Table 1. *In vivo* effects of tetracycline on the exhalation of $[^{14}C]CO_2$ from various [1-^{14}C]fatty acids

	Exhalation of $[^{14}C]CO_2$ from [1-^{14}C]fatty acids (% of radioactivity administered)		
	Palmitic acid	Octanoic acid	Butyric acid
Control	34 ± 5	25 ± 3	35 ± 4
Tetracycline	18 ± 3*	13 ± 2*	19 ± 3*

Tetracycline (0.25 mmol/kg) was administered i.p. 15 min before the administration of the [1-^{14}C]fatty acid (33 μCi/kg, 0.6 μmol/kg) given by gastric intubation. Exhalation of $[^{14}C]CO_2$ was measured for 3 hr after [1-^{14}C]palmitic acid, 20 min after [1-^{14}C]octanoic acid, and 30 min after [1-^{14}C]butyric acid. Results are means ± SEM for six mice.

* Significantly different from control mice, $P < 0.05$.

bring the pH back to 7.4. A tracer dose of [U-^{14}C]palmitic acid (150 μCi/kg; 0.16 μmol/kg) was administered by gastric intubation, in 0.2 mL of corn oil, 15 min after the administration of the tetracycline derivative, in mice fasted for 48 hr. Exhalation of $[^{14}C]CO_2$ was measured during the next 3 hr as previously described [2]. In some mice treated with tetracycline, [1-^{14}C]fatty acids of various chain lengths were used, as previously described [11].

In vivo *hepatic secretion of triglycerides*. The hepatic secretion of triglycerides was assessed by the increase in plasma triglycerides observed in fasted mice after the administration of Triton WR 1339, which blocks removal of triglycerides from the plasma [12]. Twenty-four hours-fasted mice received Triton WR 1339 (0.3 mL of a 50% solution, w/v, in 0.15 M NaCl) given i.v. (by a penis vein) 15 min after the administration of the tetracycline derivative (0.25 mmol/kg i.p.). Plasma triglycerides were measured as previously described [2]. Basal values were subtracted from those measured 3 hr after administration of Triton WR 1339 [12].

Hepatic triglycerides and mortality. Hepatic triglycerides were measured as previously described [2], 24 hr after the administration of 0.25 mmol/kg i.p. of the various tetracycline derivatives to fed mice. Mortality was assessed 24 hr after the administration of a higher dose (0.5 mmol/kg i.p.) of the tetracycline derivatives to fed mice.

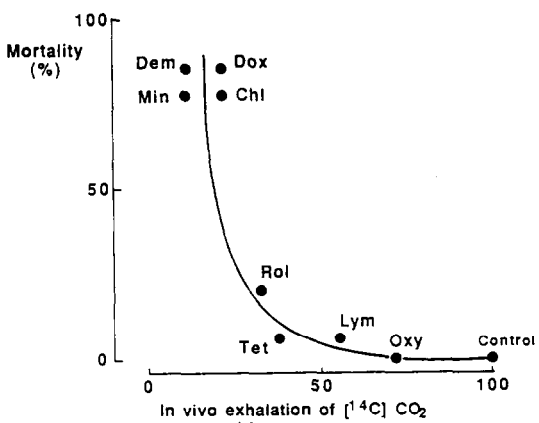

Fig. 1. Relationship between the *in vivo* oxidation rate of [U-^{14}C]palmitic acid, and mortality after administration of a high dose of various tetracycline derivatives. The plot has been drawn from results reported in Table 2. Chl: chlortetracycline; Dem: democlocycline; Dox: doxycycline, Lym: lymecycline; Min: minocycline; Tet: tetracycline; Oxy: oxytetracycline; Rol: rolitetracycline.

Results

In preliminary experiments, performed with tetracycline only, [1-^{14}C]fatty acids were used to determine the effects of this antibiotic on the oxidation of fatty acids of various chain length (Table 1). Tetracycline inhibited to the same extent the *in vivo* oxidation of [1-^{14}C]palmitic acid, [1-^{14}C]octanoic acid and [1-^{14}C]butyric acid (Table 1).

In subsequent experiments, we used [U-^{14}C]palmitic acid, which, being labeled on all carbons, measures the oxidation of the whole chain length. All tetracycline derivatives that we have studied inhibited the *in vitro* β-oxidation of [U-^{14}C]palmitic acid, and its *in vivo* oxidation to $[^{14}C]CO_2$ (Table 2). A good correlation was found between these *in vitro* and *in vivo* effects (Table 2).

The egress of triglycerides from the liver was assessed as the rate of increase in plasma triglycerides after blocking the removal of triglycerides from the plasma by the administration of Triton WR 1339 in fasted mice [12]. This egress was decreased by all tetracycline derivatives, except doxycycline (Table 2). Except for doxycycline, all derivatives increased hepatic triglycerides 24 hr after a single dose (Table 2). With doxycycline, a doubling of hepatic triglycerides could be obtained, nevertheless, by repeating the administration of doxycycline (0.25 mmol/kg i.p.) every 6 hr (Table 2).

Mortality after administration of a high dose (0.5 mmol/kg i.p.) was absent or low with oxytetracycline, lymecycline and tetracycline, intermediate with rolitetracycline, and high with chlortetracycline, doxycycline, demeclocycline and minocycline (Table 2). An inverse relationship was found between mortality and the *in vivo* oxidation rate of palmitic acid (Fig. 1).

