tubular reabsorption of urate was well known to occur in man(8) whereas evidence for tubular secretion of urate, except perhaps for one abnormal instance(9), was lacking. Such evidence has since been secured, under appropriate experimental conditions, in normal man(10), as well as in the rabbit(11) and dog(4,5,12), suggesting that the mammalian kidney does indeed possess the capacity for tubular secretion of urate. There appears to be at least one precedent for inhibition of tubular secretion of urate in man, the retention of urate caused by salicylate in low dosage, presumably by competition with urate for active tubular transport(13).

Summary. Pyrazinamide depresses C_{urate}/GFR in the Dalmatian and non-Dalmatian dog. Stop-flow studies revealed unequivocal suppression of tubular secretion of urate in the Dalmatian and gave no indication of enhancement of tubular reabsorption of urate in the non-Dalmatian.

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Studies on the Sex Difference in Toxicity of a Cholinergic Phosphorothioate.* (26791)

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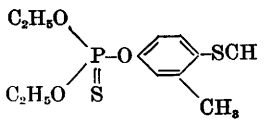
A sex difference in the acute toxicity of parathion to rats was observed in this laboratory in 1949(1). Subsequent studies have established that male rats exhibit greater resistance than females to a number of other phosphorothioates. The mechanism underlying this effect has been difficult to investigate in intact animals because the magnitude of the sex difference in susceptibility is generally relatively small. During measurements of the influence of structural changes on the toxicity of some phosphorothioates we observed a 10-fold sex difference in susceptibility of rats to 0,0-diethyl 0-(4-methylthio-m-tolyl) phosphorothioate (DMP; Bayer 29492). This compound has the following chemical structure:



The magnitude of the sex difference in susceptibility of rats to this phosphorothioate was sufficient to permit a study of factors which might be responsible for the greater resistance of male rats. The present communication describes the mammalian toxicity and anticholinesterase activity of DMP and provides evidence that the liver, under the influence of androgens, is responsible for the resistance of males toward the acute toxicity of this organic phosphate.

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TABLE I. Acute Toxicity of DMP to Mammals.

Species	Sex	Route	No. of animals	LD_{50} (mg/kg)
Rats	♀	I.P.	50	22
"	♂	"	52	200
"	♀	Oral	40	14
"	♂	"	40	95
Mice	♀	I.P.	35	25
"	♂	"	40	35
Guinea pigs	♂	"	32	30

Materials and methods. Male and female Sprague-Dawley rats weighing between 175 and 250 g were used unless otherwise specified. Young male guinea pigs (*ca* 200 g) and Carworth Farms mice (*ca* 20 g) were used. The organic phosphates, which were kindly supplied by the Chemagro Corp., Kansas City, Mo., were dissolved in a mixture of 20% ethanol and 80% propylene glycol and concentration of toxic agent was so adjusted that animals always received amounts of solution equivalent to less than 1% of their body weight. Testosterone propionate was dissolved in sesame oil (2 mg/ml) and administered subcutaneously. Cholinesterase assays were performed manometrically by the method of DuBois and Mangun(2). In the toxicity measurements animals were observed for 10 days and LD_{50} values were calculated from the mortality data by the logarithm-probability method.

Results. Table I shows the acute toxicity of DMP to rats, mice and guinea pigs. These results indicated that this compound has a relatively high and similar toxicity to mammals with the exception of male rats which exhibited a marked resistance. The 10-fold difference in susceptibility of male and female rats to DMP given intraperitoneally greatly exceeds the magnitude of the sex differences noted previously with other phosphorothioates. It was sufficiently great to permit further toxicity experiments to clarify the factors responsible for this effect.

The symptoms produced by DMP were typical of those caused by cholinergic phosphorothioates. Following administration of the compound to rats the cholinesterase activity of peripheral tissues and brain was markedly inhibited. Fig. 1 shows the inhibitory action of a sublethal dose (125 mg/kg) of DMP on cholinesterase activity of brain, submaxillary glands and serum of male rats and rate of reversal of the inhibition. Each point on the curves represents an average for tissues from 3 animals. Maximum inhibition occurred within 6 hours and persisted for about 24 hours after which gradual reversal occurred. Similar measurements performed on female rats given 13.75 mg/kg, which is the same fraction of the LD_{50} used for the assays on males, demonstrated that equitoxic doses produce the same amount of inhibition of cholinesterase and the same rate of recovery in males and females.

Phosphorothioates do not have anticholinesterase activity but they are converted to toxic metabolites *in vivo* by the liver of mammals through replacement of the sulfur of the thiophosphate linkage by oxygen(3,4,5). The resulting oxygen analogues are, therefore, directly responsible for the toxicity and anticholinesterase activity. Measurements of the toxicity of the oxygen analogue of DMP (0,0-diethyl 0-[4-methylthio-m-tolyl] phosphate) to rats (Table II) demonstrated that there is no sex difference in rats to this highly toxic compound, suggesting that the sex difference in acute toxicity of the parent compound may be due either to a difference in rate of conversion of the sulfur to the oxygen analogue or rate of detoxification of the sulfur analogue.

