

## ANTIOXIDANT ACTIVITY OF PROPYLTHIOURACIL

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**Abstract**—Propylthiouracil (PTU) has been demonstrated to reduce alcohol-induced hepatocyte damage and severe alcoholic liver disease. Although the mechanism by which the drug operates is yet to be elucidated, there is evidence that PTU may act as an antioxidant. The present study examines the reaction of PTU with oxygen free radicals and the ability of PTU to directly inhibit peroxidation of a model membrane system. PTU reacted directly with hydroxyl radicals produced by  $\gamma$ -radiolysis. The rate constant for the PTU/hydroxyl radical reaction as determined by steady state competition kinetics with *p*-nitrosodimethylaniline was  $8 \times 10^9$  L/mol/sec. PTU was less reactive towards superoxide generated by the xanthine/xanthine oxidase system, having a small but significant inhibitory effect on superoxide-induced reduction of cytochrome *c* only at a concentration of 200  $\mu$ M. The ability of PTU to protect lipids from peroxidative changes was tested in membranes prepared from linoleic acid. The rate of peroxidation induced by 40° heat decreased from 0.078 to 0.024 mM hydroperoxide/hr in the presence of 0–50  $\mu$ M PTU. However, this trend was reversed at PTU concentrations above 50  $\mu$ M. These data suggest that the protective effects of PTU against liver damage may be due to scavenging reactions with hydroxyl radicals in particular and/or its antioxidant potential.

Recent studies have indicated that free radicals and the products of their reactions may initiate pathological changes similar to those associated with alcohol (ethanol)-induced tissue damage [1]. Superoxide and hydroxyl radicals are formed during alcohol metabolism by the microsomal ethanol oxidizing system, or the reaction of acetaldehyde or purines with xanthine oxidase [2–4]. Hydroxyl radical formation may also be facilitated by increases in hepatic iron stores which are often seen in association with chronic alcoholism [5]. Hepatic lipid peroxidation has been shown to be increased after both acute and chronic ethanol consumption [6–8] and has been implicated in the subsequent development of hepatic steatosis and cirrhosis [9].

Therapeutic interventions have been largely unsuccessful in preventing or reducing alcohol-induced liver damage. However, propylthiouracil (PTU†) may be useful in this regard. Recently, a 3-year Canadian trial [10] reported decreased mortality in patients with severe alcohol-induced liver disease treated with PTU compared to controls. The mechanism of action of PTU in this context remains to be elucidated. PTU is used to treat hyperthyroidism [11] but there was no correlation between alterations in thyroid activity and successful response to the treatment of ethanol-induced liver damage [10]. PTU has also been demonstrated to reverse hypoxic liver damage resulting from an increase in the metabolic rate associated with hyperthyroidism. Similar partial ischemia has been observed in alcoholic liver damage [12]. PTU protected rat liver and isolated hepatocytes from such ischemic damage [13, 14] as well as reacting with some of the oxidizing

species derived from the respiratory burst in neutrophils [15]. Hyperthyroid patients treated with PTU have decreased serum levels of lipid peroxides measured as malondialdehyde [16]. These findings suggest that PTU may scavenge primary oxygen free radicals or may directly protect lipids from peroxidative attack.

To date there is no basic information on the reactivity of PTU towards oxygen free radicals or its potential to act as a lipid antioxidant. In the present study, the rate constant for the PTU/hydroxyl radical reaction has been measured along with the reactivity of PTU towards superoxide and its potential to act as an antioxidant in a model membrane system.

### MATERIALS AND METHODS

**Materials.** PTU (Sigma Chemical Co., St Louis, MO, U.S.A.) was recrystallized from aqueous EDTA then Milli-Q water before use in radiolysis studies or used without further purification in xanthine oxidase and lipid peroxidation studies. PNDA (AR grade) was obtained from Fluka (Switzerland). Xanthine, xanthine oxidase, superoxide dismutase and trishydroxymethyl-aminomethane were all obtained from Sigma. Linoleic acid was purchased from Serdary Research Laboratories Inc. (Ontario, Canada). All other chemicals and solvents used were "AnalaR" grade, purchased from Ajax Chemicals (Australia). Water used in solution preparation was purified by passage through a Milli-Q purification System (Millipore, Australia).

