both 60 min after Cd. This corresponds to a reduction of the Cd-content of the liver whereas that of the kidneys remains unchanged. It is doubtful whether the removal of Cd from the liver has any beneficial effect as it does not lead to reduced mortality following EDTA treatment. This can be seen from table 2 which summarizes the mortality observed in both experiments. Table 2 also shows that treatment with Na-salicylate (SA) alone exacerbates the effect of $CdCl_2$. Administration of the chelant combinations does not improve the effect achieved with one chelator alone. The results appear to depend very much on the Cd-dose and/or the time interval between chelant and metal injection, the effects being different for the different substances tested. This is corroborated to some extent by the results of Jones and Basinger[4]. It is very unfortunate, however, that these authors do not give any control figures for the mortality-rate for animals receiving $CdCl_2$ only. The reason for the apparently contrary effects of the chelators in the 2 experiments are not known. However, the results in table 2 suggest that EDTA and DMPS may act by different mechanisms.

In the meantime Schubert[3] has withdrawn the results on which our first experiment (chelate treatment 1 h after Cd) was based. In a personal communication he later suggested that a combination of DMPS and $CaNa_3$-DTPA (DTPA) instead of EDTA showed a greater effectiveness and recommended intraperitoneal treatment with 0.04 mmoles/kg DTPA and 0.2 mmoles/kg DMPS at 5 and 95 min after Cd administration. However, in our animals this treatment also proved ineffective, the mortality being 80%, as in the Cd-controls. Our results, therefore, do not confirm the results of Schubert and Derr and suggest that the mixed ligand chelate theory of Schubert[3,5] in the present form does not apply in vivo to rats. Further careful studies, especially in vivo, are needed to show why neither our experiments nor those of others[6] correspond to Schubert's potentiometric titration studies which appear to suggest superior stability of at least some mixed ligand chelates over single ligand chelates.

Table 2. Mortality of rats after $CdCl_2$ with or without subsequent treatment with single or mixed ligand chelants

$CdCl_2$	Primary chelant 0.5 mmoles/kg	Secondary chelant 2.0 mmoles/kg	Mortality 0.03 mmoles/kg $CdCl_2$ i.v. Chelant(s) after 1 h	0.1 mmoles/kg $CdCl_2$ i.p. Chelant(s) after 5 min
+	-	-	16/27= 59%	37/43= 86%
−	-	SA	0/10= 0%	0/15= 0%
+	-	SA	9/10= 90%	15/15=100%
+	-	DMPS	1/9 = 11%	14/16= 88%
+	EDTA	-	9/16= 56%	14/30= 47%
+	EDTA	SA	11/11=100%	7/15= 47%
+	EDTA	DMPS	3/10= 30%	12/15= 80%

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Anti-oxidative effect of coenzyme Q_{10}

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Summary. Coenzyme Q_{10} is effective as an anti-oxidant like α-tocopherol, especially in the heart.

The peroxidation of lipids is closely related to several pathological disorders[1-3]. To prevent lipid peroxidation in the living body, α-tocopherol has been widely used. However, a ubiquinone was also reported to have anti-oxidative activity[4] in vitro. In this experiment, we studied the anti-oxidative activity of coenzyme Q_{10} (Co Q_{10}), one of the ubiquinones, both in vivo and in vitro, comparing its activity with that of α-tocopherol.

Materials and methods. In vivo study: Female rats of the Wistar strain, weighing 200 ± 10 g, were injected i.p. with 0.2 ml of isotonic saline including Co Q_{10} (2 mg/kg, 2.5 mg/ml) or α-tocopherol (10 mg/kg, 12.5 mg/ml) for 5 successive days. On the last day, hearts and livers were isolated and their mitochondria were prepared by Hatefi's method[5]. Each mitochondrial suspension was made to contain 3.0 mg of mitochondrial protein per ml of the mannitol - sucrose mixture. Mitochondrial lipoperoxides were measured by Packer's method[6].

In vitro study: To elucidate the inhibitory action of Co Q_{10} or α-tocopherol on peroxidation of mitochondrial fatty

Table 1. Lipoperoxides in mitochondria isolated from liver or from heart of rats given CoQ_{10} or α-tocopherol in vivo

Administration		Lipoperoxides in mitochondria (nmoles/mg protein) Liver	Heart
None		16.7 ± 1.7	11.2 ± 1.5
CoQ_{10}	2 mg/kg/day, 5 days	10.9 ± 1.8	8.5 ± 0.6
α-tocopherol	10 mg/kg/day, 5 days	7.2 ± 0.8	8.0 ± 1.4

Values are mean ± SD.

Table 2. Lipoperoxides in mitochondria with or without anti-oxidants in vitro

Additions in vitro	Lipoperoxides (nmoles/mg protein)
None	16.7 ± 1.7
CoQ_{10}, 0.2 mg	12.7 ± 1.4
CoQ_{10}, 0.4 mg	11.1 ± 1.5
CoQ_{10}, 0.8 mg	9.4 ± 1.0
α-tocopherol, 0.1 mg	13.2 ± 1.3

acids in vitro, using a rat liver mitochondrial suspension prepared by the same method as that used for the in vivo experiment, 0, 0.2, 0.4 or 0.8 mg Co Q_{10} (10 mg/ml solution) or 0.1 mg α-tocopherol (10 mg/ml solution) was added to the mitochondrial suspension. Then, the lipoperoxide content of each sample was measured by the same procedure as in the in vivo experiment.

