Prevention by Catatoxic Steroids of Lithocholic Acid-Induced Biliary Concrements in the Rat[1] (36821)

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Ever since Okey (1) observed gallstone formation in cholesterol-fed guinea pigs, considerable attention has been given to the role played by this compound and its congeners, particularly the bile acids, in the pathogenesis of cholelithiasis. Special interest was aroused by dihydrocholesterol and lithocholic acid which produce gallstones quite regularly in rabbits and rats (2–4). The dihydrocholesterol-induced cholelithiasis can be inhibited by dehydrocholic acid, but since the latter does not prevent the intestinal absorption of the former, its prophylactic effect was ascribed "to prevention of bile stasis or to changes in the concentration or the pH of the bile" (2). On the other hand, the lithocholic acid-induced gallstone formation is inhibited by taurine presumably through enhancement of taurine conjugate formation (4).

Our earlier work has shown that various hormonal and anesthetic steroids as well as related vitamin D derivatives and digitalis alkaloids can be very readily inactivated by catatoxic steroids, usually, though not always, through the induction of drug-metabolizing hepatic microsomal enzymes (5). These findings suggested that the formation of biliary concrements by lithocholic acid might also be prevented by catatoxic steroids. In the work described below, these and, for control purposes, a variety of other compounds have also been tested.

Materials and Methods. Female rats (Sprague-Dawley, Canadian Breeding Farm & Laboratories Ltd., St. Constant, Que.) with an initial body weight of 100 g (range 90–110 g) were maintained exclusively on Purina Laboratory Chow and tap water, divided into 16 goups and treated as outlined in Table I.

The rats of all groups received lithocholic acid at the dose of 50 mg/100 g body weight in 1 ml peanut oil po twice daily from the fourth day to the termination of the experiment on the ninth day.

The following steroids were tested for possible protective or sensitizing effects:

PCN [3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile (Upjohn)]

CS-1[2] [9α-Fluoro-11β,17-dihydroxy-3-oxo-4-androstene-17α-propionic acid potassium salt (Searle)]

Ethylestrenol (Organon)
Spironolactone (Searle)
Norbolethone (Wyeth)
Oxandrolone (Searle)
Prednisolone acetate (Roussel)
Triamcinolone (Squibb)
Progesterone (Roussel)
Estradiol (Roussel)
Deoxycorticosterone acetate (Schering)
Hydroxydione sodium hemisuccinate (Pfizer).

All steroids were administered at the dose of 10 mg in 1 ml water (homogenized with a trace of Tween 80), po, twice daily, throughout the period of observation. Only triamcinolone was given at the individual dose of 1 mg, because of its high toxicity. L-Thyroxine (B.D.H.) and L-3,5,3′-triiodothyronine (K. & K. Laboratories) were injected (in the form of their sodium salts sc) at the dose of 200 μg in 0.2 ml water, once daily.