**PTU/hydroxyl radical rate constant determination.** Irradiations were performed in a 2500 Ci  $^{60}\text{Co}$   $\gamma$  source at 25°. Dose rates were measured by Fricke dosimetry [17] with the G-value for the formation of  $\text{Fe}^{3+}$  taken as 15.5 ions/100 eV [18]. For conversion to S.I. units ( $\mu\text{mol/J}$ ), multiply G-value by 0.1036. The dose rates varied from 57.0 to 75.31 Gy/min.

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† Abbreviations: PTU, propylthiouracil; PNDA, *p*-nitrosodimethylaniline.

In experiments where PTU/ $\text{HO}^\bullet$  reactivity was examined, solutions of 200  $\mu\text{M}$  PTU were bubbled with humidified argon, air or an 80%:20% mixture of nitrous oxide and oxygen for 30 min before commencement of irradiation. PTU/ $\text{HO}^\bullet$  rate constants were measured using competition kinetics with PNDA. PNDA was chosen as the competing species because its reaction with hydroxyl radicals can be followed by absorbance change in the visible region (440 nm) well away from the absorbance maximum of PTU (273 nm) as distinct from other competing species such as thymine, which also has absorbance in the ultra violet region [19]. Loss of PNDA in the presence of PTU was measured as the decrease in absorbance at 440 nm. The extinction coefficient ( $\epsilon$ ) for PNDA was determined to be 32,500 L/mol/cm [19].

**Reactivity of PTU with superoxide.** Superoxide radicals were produced by radiolysis of alkaline formate or the action of xanthine oxidase on xanthine. PTU solutions (200  $\mu\text{M}$ ) were irradiated in 10 mM formate/1.5  $\mu\text{M}$  EDTA adjusted to pH 10 with NaOH under humidified argon or air. In the xanthine/xanthine oxidase system, the reaction mixture contained 50  $\mu\text{M}$  xanthine and 50  $\mu\text{M}$  cytochrome *c* in 2.5 mL 0.1 M Tris/HCl, pH 7.8. Enough xanthine oxidase (7.5 units) was added to give an initial rate of cytochrome *c* reduction of about 0.05 absorbance units at 550 nm/min in the absence of PTU.  $\epsilon_{(550\text{nm})}$  for reduced cytochrome *c* was taken to be 21,000 L/mol/cm [20]. The rate of superoxide-induced cytochrome *c* reduction was determined by subtraction of the rate of cytochrome *c* reduction in the presence of 0.08 mg/mL superoxide dismutase.

**Antioxidant activity of PTU.** A chloroform solution of linoleic acid (0.292 M) was prepared and stored at  $-20^\circ$ . Vesicles were prepared by the method of Gebicki and Hicks [21]. A volume of 5 mL of 0.1 M Tris/HCl or buffered PTU solution was added to neat fatty acid left after evaporation of chloroform from 100  $\mu\text{L}$  of stock solution under a stream of nitrogen. This preparation was vortexed until all the fatty acid was removed from the walls of the vessel and a homogeneous suspension was formed. Vesicles were prepared on the day of the experiment from stock solution no more than 7 days old.

Lipid hydroperoxides were determined by measuring formation of conjugated dienes ( $\epsilon_{234\text{nm}} = 28,000 \text{ L/mol/cm}$ ) [22]. For spectrophotometry, 25  $\mu\text{L}$  linoleic acid vesicle preparation was diluted with 2 mL methanol. Absorbance was measured with a Shimadzu UV-260 spectrophotometer against a methanol blank.

## RESULTS

### Rate constant of reaction between PTU and hydroxyl radicals

Irradiation of aqueous solutions of PTU produced a loss in chromophore absorbance, due to reaction of the parent molecule with hydroxyl radicals from the radiolysis of water (Fig. 1). In the absence of oxygen, chromophore loss is decreased due to the rapid removal of hydroxyl radicals by reaction with hydrated electrons. There is also likely to be a

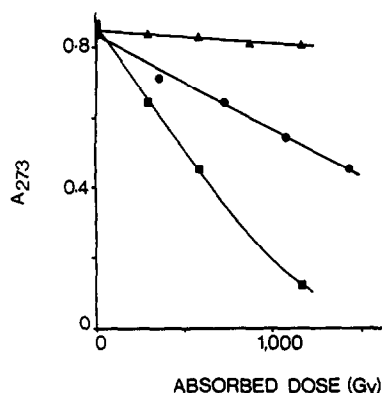
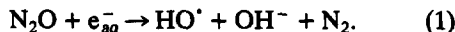


