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ANTIOXIDANTS AND LIPID PEROXIDATION: "IN VIVO" STUDIES

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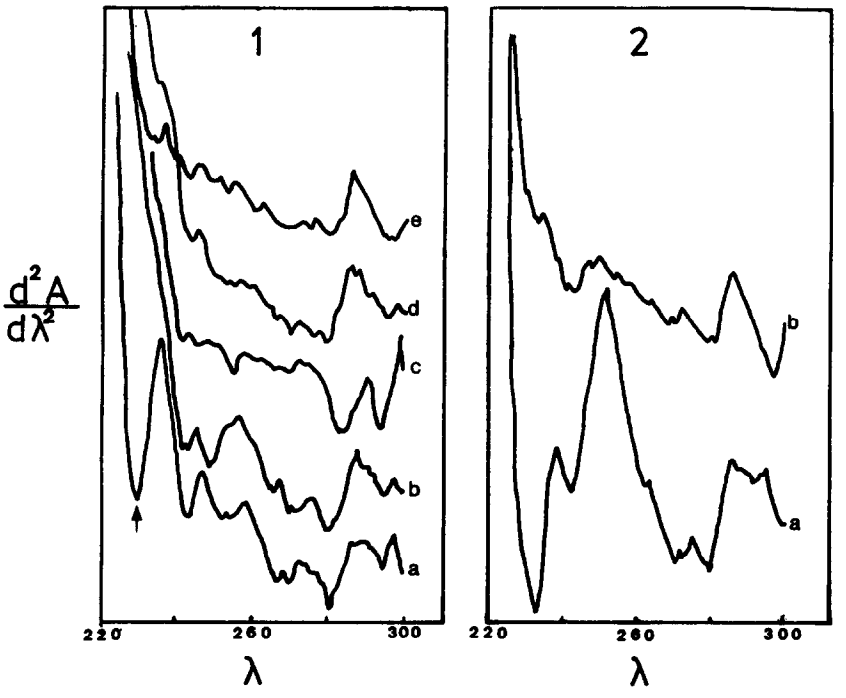
It is well known that the oxidation of many xenobiotics results in the formation of free radical intermediates the subsequent reactions of which may be inhibited by antioxidants (1). In some cases the formation of such free radical intermediates may stimulate lipid peroxidation but, until recently, the precise role of this degradative process in tissue injury "in vivo" has been difficult to evaluate: the main reason for this difficulty has been the lack of sensitive methods for measuring lipid peroxidation in experimental models "in vivo" (1,2). Recently, however, the diene difference spectrum method (3), which is the procedure best suited for evaluating peroxidative changes in polyunsaturated fatty acids (PUFA's), has been considerably improved in sensitivity and discrimination (4). In the improved procedure the conjugated diene absorption previously characterized at 233nm (2,5) has been identified with a minimum in the second derivative spectrum at 233nm; moreover, under conditions "in vivo" another absorption has been clearly recognized at 244nm. In this communication we have applied the refined diene conjugation method to a study of the liver injury produced by CCl_4 in order to investigate whether the stimulatory effect of CCl_4 on lipid peroxidation, a process well defined "in vitro" (1) can be attenuated "in vivo" by α-tocopherol or promethazine: the efficiency of these antioxidants "in vivo" during CCl_4-intoxication has been disputed (6,7).

METHODS

Male Sprague-Dawley rats intoxicated with CCl_4 (13 mmoles/kg) were treated with vitamin E (55 mg/kg) according to reference (8) and with promethazine hydrochloride (78 μmoles/kg) as described in reference (9). Animals were killed 1 h after the intoxication. Microsomal lipid extracts were scanned from 300 nm to 220 nm for UV and second-derivative spectra as previously described (3,4).

RESULTS

Figure 1 shows the second derivative spectra obtained from lipid extracts of microsomes prepared from rats treated with (a) CCl_4; (b) CCl_4 and vitamin E; (c) vitamin E; (d) mineral oil; (e) Tween 80 and ethanol. Treatments (d) and (e) are controls for the solvent used to administer CCl_4 and vitamin E respectively. Figure 2 gives corresponding results for (a) CCl_4 and (b) CCl_4 with promethazine. The presence of a minimum peak at 233 nm, which in the second-derivative spectrum unequivocally demonstrates the existence of conjugated-dienes, occurred only in rats intoxicated with CCl_4 (Fig.1a and Fig.2a). In controls or in vitamin E and in promethazine-protected animals the second derivative spectra of liver lipids did not show any signal at 233 nm (Fig.1b-e and Fig.2b). The addition of vitamin E and promethazine "in vitro" during the homogenization did not interfere with this signal if initially present (data not shown).



DISCUSSION

The measurement of the diene conjugation of microsomal lipids has been used by many investigators to determine the extent of lipid peroxidation "in vivo" (1,2). We have demonstrated here that under our experimental conditions the prior administration of vitamin E or promethazine to CCl_4-intoxicated rats completely prevents the formation of diene absorption bands that normally occurs after free radical attack on PUFA's. Our results provide direct evidence for the antioxidant activity of these substances "in vivo". The contrasting results obtained by other authors may have been due to the lack of sensitivity of the methods used in previous studies (6,7).

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