In a search for information on factors involved in the sex difference in toxicity of

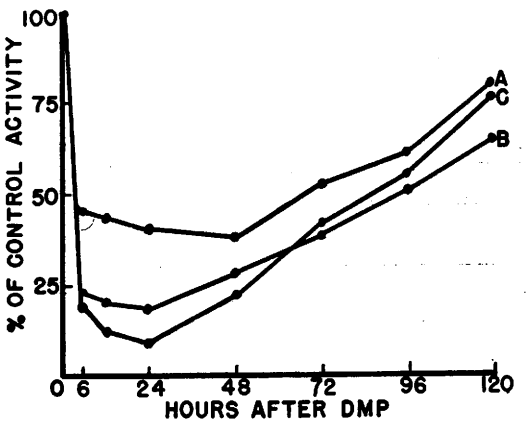

FIG. 1. Rate of decrease and recovery of cholinesterase activity of tissues from male rats after intraper. inj. of DMP (125 mg/kg). A. Brain. B. Submaxillary. C. Serum.

TABLE II. Acute Toxicity of the Oxygen Analogue of DMP to Rats.

Sex	Route	No. of animals	LD_{50} (mg/kg)
♀	I.P.	35	5.7
♂	"	30	5.8
♀	Oral	35	9.0
♂	"	40	12.0

DMP the possibility was considered that the liver, under some regulatory influence of androgens, was responsible. A number of toxicity tests were, therefore, conducted on male rats subjected to partial hepatectomy, castration, and treatment with androgens. The results are summarized in Table III. At 2 days after removal of the lateral and medial lobes of the liver the resistance of male rats to DMP was absent as indicated by a decrease in LD_{50} from 200 to 16 mg/kg. When the liver was allowed to regenerate for 14 days, the animals reacquired most of their resistance. However, when males were castrated, subjected to partial hepatectomy one week later and treated with DMP at 14 days following hepatectomy they failed to regain their resistance. This finding suggested that development of the system in the liver which accounts for the resistance of male rats to DMP is under the influence of androgens. This possibility received further support by repetition of this experiment with addition of daily subcutaneous injections of testosterone (2 mg/kg/day) from time of hepatectomy until DMP was given. The approximate LD_{50} of DMP to these animals was 150 mg/kg. Other evidence for the possible role of androgens as a factor involved in the resistance of males was obtained by measuring

TABLE III. Influence of Partial Hepatectomy, Castration and Testosterone Treatment on Acute Toxicity of DMP to Male Rats.

Treatment	No. of animals	I.P. LD_{50} (mg/kg)
None	50	200
2 days after partial hepatectomy	35	16
14 days after partial hepatectomy	32	160
14 days after partial hepatectomy and castration	24	25
Idem, and testosterone (2 mg/kg/day)	31	140
14 days after castration	32	175

the toxicity of DMP to young male rats. The LD_{50} for 23-day-old males was 15 mg/kg and at 45 days of age the value was 210 mg/kg. The resistance was, therefore, acquired within the period of puberty in male rats(6).

Additional experiments were performed on adult female rats to ascertain whether their high susceptibility could be altered by treatment with testosterone. For these measurements normal and ovariectomized female rats were given 2 mg/kg of testosterone propionate for a period of 30 days. The LD_{50} of DMP to these animals increased from 25 to about 100 mg/kg. Ovariectomy itself had no effect on the susceptibility. At 24 hours after a single dose of testosterone propionate (2 mg/kg) no effect was noted on the susceptibility of female rats to this toxic agent indicating that testosterone does not exert a direct activating effect on the system responsible for the resistance.

Discussion. The toxicity of phosphorothioates is governed by 3 biochemical reactions, namely (a) rate and extent of metabolic conversion in the liver to their oxygen analogues, (b) potency of the oxygen analogues as inhibitors of cholinesterase and (c) rate and extent of detoxification of parent compounds and oxygen analogues. Our finding that there is no sex difference in the toxicity of the oxygen analogue of DMP to rats eliminated anticholinesterase action and detoxification of the oxygen analogue from further consideration in this study. It, therefore, seemed likely that either the rate of metabolic conversion of DMP to its oxygen analogue or detoxification of the parent compound was responsible for the sex difference in susceptibility.

Previous studies in this laboratory(7) have shown that the concentration of the enzyme which catalyzes the desulfuration of phosphorothioates is higher in livers of males than of female rats. On this basis males would be expected to be more susceptible than females unless the rate of metabolic conversion to the toxic oxygen analogue by the livers of male rats is inhibited *in vivo* by some factor which is absent from *in vitro* liver systems. The biochemical reactions involved in detoxification of this phosphorothioate

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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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).