Fig. 1. Kinetics of radiation-induced loss of PTU chromophore. Initial [PTU] = 0.202 mM. (▲) Argon incubation, dose rate = 57.0 Gy/min; (●) air incubation, dose rate = 70.26 Gy/min; (■)  $\text{N}_2\text{O}:\text{O}_2 = 80\%:20\%$ , dose rate, 57.0 Gy/min. Reaction mixture diluted 1:3 with Milli-Q water for absorbance reading.

conversion of the initial unstable radical formed back to native PTU, by a mechanism similar to that found for uracil [23]. Increase in the rate of chromophore loss in the presence of nitrous oxide compared to air was indicative of a reaction between PTU and hydroxyl radicals as  $\text{N}_2\text{O}$  doubles the flux of hydroxyl radicals by the following reaction:



The rate constant for the reaction between PTU and hydroxyl radicals was determined using competition kinetics. (See Ref. 24 for a discussion of the theory of competition kinetics.) Aerated aqueous solutions of PNDA (0.047 mM) adjusted to pH 9 with dilute borate were irradiated in the presence of 0–0.245 mM PTU. Irradiation times were kept to 1–1.5 min (around 70 Gy total dose) to eliminate interference from radiolysis products of PNDA which absorb at around 420 nm [19].  $G(-\text{PNDA})$  was calculated from the initial, linear rate of radiation-induced disappearance of A (440 nm) chromophore, and was found to be independent of the initial PNDA concentration above 0.040 mM. In the absence of PTU,  $G(-\text{PNDA})$  was 1.15. This figure is in agreement with that quoted by Kraljic and Trumbore [19]. The rate of PNDA loss decreased with increasing PTU concentrations indicating competition between PNDA and PTU for the available hydroxyl radicals. Results of this experiment were transformed to allow calculation of the PTU/ $\text{HO}^\bullet$  rate constant (shown in Fig. 2). The value for the rate constant was calculated from the slope of Fig. 2 using the relationship:

$$k_{\text{PTU}/\text{HO}^\bullet} = \text{Slope} \times G(-\text{PNDA})_{[\text{PTU}]=0} \times k_{\text{PNDA}/\text{HO}^\bullet}$$

The absolute rate constant for the PNDA/ $\text{HO}^\bullet$  reaction was taken to be  $1.25 \times 10^{10} \text{ L/mol/sec}$  [25]. The rate constant calculated for the PTU/ $\text{HO}^\bullet$  reaction was  $8 \times 10^9 \text{ L/mol/sec}$ . The magnitude of this value indicates that the reaction between PTU and hydroxyl radical is diffusion controlled.

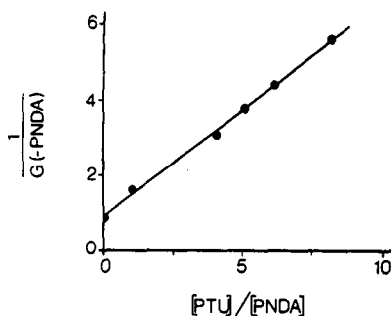


Fig. 2. Reciprocal plot of the radiation chemical yield for loss of PNDA chromophore as a function of the PTU/PNDA ratio. Initial [PNDA] = 0.046 mM; dose rate = 70.00 Gy/min.

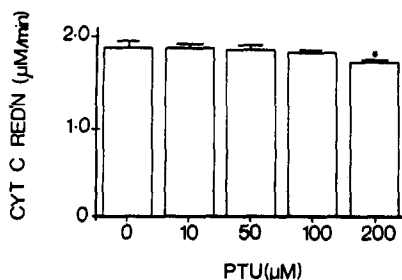


Fig. 3. Rate of superoxide-induced cytochrome *c* reduction as a function of [PTU]. (See Materials and Methods for reaction mixture). Rates are the mean  $\pm$  SD ( $N = 6$ ) for each PTU concentration after subtraction of superoxide dismutase-independent rate of cytochrome *c* reduction. \*Indicates that the rate at 200  $\mu$ M PTU is significantly different from the control rate ( $P < 0.05$  by Newman-Keuls test).

