PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 631–635 (1974)

Increased Canalicular Bile Production Induced by Pregnenolone-16α-Carbonitrile, Spironolactone and Cortisol in Rats (37864)

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In rats, spironolactone and pregnenolone-16α-carbonitrile (PCN) increase resistance to many intoxications (1) and accelerate the biotransformation and elimination of several substrates (2–5). These two steroids augment hepatic weight, cause smooth-surfaced endoplasmic reticulum proliferation in hepatocytes (6, 7), and induce mixed-function oxygenases (8, 9) and UDP-glucuronyl transferases in liver microsomes (2, 3) as well as glutathione-*S*-aryl-transferases in the cytosol of rats (4). The above changes could explain many of the protective actions of spironolactone and PCN.

Pretreatment with PCN or spironolactone produces marked choleresis in rats (2–4). This effect may result in the accelerated elimination of compounds that are mainly excreted through the bile (2–4). In the present study, we tried to clarify the mechanism(s) through which these steroids influence biliary secretion in rats. Our experiments were undertaken to find out: (a) whether choleresis depends upon increased liver weight elicited by spironolactone and PCN, (b) whether these steroids act at the canalicular or ductular level of bile formation, (c) whether increased biliary bile salt excretion plays a role in PCN- and spironolactone-induced choleresis, or (d) whether choleresis is independent of bile-salt metabolism and elimination as is the case in phenobarbital-treated rats (10, 11). Cortisol was used for comparative purposes since it increases biliary flow in dogs and rats (12, 13).

Methods. Female ARS/Sprague–Dawley rats (Madison, WI), averaging 250 g (220–280 g) and maintained *ad lib.* on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water, were given PCN [68.7 mg/kg (Upjohn)], spironolactone [84.0 mg/kg (Searle)], or cortisol acetate [80.9 mg/kg (Roussel)] in equimolar doses as micronized suspensions in 10 ml/kg water, twice daily po, for 3 days. Each study was performed 24–36 hr after the last treatment.

Operative procedures and bile collection. Two rats per day from each group were anesthetized with 45 mg/kg of pentobarbital sodium [Nembutal (Abbott)]. Their common bile ducts were then exposed through a midline abdominal incision and cannulated with PE-10 polyethylene tubes (Clay Adams Inc., NY). Half an hour later, bile was collected in previously tared tubes at 20-min intervals, for 120 min, and weighed at the end of the experiment. The body temperature of the rats was monitored with a Telethermometer (Yellow Springs Instrument Co., Yellow Springs) through the rectum and was maintained at 37.5 ± 1° by means of a heat lamp.

Biliary bile salt determinations. The total bile-salt concentration of the individual bile samples was measured according to the enzymatic method of Talalay (14) as applied by Iwata and Yamasaki (15). Hydroxysteroid dehydrogenase was prepared by homogenizing 0.3 g of the dried cells of *Pseudomonas testosteroni* (Sigma, St. Louis) in 30 ml pyrophosphate buffer (pH 10.8) at 4° for 3 min using a Polytron PT high-frequency homogenizer (Kinematica GmbH, Lucern, Switzerland). The homogenate was centrifuged at 20,000*g* for 30 min. For enzyme incubation, the supernatant fraction

was used immediately after centrifugation or after storage in deep freeze for 3 days. The incubation mixture and the experimental conditions were basically the same as those described by Javitt and Emerman (16). However, pyrophosphate buffer (pH 10.8) was used instead of potassium phosphate buffer. Sodium taurocholate (Koch-Light Laboratories, Colnbrook, Bucks, England) was applied as a standard, and the mean recovery for 35 determinations was found to be 87.8 ± 1.09% (SE). After centrifugation, the absorbancy of the incubation mixture was measured at 340 nm in a Beckmann DU spectrophotometer.

Biliary ^{14}C-erythritol clearance. To determine canalicular bile formation, 6.6 μCi/kg of ^{14}C-erythritol (Amersham/Searle, Arlington Heights, IL), sp act 2.3 mCi/nmole, was given through the jugular vein 30 min before bile duct cannulation. This single dose was sufficient to maintain a relatively high erythritol concentration which, similar to the observations of Klaassen (11), only moderately declined during the experimental period. Bile samples were collected 1 hr after the erythritol injection while blood was taken midway through the individual bile collection periods via the femoral artery. The radioactivity of bile and plasma was measured in a Packard Tricarb liquid scintillation counter after diluting 0.1 ml of the samples with 15 ml Aquasol (New England Nuclear, Boston). The biliary clearance of ^{14}C-erythritol was calculated as the product of bile flow and the ratio of ^{14}C activity in bile/plasma.

Results. Bile flow and liver weight. Biliary flow was significantly enhanced in the PCN-, spironolactone-, and cortisol-treated rats when compared with the controls (Table I). PCN and spironolactone greatly augmented hepatic weight whereas cortisol was less effective in this respect. Hence, there was no close correlation between the increase in liver weight and the acceleration of bile flow in PCN- and cortisol-treated animals.

Biliary bile-salt excretion. During the 2-hr collection period, bile flow decreased slightly (5–10% in some animals) but not significantly. The total biliary bile-salt levels during the 120-min collection period were significantly lower in the steroid-treated groups than in the controls (Table II). Yet, the total biliary bile-salt excretion rates were practically identical in all groups during this period.

