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]

After incubation at 37° for 30 min, the mixture was deproteinized by adding 1·0 ml of 0·4 N trichloroacetic acid.

Colorimetric measurement. After removal of precipitated protein by centrifugation, 1 ml KOH 1 N was added to 2·0 ml of supernatant fluid. The color was read at 400 mμ with a Beckman DU spectrophotometer and compared with suitable controls.

Studies in vitro. The effect of each drug was assayed *in vitro* with a system similar to that previously described, but with normal rat liver as the source of enzyme preparation. The drugs were added to the incubation system as solutions in alkaline potassium chloride in the case of chlorpromazine and Marsilid, and dissolved in half-and-half portions of propylene glycol and ethyl alcohol in the assay with Sedormid. In the last case a control using solvent was carried out simultaneously.

Animals. Male Wistar rats (weight 280–300 g) were used. Drugs were given through a gastric sound. The dosage was as follows: 10 mg chlorpromazine daily for each 100 g of weight for 15 days; or Marsilid, 5 mg daily for each 100 g of weight for 15 days; or Sedormid, 50 mg daily for a week. With Sedormid the rats were killed when their urine gave a strong positive reaction for porphobilinogen with Erlich's reagent.

For the two first drugs, the diminution in average weight was recorded. There was a 10% decrease for rats treated with chlorpromazine and practically no decrease in rats treated with Marsilid.

RESULTS

In Table 1 the results obtained in the experiments *in vivo* are shown. It can be seen that Sedormid causes a significant decrease in the enzyme activity, with an average of 27·3%. In Table 2 the data corresponding to the experiments *in vitro* are shown. Here also there is a decrease of enzyme activity, Although not so striking as in the experiments *in vivo*.

TABLE 1. CONJUGATION VALUES

In micromoles of *p*-nitrophenol per gram liver. All experiments performed in duplicate.

Rat No.	Normal	Treated with Sedormid	Treated with chlorpromazine	Treated with iproniazid
1	0·216	0·037	0·174	0·145
2	0·127	0·058	0·187	0·104
3	0·116	0·035	0·155	0·085
4	0·158	0·037	0·181	0·143
5	0·245	0·081	0·219	0·142
6	0·191		0·221	0·131
7	0·208			
8	0·208			
9	0·198			
10	0·126			
Average values	0·179	0·049	0·174	0·125

TABLE 2. EFFECTS OF DRUGS ON ENZYME ACTIVITY *in vitro*

Rat No.	Drug	Concentration (mM)	Decrease obtained (%)
1	Sedormid	165	48
2	Sedormid	165	68
3	Chlorpromazine	66	0
4	Chlorpromazine	132	0
5	Iproniazid	66	0
6	Iproniazid	1,320	10

Histological study. The histological alterations were systematically studied with all three drugs. The liver structure was well preserved, and the irregular modifications of little intensity, essentially degenerative, were not considered to have histopathological meaning.

DISCUSSION

The effect *in vitro* of Sedormid would suggest a direct inhibition of the enzyme activity. However, the decreases in values of conjugation are more striking *in vivo* than *in vitro*. Thus, in cases of intoxication, it would be possible to postulate either a direct inhibition by Sedormid or by some unknown metabolites or an enzymic repression. With the other drugs assayed, it is not possible to attach significance to the differences either *in vivo* or *in vitro*.

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