ratio. A similar pattern regarding initial changes in cyclic nucleotides has been reported in fibroblast-like connective-tissue cells in cell cultures when proliferation was initiated by different stimuli such as serum and a Ca^{2+}-ionophore[17,18]. The subsequent changes in the cyclic nucleotides showed a more complex pattern in the present in vivo study, however, than has been reported from experiments in vitro. Using a conventional statistical method such as Student's t-test we found a biphasic time course for each of cAMP, cGMP, and the cGMP/cAMP ratio within the observation time of 32 min. The more conservative Scheffé-test, however, did not substantiate this conclusion. It should be observed that fibroblast-like and mesothelial-cell-like cells are present in roughly equal numbers (52% and 48%), but that more fibroblast-like cells than mesothelial-cell-like cells are stimulated to proliferate with the treatment given[8]. This difference in mitogenic responsiveness between the 2 cell types is noteworthy in relation to the apparently biphasic time course for changes in cyclic nucleotides found here. The results appear to be compatible with a monophasic course for either cell type. The pattern of changes in cyclic nucleotides in the present study is quite different from what has been reported by Whitfield et al.[19] on proliferation of hepatocytes in rats in vivo. They found no alteration in the concentration of cyclic nucleotides during more than 30 min following partial hepatectomy or initiation of liver cell proliferation by i.v. injection of a mixture of triiodothyronine (T3), amino acids, glucagon, and heparin. After about 35 min they demonstrated a significant increase in the concentration of cAMP but no change in cGMP was found at all.

The time course of cyclic nucleotide changes in the present investigation is of interest from various points of view, even though it remains uncertain whether or not it is these changes that determine the subsequent DNA-synthesis and mitotic activity. One important point is that biphasic courses for both cAMP and cGMP (and the cGMP/cAMP ratio) were suggested. Without early and closely spaced observation times the results could have been very difficult to interpret. These findings may explain some of the apparently conflicting reports on changes in cAMP and cGMP in mitogenesis in previous papers[4].

1 This work was supported by the Swedish Cancer Society and the Swedish Medical Research Council (project No.04X-04498). We are indebted to Marcela Bartonek and Margareta Odenö for expert technical assistance and to Erik Leander for help with statistical matters.

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A species comparison of 2,4-dinitrotoluene metabolism in vitro[1]

R.D. Short, J.C. Dacre and C.C. Lee

Pharmacology and Toxicology, Midwest Research Institute, Kansas City (Missouri 64110, USA) and Environmental Protection Research Division, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick (Maryland 21701, USA), 2 March 1979

Summary. Postmitochondrial supernatants prepared from livers of mice, rats, rabbits, dogs, and monkeys metabolized 2,4-dinitrotoluene. The pattern of metabolites was characterized in both sexes of the species examined. In addition, the pattern of metabolites was altered by varying incubation conditions and pretreating male rats with phenobarbital or SKF 525-A.

This study was undertaken to compare the ability of livers from various species to metabolize 2,4-dinitrotoluene (2,4-DNT) and to characterize the pattern of metabolites produced. Dinitrotoluene is used as a dye intermediate. In the production of toluene diisocyanate for polyurethane foams, it is reduced to the diamine. Dinitrotoluene has also been used as a gelatinizing and waterproofing agent in the production of explosives.

In mice, rats, and dogs, the subacute toxicity of 2,4-DNT includes methemoglobinemia and atrophy of the testes with aspermatogenesis[2]. During a 24-month feeding study, rats receiving 2,4-DNT developed hepatocellular carcinomas and an increased incidence of tumors of mammary and s.c. tissues[3,4]. In contrast, mice that received 2,4-DNT mainly developed cystic renal tumors[4]. In another study, the reduced derivative of 2,4-DNT, 2,4-diaminotoluene, produced hepatocellular carcinomas in rats[5].

The results of the present study will be valuable in understanding how different species metabolize 2,4-DNT and, accordingly, will contribute to a better understanding of its toxicity.

