seems to exclude the second process. In this regard further research is being conducted in this laboratory using the isolated perfused dog spleen.

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# Metabolism of organophophorus insecticides—VI. Mechanism of detoxification of Dipterex* in the rat

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DIPTEREX, being a potent anticholinesterase agent, is widely used as an insecticide. Since mammals—including man—are subjected to possible hazards, it seemed highly desirable to investigate the mechanism of detoxification of the insecticide in the rat. The metabolism of Dipterex in the rat has been recently investigated *in vivo* by Hassan and Zayed;[1] using $^{14}C$-labelled insecticide in which the two methyl groups are $^{14}C$-labelled. The fate of Dipterex was followed during 24 hr, at the end of which 28 per cent of the administered dose could be recovered as $^{14}CO_2$ in the expired air and 32 per cent was eliminated into the urine. $^{14}C$-formate and $^{14}C$-dimethylphosphate in the urine constituted 2 per cent and 22 per cent of the injected dose respectively.

For further investigation of the metabolic pattern of Dipterex in the rat, $^{32}P$-labelled insecticide has been used. A sublethal radiodose in saline (100 mg/kg body weight) was injected (i.p.) and the urine was collected for 48 hr. $^{32}P$-activity recovered from the urine amounted to 75–85 per cent of the administered dose. The total activity proved to be products of acidic character; since it could not be recovered from Dowex (1-x8, $Cl^-$) by hydrochloric acid of pH 3·0 and was readily and completely eluted at pH 1·5. The acidic eluate was paper chromatographed in three different solvent systems (cf. Table 1). Radiometric assay of the chromatograms revealed the presence of three $^{32}P$-labelled metabolites. Two substances were identified as mono- and dimethylphosphates, as they possess *Rf* values similar to those of authentic samples run alongside as reference substances. The third metabolite, however, remained unidentified. It cannot possibly be orthophosphate or monodemethylated Dipterex (cf. Table 1).

From several chromatograms, it has been estimated that mono-methylphosphate accounts for 20–30 per cent, and dimethylphosphate for 60–70 per cent of the total metabolites output. The unidentified metabolite contributes to about 10 per cent. After acid hydrolysis of the total $^{32}P$-metabolites, only mono- and dimethylphosphates could be detected by paper chromatography. Whether the

* 0,0-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate.

TABLE 1. $R_f$ VALUES OF $^{32}$P-LABELLED METABOLITES OF DIPTEREX ELIMINATED INTO THE RAT URINE, AS WELL AS THOSE OF MONODEMETHYLATED DIPTEREX AND ORTHOPHOSPHATE

| Substance | Colour with Bandurski reagent(3) | System* A | B | C |
|---|---|---|---|---|
| monomethylphosphate | blue | 0·12 | 0·05 | 0·08 |
| unidentified metabolite | greenish blue | 0·28 | 0·09 | 0·15 |
| dimethylphosphate | blue | 0·60 | 0·17 | 0·25 |
| monodemethylated Dipterex (2) | blue | 0·48 | 0·37 | — |
| orthophosphate | yellow | 0 | 0·28 | — |

* Development time 18 hr
A = 2-propanol-ammonium hydroxide-water (75 : 24 : 1) (4)
B = n-butanol-pyridine-water (12 : 8 : 6)
C = n-butanol saturated with concentrated ammonium hydroxide.

unidentified metabolite gave—on hydrolysis—mono- or dimethylphosphate is uncertain. However, it is believed that this metabolite still contains at least one methyl group from the original compound. This lends support from the fact that it did not produce orthophosphoric acid on hydrolysis. Moreover, previous experimentation with $^{14}$C-drug,[1] showed the presence of a diffused low activity at about the position corresponding to $Rf$ 0·28 in system A.

A certain metabolite reported by Arthur and Casida[5] to be excreted in the rat urine, could not be traced in this investigation. According to these authors, this metabolite was eluted from the anion exchanger at pH 0·8 and contributed to 30–50 per cent of the total $^{32}$P-metabolites; the rest was identified as dimethylphosphate.

