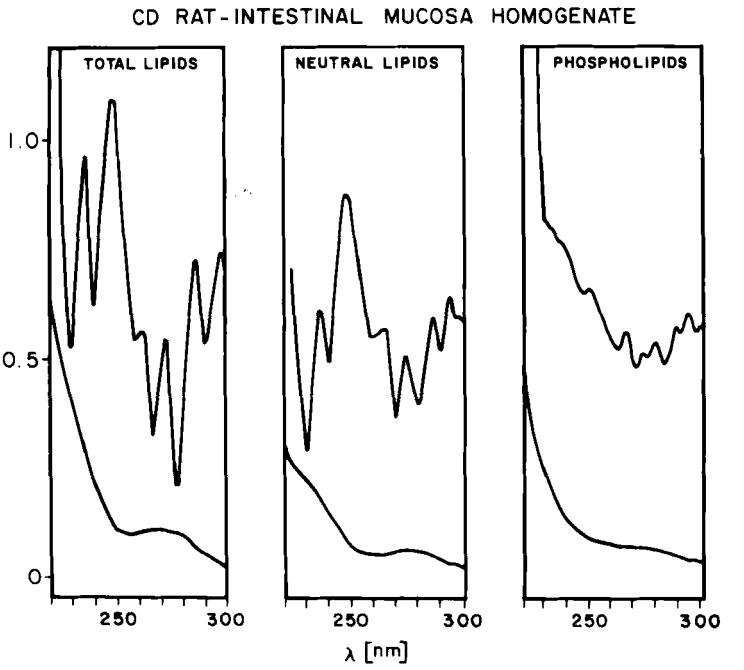
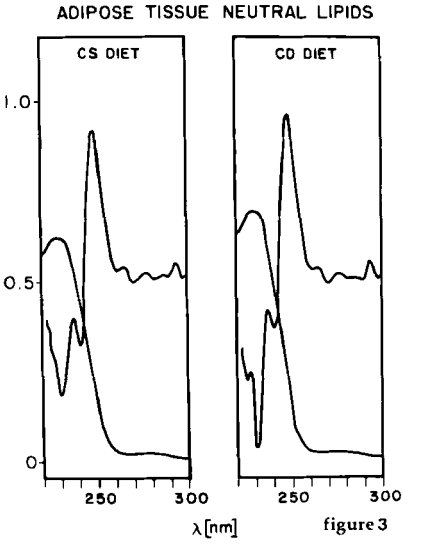


FIGURE 2



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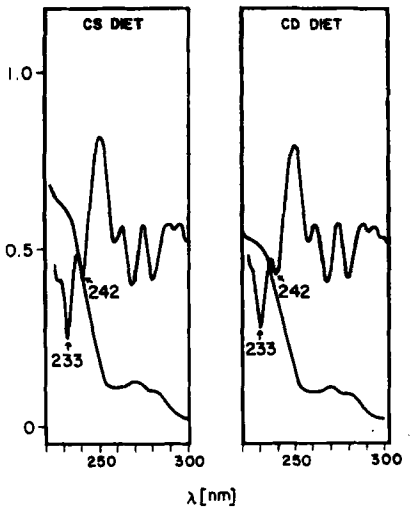

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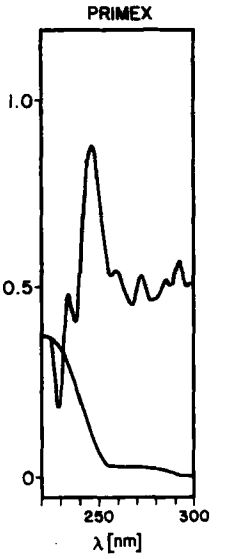