#### PTU/superoxide reactivity

Radiolysis of 200  $\mu$ M PTU in alkaline formate (dose rate = 69 Gy/min) resulted in a linear rate of chromophore loss (corresponding to 12  $\mu$ mol PTU/min) in both the presence and absence of oxygen. This was indicative of a reaction of PTU with the formate radical rather than with superoxide. Because of this spurious reactivity of PTU, formate radiolysis was an unsuitable technique for determination of PTU/superoxide reactivity.

Figure 3 shows the effect of 0–200  $\mu$ M PTU on superoxide-induced cytochrome *c* reduction. The rate of cytochrome *c* reduction at each PTU concentration is the mean of six replicates. Although there was a slight trend towards decrease in cytochrome *c* reduction with increasing PTU concentrations, only the 200  $\mu$ M PTU rate was significantly lower than the control rate ( $P < 0.05$ ; Newman-Keuls test; [26]). Control experiments showed that PTU did not directly reduce cytochrome *c*, nor did it alter the activity of xanthine oxidase at concentrations employed in the study.

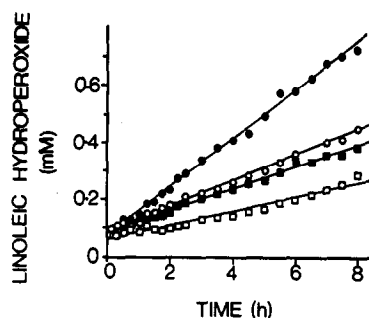


Fig. 4. Effect of PTU on kinetics of thermally induced peroxidation of linoleic acid vesicles (initial [linoleic hydroperoxide] = 0.08–0.09 mM). (●) 0; (○) 5; (■) 20; (□) 50  $\mu$ M PTU. Temperature = 40°.

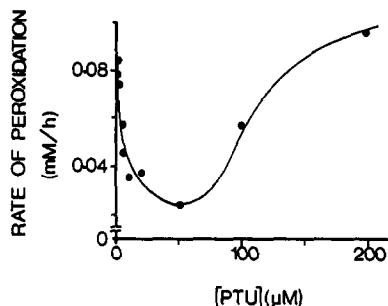


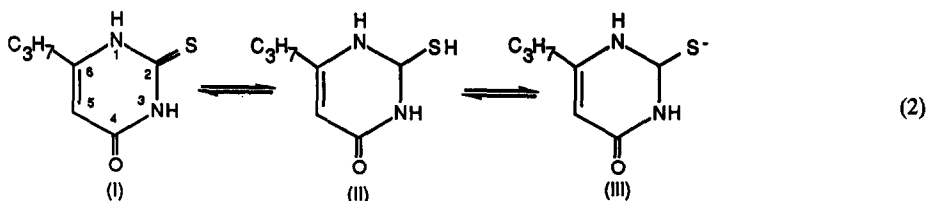
Fig. 5. Initial rate of peroxidation of linoleic acid vesicles (initial [linoleic hydroperoxide] = 0.08–0.09 mM) as a function of PTU concentration. Temperature = 40°.

#### Antioxidant activity of PTU

Figure 4 shows the antioxidant effects of PTU in a model membrane system. When vesicle preparations contained a significant initial level of hydroperoxide (approximately 1 mole %), the kinetics of hydroperoxide formation were linear in the control and PTU-containing samples over the whole course of the experiment. The rate of peroxidation decreased with increasing PTU concentrations up to 50  $\mu$ M PTU. Variation in the rate of vesicle peroxidation over a broader range of PTU concentrations is shown in Fig. 5. In this system, the antioxidant effect was gradually lost above 50  $\mu$ M PTU.

#### DISCUSSION

Although PTU has proved beneficial in the treatment of severe alcohol-related liver disease [10], its mechanism of action remains uncertain. "Functional hyperthyroidism" leading to a hypermetabolic state was demonstrated in the liver of rats chronically exposed to alcohol [13]. Centrilobular hepatic hypoxia which resolves upon alcohol withdrawal has also been observed *in vivo* [27]. These metabolic effects would be compatible with



ischemia/reperfusion injury which results in high free radical fluxes leading to pathological damage at the biochemical and cellular level [28].