Results. In vivo study: Table 1 shows the lipoperoxide content of mitochondria isolated from liver. Lipoperoxides decreased significantly with the administration of Co Q_{10} and α-tocopherol. α-Tocopherol was more effective than Co Q_{10} in the prevention of an increase in lipoperoxides under these conditions. In mitochondria isolated from heart, the quantity of lipoperoxides was significantly decreased with the administration of Co Q_{10}, or with that of α-tocopherol, compared with the control group. There was no difference between the anti-oxidative capacities of the 2 drugs administered.

In vitro study: Table 2 shows the efficiency of Co Q_{10} and α-tocopherol in the prevention of an increase in lipoperoxides in vitro. The quantity of lipoperoxides decreased significantly in proportion to the administered dosage of Co Q_{10}. In the group which received α-tocopherol, there was a significant decrease in the quantity of lipoperoxides. The administration of α-tocopherol at this dose (0.1 mg) had almost the same efficiency as the administration of 0.2 mg of Co Q_{10}.

Discussion. We studied the anti-oxidant effect of Co Q_{10} both in vivo and in vitro in comparison with α-tocopherol. Co Q_{10} was revealed to have anti-oxidative activity. It was also demonstrated that administration of relatively small doses of Co Q_{10} (2 mg/kg) had the same anti-oxidant effect as that of α-tocopherol (10 mg/kg) on heart mitochondria, though α-tocopherol was more effective than Co Q_{10} in reducing lipoperoxides in liver mitochondria. These results indicate that Co Q_{10} has anti-oxidative capacity, and that the anti-oxidative activity of Co Q_{10} and that of α-tocopherol differ from organ to organ. The data suggest that an anti-oxidant which has a different hydrophobicity from others will show differences in anti-oxidative capacity from organ to organ. As the lipid composition of cell membranes differs from organ to organ, each organ may have a different permeability to anti-oxidants.

In conclusion, Co Q_{10} is a suitable anti-oxidant for the prevention of lipo-peroxidation in vivo. Selective usage of anti-oxidants such as Co Q_{10} or α-tocopherol for patients will open a new era in their linical application.

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Ethanol-induced changes in plasma glucose, insulin and glucagon in fed and fasted rats[1]

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Summary. Orally administered ethanol produced dose-related suppression of IRI levels and elevation of IRG levels in fed and fasting rats. Plasma glucose levels were unaffected in the fasting rats, but a moderate hyperglycemic response accompanied the decline in IRI and the rise in IRG observed in the fed rats.

Ethanol can produce effects on glucose homeostasis which are dependent upon dose and state of nourishment of the subject[2]. For example, the glycemic response to moderate or large doses of ethanol is hypoglycemia in fasting subjects but hyperglycemia in fed subjects. It has been stated that the decline in insulin and the compensatory increase in glucagon in ethanol-treated fasting subjects are indirect and that they are the consequence of the fall in blood glucose. The present study reports the dose-related effects of orally administered ethanol on plasma immunoreactive insulin (IRI) and glucagon (IRG) levels taken 1 h after gavage in fed and fasting rats.

Methods. Fully nourished or fasting (18 h) male Sprague-Dawley rats weighing between 350 and 450 g were administered saline or ethanol (150, 450 or 750 ml/100 g) by orogastric intubation. 45 min after gavage each animal was anesthetized with pentobarbital i.p., the dose of pentobarbital determined by the dose of ethanol: saline, 5 mg pentobarbital/100 g; 150 mg ethanol/100 g, 3.75 mg pentobarbital/100 g; 450 mg ethanol/100 g, 2 mg pentobarbital/100 g; 750 mg ethanol/100 g, 0.8 mg pentobarbital/100 g. The level of anesthesia produced by these combinations of drugs produced sufficient anesthesia to allow cardiac puncture without discomfort to the rat. Blood samples (1 ml) were taken by cardiac puncture 1 h after gavage (15 min after pentobarbital injection) and placed in iced test tubes containing 100 µl benzamidine (0.5 M) and 100 µl EDTA (12 mg/ml) to prevent proteolysis and coagulation, respectively. Plasma samples were subsequently analyzed in duplicate for IRI[3], IRG[4] and glucose[5] levels. Antiserum 30K (Roger Unger, Dallas, Texas), which reacts with no more than 2% of plasma gut GLI, was used for IRG determinations. Statistical analysis consisted of Students t-test for unpaired (group) data.

Results and discussion. As shown in figure 1, ethanol p.o. produced a decline in plasma IRI levels in fasted rats at 1 h after each dose of ethanol. Plasma IRG levels were not changed from control (saline-treated) levels by 150 mg ethanol/100 g but IRG levels after the intermediate (450 mg/100 g) and high (750 mg/100 g) doses of ethanol were elevated significantly ($p < 0.05$; $p < 0.01$). Plasma glucose levels were not different among the 4 groups of fasted rats.

Fed rats (saline-treated) had basal levels of IRI (4-fold higher) and IRG (3-fold lower) that were significantly different from those of fasted rats (figure 2) and are appropriate for their different state of nourishment. Administration of 150 mg ethanol/100 g p.o. produced no changes in glucose, IRI or IRG levels in fed rats. An oral dose of 450 mg ethanol/100 g produced significant eleva-