have not been investigated. It, therefore, remains to determine the exact biochemical mechanism responsible for the sex difference in toxicity of DMP and other phosphorothioates. However, through the use of partially hepatectomized male rats the liver was found to be the organ directly responsible for the sex difference in susceptibility. Experiments with partially hepatectomized, castrated and testosterone-treated male rats, as well as weanling males, provided information suggesting that synthesis of some component of a system in liver which affects the toxicity of phosphorothioates to male rats is stimulated by testosterone. These findings may, therefore, serve as a basis for further studies aimed at identification of the exact biochemical process responsible for the sex difference in susceptibility of rats to phosphorothioates.

Summary. Measurements of the acute toxicity of DMP, a new cholinergic phosphorothioate, demonstrated that it has a high toxicity to female rats, male and female mice and male guinea pigs as evidenced by intraperitoneal LD_{50} values ranging from 22 to 35 mg/kg. An exception was noted in the case of male rats to which the intraperitoneal LD_{50} was 200 mg/kg. DMP inhibits cholinesterase activity of brain and peripheral tissues *in vivo*. The LD_{50} of the oxygen analogue of DMP was 5.8 mg/kg to male rats and there was no sex difference in susceptibility. The 10-fold resistance of male rats to the toxicity of DMP was absent at 2 days after partial hepatectomy. At 14 days after partial hepatectomy males regained their resistance unless they were castrated before hepatectomy. Injection of testosterone caused redevelopment of resistance in hepatectomized, castrated rats. The results of these experiments indicate that some system in the liver is responsible for the resistance of male rats to DMP and that androgens govern the development of this system.

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Received June 8, 1961. P.S.E.B.M., 1961, v107.

Coenzyme Q Metabolism in Pantothenic Acid Deficiency.* (26792)

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Though the origin of the substituted benzoquinone ring of the coenzyme Q molecule still remains uncertain, the mechanism of biosynthesis of the isoprenoid side chain appears to be well established. Incorporation of intraperitoneally administered labeled mevalonic acid into rat liver coenzyme Q has been reported by Gloor and Wiss(1,2); Dialameh and Olson(3) have since observed that acetate-C^{14} also serves as a precursor of the isoprenoid side chain, *in vivo,* in the rat. Biosynthesis of the side chain of coenzyme Q from these 2 labeled precursors, both *in vivo* (4-6) and *in vitro*(6,7) have been confirmed by others. The well known involvement of coenzyme A in biosynthesis of isoprene units and of mevalonic acid from 2 carbon fragments, suggested a study of the relation of coenzyme Q metabolism to pantothenic acid status of the animal. Observations on endogenous tissue levels of coenzyme Q as well as on its biosynthesis *in vivo* and *in vitro* from labeled precursors in pantothenic acid de-

* Preliminary report has been published(18).

TABLE I. Intracellular Distribution of Coenzyme Q.

Group*	Pantothenic acid, μg/g	Coenzyme Q, μg/g: Whole liver	Nuclei	Mitochondria	Microsomes	Supernatant	Succinoxidase activity, μl O_2/hr/g
P-supplemented	133.7 ± 6.1	127 ± 7.6	37 ± 4.1	48 ± 2.7	19 ± 3.1	7 ± 1.6	1284 ± 69
P-deficient	79.6 ±11.2	103 ± 3.1	33 ± 5.6	36 ± 4.1	17 ± 4.1	6 ± 2.4	963 ± 74

Results are avg of 4 independent observations ± stand. error of mean, and are expressed on fresh weight basis.

* P = Pantothenic acid.

ficiency are presented and discussed in the light of other reported observations.

Experimental. Induction of deficiency. Deficiency of pantothenic acid was induced by feeding weanling male rats (Wistar strain) a purified casein ration, devoid of the vitamin, for 8 weeks (for details, cf. 18). The control group received a supplement of 20 mg calcium pantothenate, per kg of diet, throughout the experimental period. To one batch of rats fed the deficient diet for 8 weeks, calcium pantothenate (10 mg/rat) was administered intraperitoneally, and the animals were sacrificed at intervals of 0, 8, 24 and 48 hours from time of administration.