Discussion

Tetracycline has been shown previously to increase hepatic triglycerides as a consequence of (i) decreased oxidation of fatty acids in the liver [2] and (ii) decreased egress of lipoproteins from the liver [3–5]. The present report shows that, with the exception of doxycycline, all other tetracycline derivatives that we studied, also produced these two effects (Table 2). Doxycycline was an exception in that it likewise decreased β-oxidation, but did not decrease the egress of lipids from the liver (Table 2). It is noteworthy that this derivative did not increase hepatic triglycerides after a single dose in this study (Table 2) and in a previous study [13]. These observations are consistent with the view that decreased egress of lipids from the liver is an important factor in the hepatic accumulation of fat induced by tetracycline [3–5]. Nevertheless, deposition of triglycerides in the liver could also be obtained by repeating the doses of doxycycline (Table 2), showing that decreased β-oxidation can also lead to fat accumulation.

Both the degree of inhibition of the mitochondrial oxidation of fatty acids, and the degree of inhibition of the egress of triglycerides from the liver differed markedly with different derivatives (Table 2). These two effects varied independently of each other (Table 2), suggesting that they may be mediated by different physico-chemical properties. The molecular mechanisms responsible for these two effects remain unknown [2–5], however, and are not clarified by the present study.

As opposed to macrovacuolar steatosis, microvesicular steatosis is a severe disease, potentially leading to liver

Table 2. *In vitro* and *in vivo* effects of various tetracycline derivatives

	In vitro formation of acid-soluble β-oxidation products from [U-^{14}C]palmitic acid in the presence of various tetracyclines (1 mM) (nmol/mg protein/min)	*In vivo* exhalation of [^{14}C]CO^2 from [U-^{14}C]palmitic acid after administration of 0.25 mmol/kg of various tetracyclines (% of radioactivity administered)	*In vivo* increase in plasma triglycerides 3 hr after administration of Triton WR 1339 given 15 min after 0.25 mmol/kg of various tetracyclines (mg/mL)	Hepatic triglycerides 24 hr after administration of 0.25 mmol/kg of various tetracyclines (mg/whole liver)	Mortality 24 hr after administration of 0.5 mmol/kg of various tetracyclines (%)
Control	6.4 ± 0.3	18 ± 1	8.1 ± 0.6	11 ± 2	0
Oxytetracycline	6.0 ± 0.4*	13 ± 2†	5.1 ± 0.4†	38 ± 12†	0
Lymecycline	2.2 ± 0.3†	10 ± 3†	6.2 ± 0.4†	37 ± 9†	7
Tetracycline	2.7 ± 0.4†	7 ± 1†	4.6 ± 0.5†	41 ± 7†	6
Rolitetracycline	4.2 ± 0.3†	6 ± 1†	4.5 ± 1.0†	24 ± 9†	21
Chlortetracycline	0.6 ± 0.1†	4 ± 1†	6.4 ± 0.6†	91 ± 11†	79
Doxycycline	1.8 ± 0.2†	4 ± 2†	8.2 ± 0.6	12 ± 2‡	86
Demeclocycline	1.3 ± 0.5†	2 ± 1†	6.5 ± 0.4†	62 ± 9†	86
Minocycline	0.3 ± 0.1†	2 ± 1†	5.7 ± 1.0†	20 ± 4†	79

Results are means ± SEM for, respectively, 4–20 determinations (*in vitro* formation of β-oxidation products), 4–12 mice (*in vivo* exhalation of labelled CO_2), 10 mice (*in vivo* increase in plasma triglycerides after Triton WR 1339), 6–17 mice (hepatic triglycerides), or 14–19 mice (mortality).

* Not significantly different from control mice. However, with a higher concentration (3 mM) of oxytetracycline, this formation was significantly ($P < 0.05$) decreased from 6.5 ± 0.2 nmol/mg protein/min in control, to 3.9 ± 0.2 with oxytetracycline (means ± SEM for three determinations).

† Significantly different from control values, $P < 0.05$.

‡ Not different from control mice. However, after administration of four repeated doses (0.25 mmol/kg i.p. every 6 hr), hepatic triglycerides 24 hr after the first dose were significantly ($P < 0.05$) increased, from 16 ± 2 mg/whole liver in control mice to 35 ± 5 in treated mice (mean ± SEM for 12 mice).

failure, coma and death in humans [1]. Several drugs which produce this severe form of fat accumulation have been shown to decrease the mitochondrial oxidation of fatty acids [2, 14–18]. In the present study, there was an inverse relationship between the residual *in vivo* oxidation rate of palmitic acid and mortality after high doses (Fig. 1). This might indicate a possible causal relationship between severe inhibition of fatty acid oxidation and mortality.

In conclusion, our observations can be summarized as follows: (i) Oxytetracycline, limecycline, rolitetracycline, chlortetracycline, demeclocycline and minocycline, like tetracycline, decrease the β-oxidation of fatty acids, decrease the egress of triglycerides from the liver, and lead to the accumulation of triglycerides in the liver. (ii) Doxycycline only inhibits β-oxidation, without decreasing the egress of lipids from the liver; this derivative does not lead to hepatic fat accumulation, unless doses are repeated. (iii) Severe inhibition of fatty acid oxidation by some derivatives was associated with high mortality, suggesting a possible cause-and-effect relationship.

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