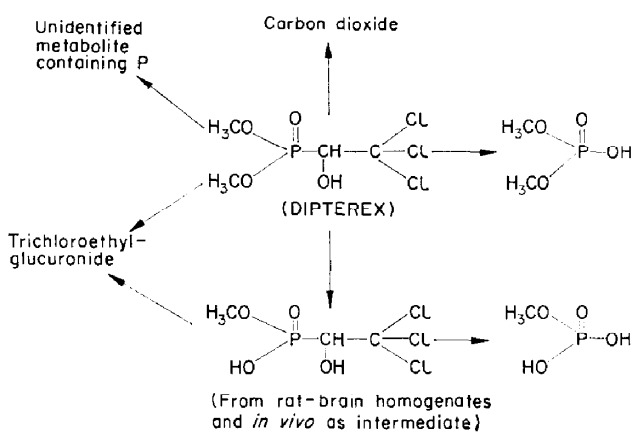


Figure 1 illustrates a scheme suggested for the metabolic pathways of Dipterex in the rat. The production of monomethylphosphate substantiates the formation of monodemethylated Dipterex as intermediate; being its immediate precursor. In this connection, monodemethylated Dipterex was shown to be formed as a metabolite of Dipterex in rat-brain homogenates.[2] The possibility that dimethylphosphate may constitute a precursor for monomethylphosphate is excluded; since dimethylphosphate is excreted quantitatively unchanged in the urine when injected into the rat.[5] Trichloroethanol produced on hydrolyisis of the phosphonate bond is excreted as trichloroethylglucuronide.[6]

It may be concluded that esterase and phosphatase actions proceed independently; yet products formed by hydrolysis through phosphatases are further acted upon by esterases, so that metabolites

with intact phosphonate bond never find their way into the urine. This is again supported by the work done by Arthur and Casida,[6] who believed that the low toxicity of Dipterex to mammals is ascribed to rapid hydrolysis of the phosphonate bond by serum esterases.

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## Effect of Sedormid chlorpromazine, and iproniazid on the activity of UDP-glucuronate glucuronyl transferase in rat liver: A histological study

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NUMEROUS studies have shown that conjugations with glucuronic acid constitute a major mechanism of detoxication in mammalian organisms. Dutton and Storey[1] have studied the transfer of glucuronic acid from uridine diphosphate glucuronic acid to *o*-aminophenol. Experiments *in vivo* with rabbits and other animals[2] have demonstrated the fate of aniline used as a toxic substance. The partial urinary excretion of aniline as N-glucuronide was demonstrated.

With relation to physiological substances that are toxic over normal levels, bilirubin has been most studied. Among the many investigations establishing its metabolism, the glucuronide determinations in the bile of rats injected i.v. with bilirubin[3] and the postulations of Billing and Lathe[4] on the conjugation of bilirubin with glucuronic acid as the most important mechanism of excretion must be quoted.

Read *et al.*[5] reported the reversible histological changes and the bile retention produced by chlorpromazine in human beings. The isolation and characterization of chlorpromazine conjugated with glucuronic acid in human urine was carried out by Lin *et al.*[6] Some increased blood bilirubin values have been reported[7] during therapy with iproniazid. With relation to Sedormid, a decreased content in bilirubin glucuronide was detected in bile of rats treated with this substance.[8]

The present paper shows the effects of two icterogenic drugs: Marsilid (isopropyl nicotinic acid hydrazide) and chlorpromazine [10-(3-dimethylamino-*n*-propyl-2-chlorophenothiazine)]; and a porphyriogenic drug: Sedormid (allyl-isopropyl-acetyl-carbamide) on the liver glucuronyl transferase activity, both *in vivo* and *in vitro*.

## MATERIALS AND METHODS

*Uridine diphosphate glucuronic acid* (UDP-glucuronic acid). A liver extract prepared according to Grodsky and Carbone[9] was employed as UDP-glucuronic acid source; for *p*-nitrophenol, commercial material was recrystallized from ethanol–water. Tissue extracts for enzymic solution were prepared by homogenizing 2 g exsanguinated rat liver in 10 ml alkaline solution of KCl.[9] The homogenate was centrifuged at 8,500 *g* for 15 min. The glucuronyl transferase activity was followed by the modified method of Isselbacker and Pinkus.[10]

*Incubation system.* The incubation mixture, final volume 4·25 ml, contained 0·40 ml Tris buffer, 0·5 M (pH 7·5); 0·1 ml EDTA,[11] pH 7·0, which produces an activation of rat liver UDP-glucuronyl transferase; 0·25 ml ascorbic acid (25 mg/50 ml); 0·20 ml *p*-nitrophenol, 0·7 mM; 1·0 ml of liver homogenate supernatant (from treated rat liver) representing 0·2 g tissue; 1·3 ml alkaline KCl solution (KCl 0·154 M–$KHCO_3$ $3{\cdot}2 \times 10^{-4}$ M); 1·0 ml boiled liver extract.[9]