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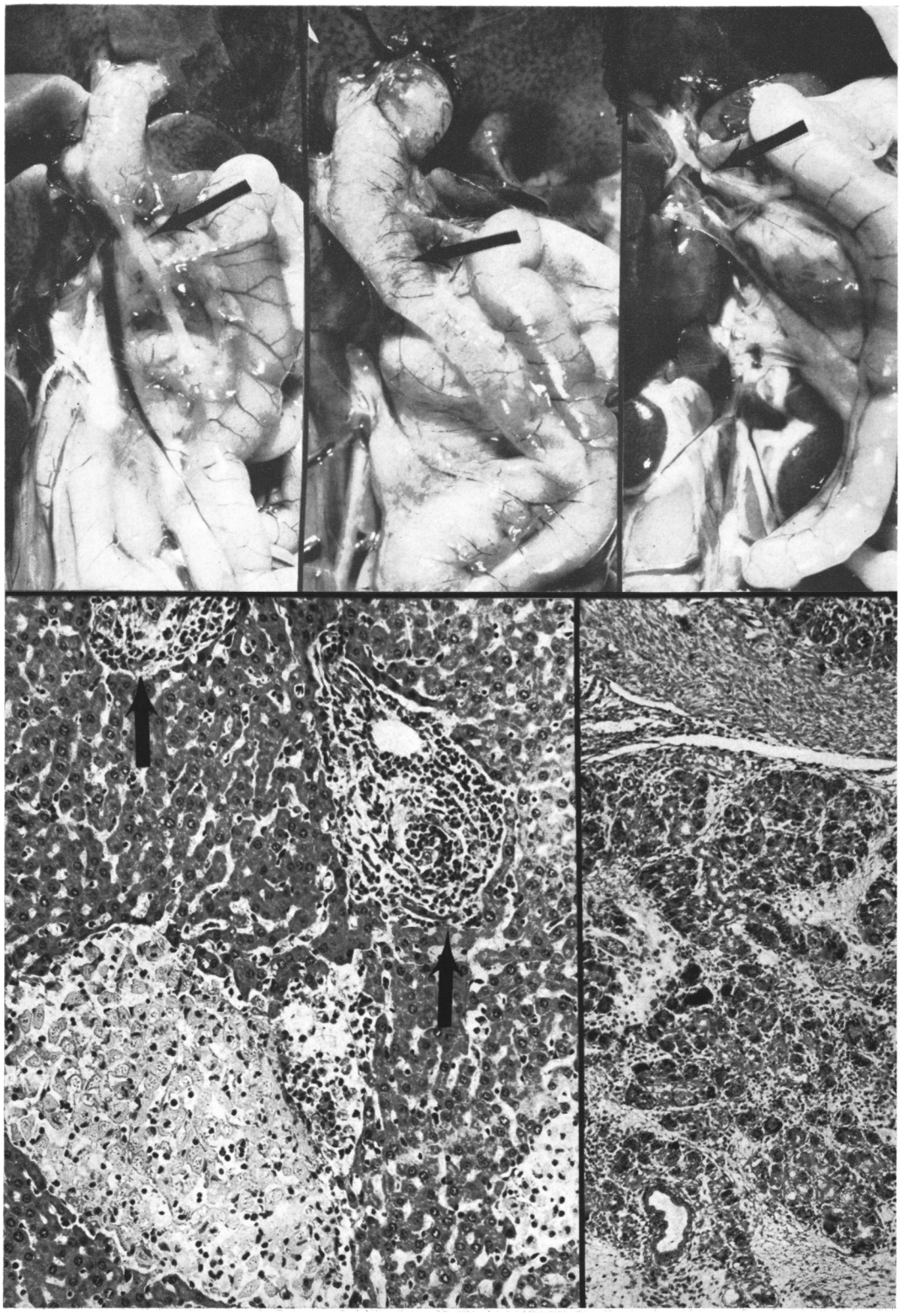
[2] For catatoxic steroid No. 1 (manufacturer's code No.: SC-11927); the first nonhormonal steroid shown to possess catatoxic activity.



For control purposes, two classic nonhormonal microsomal drug-metabolizing enzyme inducers were tested, namely, phenobarbital sodium (B.D.H.) and diphenylhydantoin (Eastman), both at the dose of 6 mg in 1 ml water, po, twice daily like the steroids, from the first to the last day of the experiment.

Results and Discussion. All the controls receiving lithocholic acid alone began to lose weight and became icteric within the first 3 days. At autopsy, the most striking change was a considerable dilatation of the common bile duct (especially its proximal portion) and the main bile ducts of the individual lobes, just above their entry into the choledochus. These ducts were usually filled with concretions, which sometimes formed uninterrupted ramifying solid casts totally obstructing the lumen of the principal bile duct system. The distal portion of the common bile duct, which traverses the pancreas, remained almost normal except in the most severely affected animals. The hepatic parenchyme macroscopically exhibited a diffuse yellowish discoloration, which was mostly due to icterus but, in addition, partly circumscribed white areas could also be seen with the naked eye. Histologically, in addition to moderate but diffuse infiltration with small fat droplets, circumscribed areas of irregularly distributed focal necroses were noted throughout the hepatic parenchyme without any regular relationship to the central, portal or midzonal regions. The dilated intrahepatic bile ducts were often filled with homogeneous bile thrombi and surrounded by innumerable newly formed bile ductules. The peripheral portion of the choledochus also showed intense proliferation of its epithelial lining often covering complex folds of almost papilloma-like aspect (Fig. 1). Around the common bile duct, the pancreatic tissue exhibited inflammatory changes with edema, proliferation of fibroblasts but little if any leukocytic infiltration. All these changes were aggravated by prednisolone acetate and triamcinolone, although this is not evident in Table I because the glucocorticoids caused 100% mortality before the termination of the experiment. Hence, it may be concluded that glucocorticoids actually aggravate the toxicity of lithocholic acid. On the other hand, PCN and CS-1—which when previously tested against other toxicants, had been found to be the most active among the catatoxic steroids—also showed a particularly high protective potency against lithocholic acid as regards all the parameters measured (Table I). The effect of the other steroids in this series was not particularly striking. Curiously, diphenylhydantoin, a classic microsomal enzyme inducer, did not prove to be potent against lithocholic acid poisoning, and phenobarbital protected only against some of its toxic effects. Unexpectedly, thyroxine and triiodothyronine exerted a marked prophylactic action.