Determinations. The livers were quickly excised, chilled in isotonic sucrose (0.25 M) and made into 10% homogenates in the same, using a Potter-Elvehjem type homogeniser. Separation of the homogenate into the subcellular fratcions was carried out by the procedures of Schneider and Hogeboom(8) and of Palade and Siekevitz(9). The nuclei and mitochondria were separated at 700 × g and at 5000 × g for 10 minutes each, in a PR-2 International refrigerated centrifuge, and the microsomes were sedimented at 105,000 × g for 60 minutes in a Spinco model L preparative ultracentrifuge. The supernatant fraction was further freeze-dried. Total pantothenic acid content(10) and succinoxidase activity(11) of liver were assayed essentially as detailed by the respective authors, while coenzyme Q in the unsaponifiable liver lipids was chromatographically purified by the method of Festenstein *et al.*(12) with minor modifications. Absorption of the oxidised and reduced forms (after addition of potassium borohydride) at 272 mμ was read on a Beckman model DU Spectrophotometer(13).

Studies on in vivo *and* in vitro *incorporation of labeled precursors.* Four animals from each group were fasted overnight, intraperitoneally administered either 0.5 ml acetate *U*-C^{14} (20 μc) or mevalonate-2-C^{14} (10 μc), and were sacrificed 4 hours later. The livers were removed, and coenzyme Q was isolated from the unsaponifiable lipids by chromatography on alumina. The fractions containing the coenzyme Q were rechromatographed and the radioactivity of 2 samples pooled together was counted at infinite thinness on stainless steel planchets employing the Tracerlab SC-16 windowless flow gas counter in conjunction with Tracerlab SC-51 autoscaler. After counting, the coenzyme Q in the planchets was determined spectrophotometrically.

For studies on *in vitro* incorporation, liver slices, prepared using a Stadie tissue slicer (A. H. Thomas Co., U.S.A.), and weighing 500 mg from animals of both groups were incubated in Warburg flasks open to atmosphere and containing 10 μM mevalonate-2-C^{14} (10 μc) along with 3 μM disodium salt of adenosine triphosphate, and 60 μM glucose in 4.0 ml of Krebs-Ringer phosphate buffer, pH 7.2. At the end of incubation period, the

TABLE II. Effect of Administration of Pantothenic Acid to Deficient Rats.

Hr after administration	Coenzyme Q, μg/g: Whole liver	Mitochondria	Succinoxidase activity, μl O_2/hr/g
0	103 ± 3.1	36 ± 4.1	963 ± 74
8	111 ± 4.7	40 ± 3.6	1048 ± 42
24	124 ± 6.3	47 ± 1.4	1213 ± 36
48	129 ± 3.1	45 ± .9	1246 ± 98

Calcium pantothenate (10 mg) was inj. intraper. to deficient rats and animals sacrificed 0, 8, 24 and 48 hr later.

Results are avg of 4 independent determinations ± stand. error of mean and are expressed on fresh liver weight.

TABLE III. Incorporation of Acetate-C^{14} and Mevalonate-2-C^{14} into Rat Liver Coenzyme Q *In Vivo*.

Group*	Incorporation into coenzyme Q from Acetate-C^{14} Total counts, cpm	Acetate-C^{14} Specific activity, cpm/mg	Mevalonate-2-C^{14} Total counts, cpm	Mevalonate-2-C^{14} Specific activity, cpm/mg
P-deficient	144 ± 42	831 ± 62	654 ± 35	3316 ± 269
P-supplemented	336 ± 71	1864 ± 221	957 ± 104	4456 ± 492

Four animals from each group were fased overnight and administered intraper. either acetate-*U*-C^{14} (20 μc) or mevalonate-2-C^{14} (10 μc) and were sacrificed 4 hr later.

Results are avg of 4 independent determinations ± stand. error of mean.

* P = Pantothenic acid.

suspending medium was decanted off, the slices washed twice with Krebs-Ringer phosphate buffer, and radioactivity and spectrophotometric determinations of coenzyme Q in pooled slices carried out as described earlier.

Results and discussion. The changes in liver stores of coenzyme Q and its intracellular distribution in pantothenic acid deficiency are presented in Table I. A decrease in level of coenzyme Q, particularly marked in the mitochondrial fraction, is paralleled by reduction in succinoxidase activity. This parallelism between mitochondrial coenzyme Q and succinoxidase activity would be consistent with the reported participation of coenzyme Q in the succinoxidase system(14-17).

Administration of a single dose of calcium pantothenate to deficient animals restores the coenzyme Q levels, with concomitant increase in succinoxidase activity, to almost normal by the end of 24 hours (Table II).