Material and methods. In the present study, CD rats, CD-1 mice, New Zealand albino rabbits, beagle dogs, and rhesus monkeys were used. The animals were killed, and their livers were removed, weighed, and homogenized in 6 vol. of 1.15% KCl containing 50 mM tris HCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 × g for 30 min, and the resulting postmitochondrial supernatant was used for all incubations, which were conducted in 25-ml Erlenmeyer flasks. The reaction mixture contained 5 mM glucose-6-phosphate; 5 mM magnesium chloride; 0.8 mM nicotinamide adenine dinucleotide phosphate; 1 mM 2,4-dinitrotoluene [ring-^{14}C (U)] (^{14}C-2,4-DNT) which was purchased from New England Nuclear (Boston, Massa-

Table 1. Metabolism of [^{14}C] 2,4-DNT by postmitochondrial supernatants of livers from male rats

Pretreatment	Room air			Flushed with nitrogen		
	Dinitrobenzyl alcohol	Aminonitro-toluene	Origin	Dinitrobenzyl alcohol	Aminonitro-toluene	Origin
None	9.7±0.6[a]	2.7±0.6	2.4±0.1	6.8±1.0[b]	9.5± 3.0	2.2±0.2
Phenobarbital	22.5±2.4[c]	1.4±0.4	8.3±1.3[c]	8.3±2.1[b]	39.7±13.2[b,c]	11.2±0.7[b,c]
SKF 525-A	4.8±0.5[c]	1.3±0.5	3.3±1.3	3.9±1.1	13.4± 1.7[b]	3.4±0.7

[a] All values represent the mean±SE (nmoles/mg protein) for 4 determinations. [b] Significantly different from room air group with same pretreatment ($p<0.05$, 2-sample rank test)[9]. [c] Significantly different from non-pretreated group with same atmosphere ($p<0.05$, 2-sample rank test)[9].

Table 2. Metabolism of [^{14}C]2,4-DNT by postmitochondrial supernatant of liver from various species

Species	Sex	Room air			Flushed with nitrogen		
		Dinitrobenzyl alcohol	Aminonitro-toluenes	Origin	Dinitrobenzyl alcohol	Aminonitro-toluenes	Origin
Mouse	Male	11.0±0.8[a]	4.0±0.2	3.9±0.1	8.4±0.7	10.3±2.8	3.8±0.2
	Female	16.6±0.7	1.3±0.1	3.4±0.5	13.0±1.8	5.1±2.0	3.1±0.3
Rat	Male	11.1±0.6	1.8±0.5	3.8±0.5	4.6±1.6	30.4±9.4	4.2±0.3
	Female	8.0±1.0	1.3±0.1	1.8±0.2	6.1±0.6	3.1±0.8	1.8±0.2
Rabbit	Male	21.5±1.3	7.6±0.4	5.4±0.2	16.8±0.6	20.3±2.1	5.1±0.2
	Female	28.8±1.5	8.3±0.4	6.4±0.4	24.4±1.1	13.7±0.7	5.2±0.7
Dog	Male	17.1±1.0	0.7±0.1	1.5±0.2	8.6±3.4	8.0±5.7	1.1±0.3
	Female	14.0±1.5	0.6±0.1	1.6±0.2	11.4±1.5	1.1±0.3	1.2±0.3
Monkey	Male	20.1±7.6	15.8±1.7	7.2±2.6	13.4±7.3	55.8±5.4	13.9±5.1
	Female	25.0	17.3	10.6	26.8	22.1	9.8

[a] All values represent the mean±SE (nmoles/mg protein) for 3 to 4 observations or individual value.

chusetts); and 0.5 ml of the postmitochondrial supernatant, for a final volume of 2.5 ml.