Changes typical of ischemia/reperfusion injury demonstrated during chronic or acute alcohol challenge include: (a) alteration in the redox state of the cell (increase in NADH:NAD<sup>+</sup> ratio) favouring the xanthine oxidase reaction [29]; (b) conversion of xanthine dehydrogenase to xanthine oxidase [12, 30]; (c) production of superoxide and hydroxyl radicals [2]; and (d) peroxidation of hepatic membrane lipids which can be inhibited by allopurinol, a xanthine oxidase inhibitor [4].

Involvement of PTU in the modulation of free radical activity during alcohol damage has been suggested [31], although the evidence presented was only circumstantial. The present study is the first to address directly the reactivity of PTU towards those radical products most likely to be formed during alcohol challenge namely superoxide, hydroxyl radicals and intermediates produced during lipid peroxidation.

Exposure of PTU to radiolytic oxidation resulted in the loss of PTU chromophore absorbance proportional to the flux of hydroxyl radicals produced (Fig. 1). A likely mechanism for the loss of PTU chromophore would be the addition of HO<sup>•</sup> at C<sub>5</sub> with a concomitant saturation of the double bond between C<sub>5</sub> and C<sub>6</sub> analogous to that proposed for uracil [23, 32]. (See Structure I, Reaction 2, for numbering of the pyrimidine ring.) Reaction of the imino intermediate with hydroxyl radical has also been suggested as the mechanism for a similar thione-containing compound, thiourea [33]. Similar canonical forms might be expected for PTU as shown in (2) above, where (I) is the thione form, (II) the imino form and (III) the thiol ion of PTU. We have detected 1 mole % of freshly prepared PTU in the imino/thiol form on the basis of reaction with Ellman's reagent [34]. The pK<sub>a</sub> for ionization of the imino group of PTU was 7.8 [35] so at pH 9 (where the PNDA competition experiments are carried out), all the fractions of PTU in the imino form will be ionized. The reactivity of these species with hydroxyl radicals will be independent of the extent of ionization as the sulphydryl/ and thiol/hydroxyl radical rate constants are equally high (both between 1 and 2 × 10<sup>10</sup> L/mol/sec [36]). However, the experimental design employed in the present study allows the PTU/hydroxyl radical rate constant to be calculated without any *a priori* assumptions about the chemistry of the PTU/HO<sup>•</sup> reaction. Its magnitude (8 × 10<sup>9</sup> L/mol/sec) indicates that PTU is a highly efficient hydroxyl radical scavenger.

PTU at a concentration of 200 μM caused a small but significant decrease in the rate of superoxide-induced cytochrome *c* reduction (Fig. 3). Similar concentrations of PTU were found to inhibit superoxide-induced oxidation of *o*-dianisidine by 90% [37]. A rate constant for the PTU/superoxide reaction cannot be determined accurately with the xanthine/xanthine oxidase system because of PTU solubility limitations. However, from the data in Fig. 3 the upper limit of the PTU/superoxide rate constant is 10<sup>4</sup> L/mol/sec with respect to cytochrome *c* (if self-dismutation of superoxide is neglected). By comparison, thiourea was found to react with superoxide with a rate constant of 1.1 × 10<sup>3</sup> L/mol/sec (determined by competition kinetics with cytochrome *c*) [38].

PTU treatment has been shown to lower the serum lipid hydroperoxide levels in hyperthyroid subjects. However, it was not possible to ascertain whether the PTU was having a direct effect on the intermediates involved in lipid peroxidation or whether it was exerting its effect at a metabolic level [16]. In the present study, PTU was shown to possess endogenous antioxidant properties when included in a peroxidizable model membrane system at concentrations at or below 50 μM. This concentration approximates serum levels during most therapeutic regimens [11]. The species responsible for the antioxidant activity is most probably the imino intermediate (PTU-SH) shown in Reaction 2, which may react with the alkyl, alkoxy or peroxy radicals in the peroxidative chain reaction, i.e.:



where L<sup>•</sup>, LO<sup>•</sup>, and LOO<sup>•</sup> are alkyl, alkoxy and peroxy radicals, respectively, and LH, LOH and LOOH are the unoxidised, alcohol and hydroperoxide forms of linoleic acid, respectively. Reactions 4 and 5 are most likely to operate at the higher initial concentrations of linoleic hydroperoxide (Fig. 4) where the rate determining step in the peroxidative chain reaction is the decomposition of preexisting hydroperoxides, rather than the *de novo* initiation of alkyl radicals.