That the decrease in liver stores of coenzyme Q in pantothenic acid deficiency may be due to decreased synthesis, because of the involvement of coenzyme A in biosynthesis of the isoprenoid side chain, appears to be fully borne out by studies on incorporation of labeled precursors into coenzyme Q, both *in vivo* and *in vitro*. The deficient animals show lesser incorporation of the label into coenzyme Q, more particularly seen with acetate (Table III). *In vitro* incorporation of mevalonate-C^{14} into coenzyme Q is far less than *in vivo*, and is possibly due to the lack of certain essential cofactors in the *in vitro* system, as has also been suggested by Phillips(7) (Table IV).

TABLE IV. Incorporation of Mevalonate-2-C^{14} into Rat Liver Coenzyme Q *In Vitro*.

Group*	Incorporation into coenzyme Q Total counts, cpm	Specific activity, cpm/mg
P-deficient	316 ± 24	814 ± 47
P-supplemented	467 ± 29	1162 ± 113

Liver slices (500 mg) were incubated in a system containing 10 μM mevalonate-2-C^{14} (10 μc) along with 3 μM disodium salt of adenosine triphosphate, and 60 μM glucose in 4.0 ml Krebs-Ringer phosphate buffer, pH 7.2 for 3 hr.

Results are avg of 4 independent determinations ± stand. error of mean.

* P = Pantothenic acid.

Summary. 1. Pantothenic acid deficiency results in decreases in coenzyme Q and succinoxidase activity of liver. Administration of pantothenic acid to the deficient animals results in a restoration to normal by the end of 24 hours. 2. The deficient animals show decreased incorporation of labeled acetate and mevalonate into liver coenzyme Q, indicating a decreased synthesis of the coenzyme Q as the possible cause of lowered liver levels of coenzyme Q in pantothenic acid deficiency.

Grateful acknowledgement is made to Dr. O. Wiss, Hoffmann la Roche Ltd., Basle, Switzerland for the kind gift of mevalonic acid-2-C^{14} used in these studies. Our thanks are also due to the Indian Council of Medical Research for financial support and to Dr. V. Subrahmanyan, Director of this Institute, for his interest and encouragement.

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Received June 9, 1961. P.S.E.B.M., 1961, v107.

Effect of Vanadium Administration on Coenzyme Q Metabolism in Rats. (26793)

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During studies on the *in vitro* biosynthesis of cholesterol, Curran(1) observed a marked inhibitory effect of vanadium ions on incorporation of acetate-C^{14} into cholesterol by liver homogenates. Similar observations on *in vivo* biosynthesis of cholesterol and of phospholipids in vanadium fed animals have been reported by others(2-4).

While Azarnoff and Curran(5) indicated that the inhibition occurred between the stages of mevalonic acid and β-methyl crotonate, Wright *et al.*(6) suggested that vanadium probably interfered with maintenance of adenosine triphosphate levels in the system and that this may impair the synthesis of phosphorylated intermediates from mevalonic acid. This view is further substantiated by the report that exogenous adenosine triphosphate enhances the synthesis of cholesterol from mevalonic acid(7) and that inhibitors of oxidative phosphorylation decrease it(8). A similar inhibition of acetate incorporation into phospholipids *in vitro* by fluoride, cyanide, dinitrophenol and iodoacetate has been shown earlier by Kline and De Luca(9).

That the decreased synthesis of cholesterol may be due to a lowering in tissue stores of coenzyme A is suggested by the recent work of Mascitelli-Coriandoli and Citterio(10), who have observed considerable reductions in hepatic coenzyme A in rats administered vanadium either orally or parenterally. Other workers(4,11) have suggested that the metabolism of sulfur amino acids may be interfered with by vanadium, in which case a shortage might be postulated of the sulfhydryl reserves of the cell, notably of cysteine, that are needed for biosynthesis of coenzyme A.

The well known involvement of coenzyme A in biosynthesis of isoprene units and of mevalonic acid from 2 carbon fragments and the reported incorporation(12-15) of these into the isoprenoid side chain of coenzyme Q prompted a study of the effect of vanadium administration on coenzyme Q metabolism.

Experimental. Male weanling rats (Wistar strain) 40-50 g in weight were fed a purified 10% casein diet replete in all respects (cf. 16) for a period of 8 weeks. To one group of rats, ammonium vanadate (0.6 mg/rat/week) was administered intraperitoneally while control rats received an equal amount of distilled water. At the end of 8 weeks, some animals from the vanadium ad-