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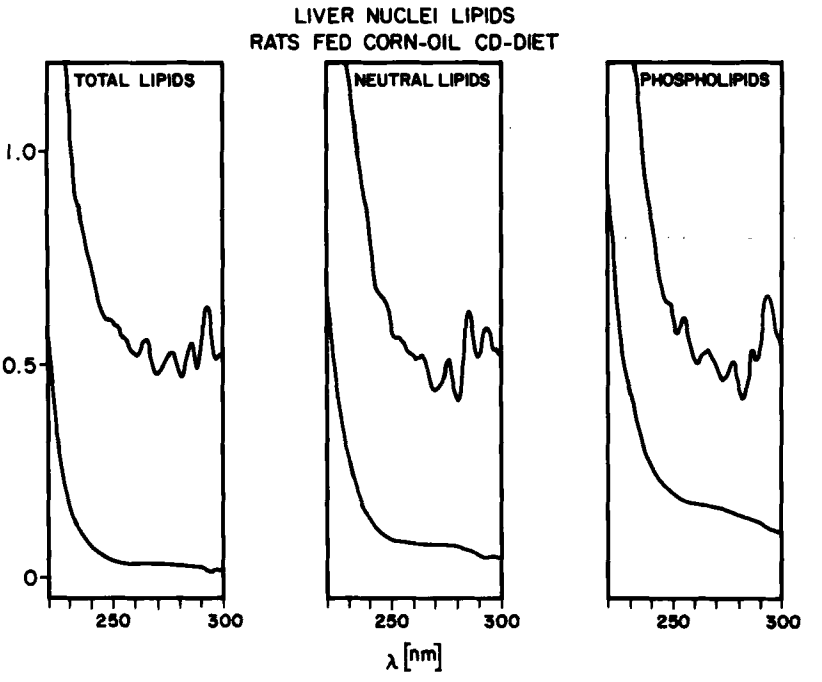

FIGURE 6

FIGURES 1–6 Absorption (lower curves) and second derivative absorption sepectra (upper curves) of lipid fractions obtained as indicated in Materials Methods and Results.

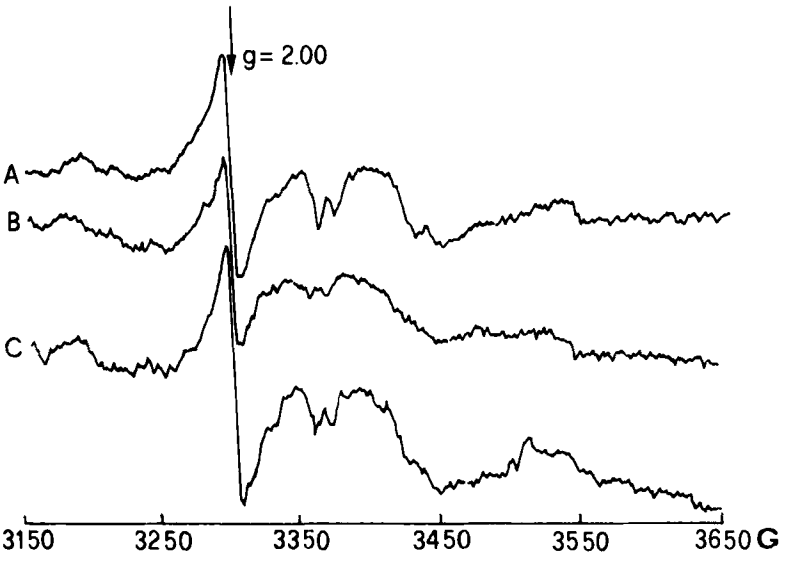

FIGURE 7 ESR spectra of livers taken at instrument setting of 500 G scan width, 5 G modulation amplitude, 2 mW microwave power.
A strong absorption (g = 2.003) due to free radical species is shown.

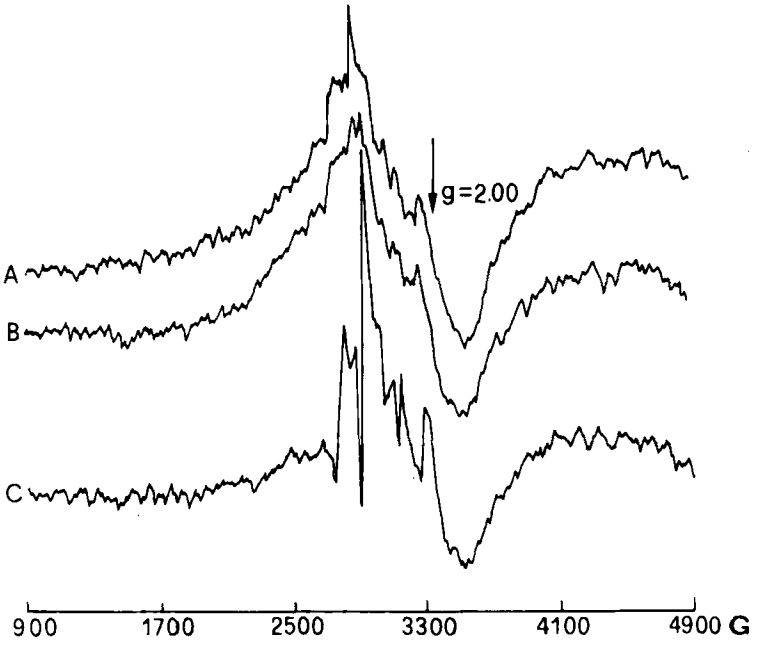

FIGURE 8 ESR spectra of livers taken at instrument setting of 4000 G scan width, 5 G modulation amplitude, 50 mW microwave power. High spin heme iron is evident (g value $\geqq$ 3) and low spin heme iron (g = 2.8, 2.2 and 1.96) are shown.