Thiyl radical intermediates may also act as pro-oxidants if present at appropriate concentrations. The rate constant for initiation of the peroxidation of linoleic acid by the cysteine radical (Cys-S<sup>•</sup>) was

found to be  $10^6$ – $10^7$  L/mol/sec [39]. We found that partitioning of PTU into the vesicle membrane is favoured, the octanol:water partition coefficient for PTU being 4.95. On this basis, most PTU-S' formed in Reactions 3–5 will reside in the membrane and be available for reaction with linoleic acid. Thus, induction of, rather than protection from, peroxidation may be favoured at the higher PTU levels used in the study (Fig. 5).

As the distribution of PTU into human body tissues has not been fully determined, it is not possible to estimate the actual hepatic levels which would be attained during therapy for alcohol-related liver injury. The data presented here indicate that the PTU molecule will act as a highly efficient scavenger of hydroxyl radicals and an efficient inhibitor of lipid peroxidation at the free drug levels commonly attained in serum during such therapeutic use (at a dose of 300 mg/day) [11].

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## REFERENCES

- Reinke LA, Lai EK, DuBose CM and McCay PB, Reactive free radical generation *in vivo* in heart and liver of ethanol-fed rats: correlation with radical formation *in vitro*. *Proc Natl Acad Sci USA* **84**: 9223–9227, 1987.
- Nordmann R, Ribiere C and Rouach H, Involvement of oxygen free radicals in the metabolism and toxicity of ethanol. *Prog Clin Biol Res* **241**: 201–213, 1987.
- Cederbaum AI, Microsomal generation of hydroxyl radicals: its role in microsomal ethanol oxidising system (MEOS) activity and requirement for iron. *Ann N Y Acad Sci* **492**: 35–49, 1987.
- Kato S, Kawase T, Alderman J, Inatomi N and Lieber CS, Role of xanthine oxidase in ethanol-induced lipid peroxidation in rats. *Gastroenterology* **98**: 203–210, 1990.
- Irving MG, Halliday JW and Powell LW, Alcoholism and increased hepatic iron stores. *Alcoholism Clin Exp Res* **12**: 7–13, 1988.
- Shaw S, Jayatilake E, Rose WA, Gordon ER and Lieber CS, Ethanol-induced lipid peroxidation: potentiation by long-term alcohol feeding and attenuation by methionine. *J Lab Clin Med* **98**: 417–424, 1981.
- Comporti M, Lipid peroxidation and cellular damage in toxic liver injury. *Lab Invest* **53**: 599–623, 1985.
- Reitz RC, Possible mechanism for peroxidation of lipids due to chronic ethanol ingestion. *Biochim Biophys Acta* **380**: 145–154, 1975.
- Poli G, Albano E and Diazanni MU, The role of lipid peroxidation in liver damage. *Chem Phys Lipids* **45**: 117–142, 1987.
- Orrego H, Blake JE, Blendis LM, Compton KV and Israel Y, Long-term treatment of alcoholic liver disease with propylthiouracil. *N Engl J Med* **317**: 1421–1427, 1987.
- Cooper DS, Antithyroid drugs. *N Engl J Med* **311**: 1353–1362, 1984.
- Younes M and Strubelt O, Enhancement of hypoxic liver damage by ethanol: involvement of xanthine oxidase and the role of glycolysis. *Biochem Pharmacol* **36**: 2973–2977, 1987.
- Israel Y, Kalant H, Orrego H, Khanna JM, Videla L and Phillips JM, Experimental alcohol-induced hepatic necrosis: suppression by propylthiouracil. *Proc Natl Acad Sci USA* **72**: 1137–1141, 1975.
- Gonzalez-Reimers CE, Santolaria-Fernandez FJ, Gonzalez-Hernandez T, Batista-Lopez N, Gomez-Sirvent JL, Perez-Delgarno MM and Ferres-Torres R, Effect of propylthiouracil on liver cell development in the male albino mouse: protective effect against ethanol-induced alterations. *Drug Alcohol Depend* **21**: 11–18, 1988.
- Imamura M, Aoki N, Saito T, Ohno Y, Maruyama Y, Yamaguchi J and Yamamoto T, Inhibitory effects of antithyroid drugs on oxygen radical formation in human neutrophils. *Acta Endocrinol* **112**: 210–216, 1986.
- Videla LA, Sir T and Wolff C, Increased lipid peroxidation in hyperthyroid patients: suppression by propylthiouracil treatment. *Free Rad Res Commun* **5**: 1–10, 1988.
- Weiss J, Allen AO and Schwartz WA, The use of Fricke ferrous sulphate dosimeter for Gamma-ray doses in the range 4–40 kRad. *Proc 1st Int Conference Peaceful Uses Atomic Energy* **14**: 179–181, 1955.
- Allen AO, *Radiation Chemistry of Water and Aqueous Solutions*, p. 22. Van Nostrand, New York, 1961.
- Kraljic I and Trumbore CN, *p*-Nitrosodimethylaniline as an hydroxyl radical scavenger in radiation chemistry. *J Am Chem Soc* **87**: 2547–2550, 1965.
- Massey V, Microestimation of succinate and the extinction coefficient of cytochrome C. *Biochim Biophys Acta* **34**: 255–256, 1959.
- Gebicki JM and Hicks M, Preparation and properties of vesicles enclosed by fatty acid membranes. *Chem Phys Lipids* **16**: 142–160, 1976.
- Pitt GAJ and Morton RA, Ultra-violet spectrophotometry of fatty acids. *Prog Chem Fats Lipids* **4**: 227–278, 1957.
- Shragge PC, Varghaese AJ, Hunt JW and Greenstock CL, Radiolysis of uracil in the absence of oxygen. *Radiat Res* **60**: 250–267, 1974.
- Hicks M and Gebicki JM, Rate constants for the reaction of hydroxyl radicals with tris, tricine and hepes buffers. *FEBS Lett* **199**: 92–94, 1986.
- Dorfman LM and Adams GE, *Reactivity of Hydroxyl Radicals in Aqueous Solutions*, p. 58. US Department of Commerce, National Bureau of Standards, 1973.
- Zar JH, *Biostatistical Analysis*, pp. 151–155. Prentice-Hall, New Jersey, 1974.
- Hadengue A, Moreau R, Lee SS, Gaudin C, Reuff B and Lebrec D, Liver hypermetabolism during alcohol withdrawal in humans. *Gastroenterology* **94**: 1047–1055, 1988.
- Bulkley GB, Free radical-mediated reperfusion injury: a selective review. *Br J Cancer* **55** (Suppl VIII): 66–73, 1987.
- Lieber CS, Alcohol and the liver: metabolism of ethanol, metabolic effects and pathogenesis of injury. *Acta Med Scand* **703** (Suppl): 11–55, 1985.
- Sultatos LG, Effects of acute ethanol administration on the hepatic xanthine dehydrogenase/xanthine oxidase system in the rat. *J Pharmacol Exp Ther* **246**: 946–949, 1988.
- Saunders JB, Treatment of alcoholic liver disease. *Baillieres Clin Gastroenterol* **3**: 39–65, 1989.
- Schuchmann MN and von Sonntag C, The radiolysis of uracil in oxygenated aqueous solution. A study by