For aerobic incubations, all components were added and the reactions were started by adding 50 µl of 95% ethanol containing ^{14}C-2,4-DNT. The flasks, which remained open to room air, were placed on a shaking water bath at 37 °C for 1 h. For incubations with reduced oxygen concentrations, all components except the substrate were added, and nitrogen was blown into the flask for 30 sec. Reactions were started by adding the substrate, and the flasks were immediately sealed with a rubber stopper and incubated according to the above methods. After incubation, 2.5 ml of acetone was added, and the mixture was cooled on ice for 10–15 min and then centrifuged. The supernatants were co-chromatographed with standards on silica gel TLC plates in a solvent system of benzene and ethyl acetate (4:1). The standards, with R_f-values in parentheses, were: 2,4-DNT (0.83), 2-amino,4-nitrotoluene (0.52), 2-nitro,4-aminotoluene (0.52), 2,4-dinitrobenzyl alcohol (0.33), and 2,4-diaminotoluene (0, i.e., remained at origin). The standards were located under UV light, and radioactivity was quantified using liquid scintillation counting. For convenience of presentation, the radioactivity associated with a standard is assumed to be chemically identical to the standard. Control reactions, which contained 1.15% KCl instead of the postmitochondrial supernatant, were conducted with all experiments. Radioactivity associated with a standard in control reactions was subtracted from the radioactivity obtained with the corresponding standard in enzymatic reactions. Using bovine serum albumin, protein concentrations were determined[6], and the results were expressed as nmoles product/mg protein.

In an effort to characterize these reactions, male rats were pretreated with either phenobarbital (80 mg/kg i.p. daily for 4 days and sacrificed 24 h after last dose) or SKF 525-A (50 mg/kg i.p. 1 h before sacrifice). These pretreatments are known to alter the liver's ability to metabolize many chemicals[7]. Livers from these animals were used for incubations as previously described.

Results. Pretreatment with phenobarbital increased the formation of dinitrobenzyl alcohol by livers from male rats (table 1). In contrast, pretreatment with SKF 525-A reduced the amount of this metabolite. These observations suggest that 2,4-DNT is metabolized to dinitrobenzyl alcohol by the hepatic microsomal mixed function oxidase system[7]. Phenobarbital pretreatment also increased the amount of material that remained at the origin. When a nitrogen flush was used to reduce the oxygen concentration, the formation of reduced metabolites increased and the production of dinitrobenzyl alcohol decreased.

The production of dinitrobenzyl alcohol was generally greater in rabbits, dogs, and monkeys than in mice and rats (table 2). The formation of this metabolite decreased when the oxygen concentration was reduced. More aminonitrotoluenes and metabolites that remained at the origin during incubation following a nitrogen flush were produced by male monkeys than by any of the other animals tested. After incubation, radioactivity which corresponded to 2,4-DNT represented 70–90% of the total radioactivity. There were various sex differences in the metabolism of ^{14}C-2,4-DNT; however, the only consistent difference was an increased formation of aminonitrotoluenes by males following a nitrogen flush. Although there was a dramatic sex difference for the production of this metabolite in rats (table 2), this value for males in another experiment (table 1) was lower.

Discussion. The major urinary metabolites of 2,4-DNT have been reported to be 2,4-dinitrobenzyl alcohol, 2,4-diaminotoluene, monoaminonitrotoluenes, and conjugates of these compounds[9]. In the present study, radioactivity which co-chromatographed with some of these compounds was produced by livers from various species during an in vitro incubation with 2,4-DNT. In general, livers of rabbits, dogs, and monkeys produced more dinitrobenzyl alcohol

under aerobic conditions, whereas livers of monkeys, and males of all species tested, produced more aminonitrotoluenes after nitrogen flush. In addition, the livers of monkeys produced more metabolites which remained at the origin after nitrogen flush.

In summary, the present study demonstrates that 2,4-DNT is metabolized in the liver by a postmitochondrial supernatant. The pattern of metabolites produced is altered by pretreating male rats with phenobarbital or SKF 525-A and by varying incubation conditions in all the species examined. In addition, the metabolic profile of 2,4-DNT was characterized in several species. A comparison of species' differences in toxicity[2,3] and the corresponding metabolic profile does not clearly indicate what role metabolism plays in toxicity.

1 This research was supported by the US Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21701, under Contract No. DAMD-17-74-C-4073. The authors thank Ms Mary Sawyer for competent technical assistance.