It would be premature to speculate about the underlying protective mechanisms, but on the basis of earlier experiments with many other lipid soluble toxicants, it is probable that the most active catatoxic steroids, namely PCN and CS-1 act through the induction of appropriate hepatic drug-metabolizing enzymes. Further research will be required to verify this suspicion and to explore other

FIG. 1. Prevention by PCN of lithocholic acid-induced bile concrement formation. All three animals were given the same lithocholic acid treatment. The first two (left and middle) served as controls; they received ethylestrenol which failed to protect the choledochus against the lithocholic acid-induced dilatation and concrement formation. The animal on the right was completely protected by PCN. The arrows indicate the point at which the choledochus enters the virtually transparent margin of the pancreas. (top left) Dilatation due to bile concrement formation limited to the hepatic hilum. (top middle) The dilatation and concrement formation continue throughout the pancreatic portion of the choledochus. (top right) Essentially normal appearance of the choledochus and its main branches, owing to protection by PCN. (bottom left) General view of the hepatic parenchyme showing two well-circumscribed necrotic foci (light areas) and inflammatory infiltrates (arrows) around proliferating bile ductules (×120). (bottom right) Diffuse edematous inflammatory reaction in the pancreas next to the duodenum, which is near the upper margin of the field (×50). All sections stained by the PAS technique.

TABLE I. Factors Influencing Lithocholic Acid Intoxication.

Treatment[a]	Hepatic lesions[b]	Choledochus lesions[b]	Icterus[b]	Final body wt[c] (g)	Mortality[b]
None	14/16	14/16	15/16	86 ± 3	9/16
PCN	0/10[f]	0/10[f]	0/10[f]	123 ± 4[f]	0/10[f]
CS-1	4/10[d]	0/10[f]	0/10[f]	115 ± 4[f]	0/10[f]
Ethylestrenol	8/10 NS	10/10 NS	6/10 NS	90 ± 7 NS	0/10[f]
Spironolactone	8/10 NS	5/10 NS	8/10 NS	87 ± 8 NS	4/10 NS
Norbolethone	10/10 NS	9/10 NS	9/10 NS	97 ± 6 NS	2/10 NS
Oxandrolone	10/10 NS	10/10 NS	9/10 NS	84 ± 3 NS	7/10 NS
Prednisolone ac	10/10 NS	1/10[f]	10/10 NS	81 ± 5 NS	10/10[dg]
Triamcinolone	10/10 NS	4/10[d]	10/10 NS	72 ± 4[eg]	10/10[dg]
Progesterone	5/9 NS	5/9 NS	6/9 NS	92 ± 5 NS	4/9 NS
Estradiol	7/10 NS	5/10 NS	10/10 NS	76 ± 4 NS	9/10 NS
DOC ac	10/10 NS	8/10 NS	10/10 NS	87 ± 5 NS	4/10 NS
Hydroxydione	10/10 NS	9/10 NS	9/10 NS	85 ± 5 NS	7/10 NS
Thyroxine	4/9[d]	1/9[f]	1/9[f]	119 ± 4[f]	1/9[d]
Triiodothyronine	0/10[f]	1/10[f]	0/10[f]	120 ± 4[f]	0/10[f]
Phenobarbital	8/10 NS	2/10[f]	1/10[f]	114 ± 4[f]	1/10[d]
Diphenylhydantoin	10/10 NS	10/10 NS	10/10 NS	82 ± 2 NS	7/10 NS

[a] The rats of all groups received lithocholic acid as described in the Materials and Methods section.

[b] Hepatic and choledochus lesions as well as icterus were estimated on the day of death, and mortality (positive/total) was listed at autopsy on day 9 ("exact probability test").

[c] Final body weight was registered on day 8 of the experiment (Student's t test), since after that mortality was 100% in the groups given glucocorticoids.

[d] $p < .05$, [e] $p < .01$, [f] $p < .005$ significance in comparison with the first group. Superscript letters d, e, and f indicate a decrease (except the three followed by g, an increase) in the toxicity of lithocholic acid.

possibilities (*e.g.*, increased conjugation with taurine and enhanced excretion through the bile or accelerated biodegradation at sites other than hepatic microsomes).

Summary. Experiments on rats indicate that lithocholic acid-induced changes in the liver and choledochus, as well as the associated bile concrement formation, icterus, loss of body weight and mortality, are all inhibited or abolished by certain catatoxic steroids (PCN and CS-1) as well as by thyroxine and triiodothyronine. Prednisolone and triamcinolone have an opposite effect. Among the classic hepatic microsomal enzyme inducers, diphenylhydantoin is ineffective, and phenobarbital is much less potent than PCN or CS-1 in influencing the toxicity of lithocholic acid.

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