spholipids, and irrespective of which diet was fed to the animals. The findings were hardly consistent with the possibility of an ongoing peroxidation of cellular-membrane phospholipids, and suggested instead a dietary derivation of the conjugated dienes. Analyses made on fat extracted from samples of the diets revealed indeed conjugated dienes in both the control CS and CD diet (Figure 4). The fat of two purified diets consists of 5% corn oil and 10% Primex, a mixture of partially hydrogenated soya-bean and palm oils.[11] Analyses made on samples of the two fats, obtained from the diet supplier, showed abundant presence of conjugated dienes in Primex (Figure 5), but not in corn oil. Groups of 3–5 rats each were then fed for 1 to 7 days CS or CD diets prepared with 15% corn oil, and no Primex. No conjugated dienes were detected in total, neutral and phospholipids, of liver microsomes and nuclei (Figure 6) prepared from these rats.

Figure 7 shows typical ESR liver spectra obtained at instrumental settings that illustrate free radicals absorbing at g value of 2.003. The signal can be attributed to ubiquinon and flavins radicals, which are observed in any biological tissue and, in particular in the liver.[12] No significant spectrum differences were noted as result of the animal treatment, as shown in Figure 7 (A, liver of a rat fed the CS diet, and B and C of rats fed the CD diet for 3 weeks or 3 months). No significant spectrum differences were observed also at instrumental settings that would detect high ($g \geqslant 3$) and low g ($g = 2.8$, 2.2 and 1.96) spin heme iron (Figure 8; A, rat fed the CS diet, and B and C, rats fed the CD diet for 3 weeks or 3 months).

DISCUSSION

Partial hydrogenation of polyunsaturated oils generates a wide range of unusual positional and geometric fatty-acid isomers, a fraction of which ends up with having carbon centred, rather than oxygen centered, conjugated dienes.[13] The isomers are fairly stable, and are readily absorbed and assimilated in rat and human tissues.[14] The purified CD and CS diets used for carcinogenesis studies in our and other laboratories[4–6] contain a mixture of partially hydrogenated vegetable oils as one of their components.[11] In agreement with previous results from other laboratories,[4–6] we have detected conjugated dienes in total lipids extracted from liver subcellular organelles of rats fed the CD diet. However, our results show that dietary fat, and not a peroxidation of cellular membrane lipids, is the source of the observed conjugated dienes. We based this conclusion mainly on finding conjugated dienes in tissue total and neutral lipids, but not phospholipids, of rats fed both the control CS, as well as the CD diet; presence of conjugated dienes in the diets themselves, and in the partially hydrogenated fat used in their preparation; and, absence of diene conjugates in lipids extracted from liver subcellular organelles of rats, fed CD and CS diets prepared exclusively with corn oil. The last results include analyses made on lipids extracted from liver nuclei. Thus, to the extent that alterations in liver DNA occur in rats fed a CD diet,[7] their cause would have to reside in processes other than a peroxidation of nuclear membrane lipids and radicals generated therefrom. In a separated but related study,[35] bulky/hydrophobic liver DNA-adducts were not detected in rats fed a CD diet, such as might presumably be formed by interactions of products of membrane lipid peroxidation with cellular DNA.[16,17] It has been postulated that, in these rats, peroxidation of nuclear membrane lipids is sparked by free radicals, generated as an effect of the diet, since conjugated dienes were not observed in animals

treated simultaneously with a free radical trapping agent.[3] Free radicals are known to play a role in tumor induction and promotion, and the literature reports ESR observable changes during tumor promotion and growth.[18-21] However, we failed to observed any significant alteration of the free radical and heme iron ESR spectra of livers of rats fed the CD diet. It seems however possible that, if present, the radicals might be of species not detectable under the instrumental settings and conditions used in the present study.

Acknowledgements

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