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Modification by levo-propranolol of tremors induced by harmine in mice

S.K. Kulkarni and P.N. Kaul

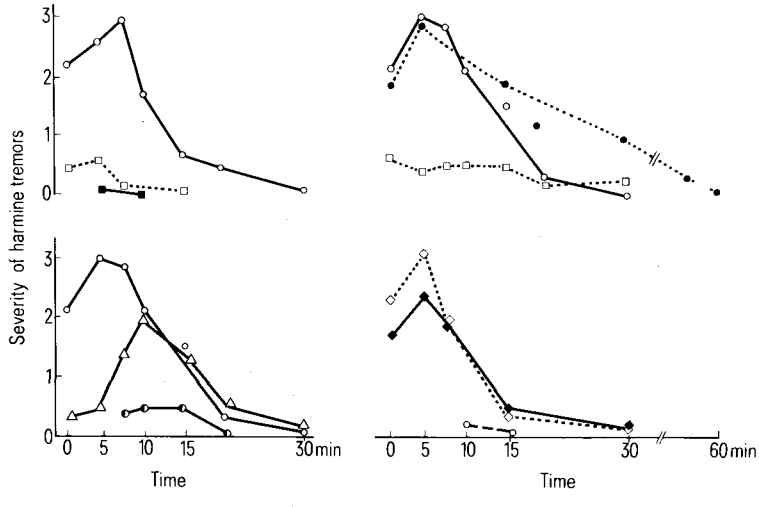
Department of Pharmaceutical Sciences, Panjab University, Chandigarh 160014 (India) and University of Oklahoma Health Sciences Centre, College of Pharmacy, Oklahoma City (Oklahoma, USA), 6 March 1979

Summary. It has been recently reported that the levo isomer of propranolol possesses anti-serotonin properties in animals. Since harmine-induced behavioural changes in mice are reported to be mediated through central serotonergic receptors, an attempt was made to test whether l-propranolol would also modify harmine-induced responses by virtue of its anti-serotonergic or anti-adrenergic property. The results indicated that l- and dl-propranolol inhibited central serotonin receptor mediated responses to harmine in mice, a finding that is analogous to other recent observations.

Harmine-induced tremors in animals are believed to be serotonergic in origin because of its structural similarity to serotonin[1,2]. The recent observations that dl-propranolol had beneficial effects in schizophrenia[3] and that l-propranolol inhibited the behavioural responses of rats to increased serotonin levels in the central nervous system[4] prompted us to test whether l-propranolol would also have similar effects on the harmine-induced (serotonergic receptor mediated) responses in mice.

Quipazine, 2-1(1-piperazinyl)-quinoline, has been reported to stimulate serotonin receptors both in the central and peripheral nervous systems[5,6]. The behavioural responses to quipazine are antagonized by methysergide, BOL 148 and cyproheptadine[5]. Since quipazine and harmine possibly act by the same mechanism, namely the stimulation of serotonergic receptors, in the present study, we also investigated the effects of quipazine on harmine-induced responses and their modification by propranolol.

Male albino Swiss-Webster mice (25–30 g) maintained on a 12-h light and dark cycle and ad libitum food and water were used. All drugs were dissolved in distilled water or saline in concentrations such that the i.p. injection volume 1 ml/100 g of mice was kept constant. Each group consisted of 8–10 animals.



Modification by levo-propanolol of harmine (H) and quipazine (Q) effects in mice. ○——○, harmine (15 mg/kg); □····□, levo-propranolol (20 mg/kg)+H; ■——■, levo-propranolol (40 mg/kg)+H; ●····●, H+Q (20 mg/kg); □····□, levo-propranolol (20 mg/kg)+H+Q; ◐——◐, cyproheptadine (0.5 mg/kg)+H; △——△, cyproheptadine+H+Q; ◇····◇, practolol (20 mg/kg)+H; ◆——◆, phenoxybenzamine (15 mg/kg)+H; ○- - - -○, dl-propranolol (20 mg/kg)+H.