- product analysis and pulse radiolysis. *J Chem Soc Perkin Trans II*: 1525–1531, 1983.
33. Charlesby A, Fydeler PJ, Kopp PM, Keene JP and Swallow AJ, In: *Pulse Radiolysis* (Eds. Ebert M, Keene JP, Swallow AJ and Baxendale JH), pp. 193–201. Academic Press, New York, 1965.
34. Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**: 70–77, 1959.
35. Garrett ER and Webber DJ, Metal complexes of thiouracils I: stability constants by potentiometric titration studies and structures of complexes. *J Pharm Sci* **59**: 1383–1398, 1970.
36. von Sonntag C, *The Chemical Basis of Radiation Biology*, p. 355. Taylor and Francis, London, 1989.
37. Wilson R, McKillop JH, Chopra M and Thompson JA, The effect of antithyroid drugs on B and T cells *in vitro*. *Clin Endocrinol* **28**: 389–397, 1988.
38. Kelner MJ, Bagnell R and Welch KJ, Thioureas react with superoxide radicals to yield a sulfhydryl compound. *J Biol Chem* **265**: 1306–1311, 1990.
39. Schöneich C, Asmus K-D, Dillinger U and Bruchhausen Fv, Thiyl radical attack on polyunsaturated fatty acids: a possible route to lipid peroxidation. *Biochem Biophys Res Commun* **161**: 113–120, 1989.