Biliary ^{14}C-erythritol clearance and bile-salt independent fraction of bile. Erythritol is supposed to enter the bile only at the canalicular level; thus, canalicular bile formation can be estimated on the basis of biliary ^{14}C-erythritol clearance (17, 18).

The bile to plasma ratio of ^{14}C-erythritol was close to 1 in the control and steroid-treated groups, indicating that the increased bile volume produced by the steroids was of canalicular origin and that ductular secretion or reabsorption had little if any influence on the composition of bile in these rats (Table III).

The correlation between biliary ^{14}C-erythritol clearance and bile-salt excretion, calculated according to the method of least squares (19), is shown in Fig. 1. Extrapolation of the regression line to 0 bile acid se-

TABLE I. Influence of PCN, Spironolactone, and Cortisol on Liver Weight and Biliary Flow (Means ± SE).

Treatment	Number of animals	Body weight (g)	Liver weight (g)	Bile flow[a] (μl/min/kg)
None	17	250.9 ± 4.5	7.2 ± 0.15	51 ± 2
PCN	18	261.0 ± 3.1	10.6 ± 0.24** (48.5%)[b]	96 ± 3** (88%)
Spironolactone	13	259.2 ± 3.9	10.7 ± 0.17* (50%)	76 ± 4** (49%)
Cortisol	14	244.1 ± 3.7	7.8 ± 0.22* (9%)	72 ± 3** (41%)

[a] Mean of six bile collections.

* Significantly different ($P < 0.05$) from controls.

** Significantly different ($P < 0.001$) from controls.

[b] Figures in brackets indicate a mean increase in liver weight or bile flow, expressed as a percentage of the control values.

TABLE II. Biliary Bile-Salt Concentration and Bile-Salt Excretion in Control and Steroid-Treated Rats (Means ± SE).

Treatment	Bile salt concentration[a] (μmoles/ml)	Bile salt excretion[a] (μmoles/min/kg)
None	27.2 ± 1.14	1.36 ± 0.090
PCN	12.9 ± 0.85*	1.26 ± 0.098
Spironolactone	17.1 ± 1.02*	1.30 ± 0.083
Cortisol	22.1 ± 0.85**	1.58 ± 0.098

[a] Mean of three bile collections (0–20, 40–60, and 80–100 min). The number of animals was the same as in Table I.

* Significantly different ($P < 0.001$) from controls.

** Significantly different ($P < 0.005$ from controls.

cretion results in intersection of the y axis, which arbitrarily defines a bile-salt independent fraction of canalicular flow. This extrapolation demonstrates an increase in the bile-salt independent fraction from 16.4 μl min^{-1} kg^{-1} to 68.9, 30.5, and 26.5 μl min^{-1} kg^{-1} in the PCN-, spironolactone-, and cortisol-treated rats, respectively.

Discussion. Except for primary bile acids and salts, little is known about the mechanism through which steroids exert a choleretic action. Bile-salt-induced biliary secretion depends on the active excretion of bile-salt molecules into the canaliculi and on subsequent osmotic flow of water and electrolytes. On the other hand, Macarol *et al.* (13) showed that, in dogs, cortisol elicits a choleretic response which is of hepatocellular origin but which is not mediated through the osmotic action of bile salts. The effects of PCN and spironolactone on bile flow are similar to those of cortisol, because the former have no influence on bile-salt excretion but nevertheless enhance bile flow, which is of canalicular origin. Thus, these steroids accelerate biliary flow by augmenting the bile-salt independent fraction of canalicular bile.

Since, in preliminary experiments, no significant change in bile flow was detected 1, 5, or 24 hr after administration of a single large dose of PCN or spironolactone, the presently shown steroid-induced increase in biliary flow was probably not due to simple osmotic choleresis following the excretion of these steroids or their metabolites into the bile. As a rule, at least 2 days of treatment were necessary to enhance bile flow. Under this condition, almost identical values were obtained 1, 5, and 24 hr after the last steroid administration (G. Zsigmond, unpublished observations).

In general, accelerated biliary flow could be related to increased hepatic weight. However, it should be noted that PCN has a more pronounced effect on bile flow than on liver weight. Furthermore, cortisol only affects biliary secretion. These examples show that, although increased hepatic weight may play a role in enhancing bile clearance, the effect of the steroids cannot be explained by this mechanism alone.

In rats and rabbits, inhibitors of Na^+K^+-dependent ATPase may decrease the bile-salt independent fraction of canalicular bile (20, 21). This suggests that active sodium transport may influence fluid transport through the canalicular membrane. It is possible that the increase in biliary flow, induced by PCN and spironolactone, may be associated with enhanced activity of Na^+K^+-

TABLE III. Bile to Plasma ^{14}C-Erythritol Ratio and Biliary ^{14}C-Erythritol Clearance in Control and Steroid-Treated Rats (Means ± SE).

Treatment	Number of animals	Bile:plasma ^{14}C-erythritol ratio	Biliary ^{14}C-erythritol clearance (μl/min/kg)
None (36)[a]	6	1.02 ± 0.012	56.0 ± 4.20
PCN (44)	8	1.06 ± 0.027	98.3 ± 3.98*
Spironolactone (25)	5	1.03 ± 0.027	82.3 ± 6.57**
Cortisol (40)	7	1.01 ± 0.009	66.9 ± 5.10

[a] Figures in brackets indicate number of determinations.

* Significantly different ($P < 0.001$) from controls.

** Significantly different ($P < 0.01$) from controls.

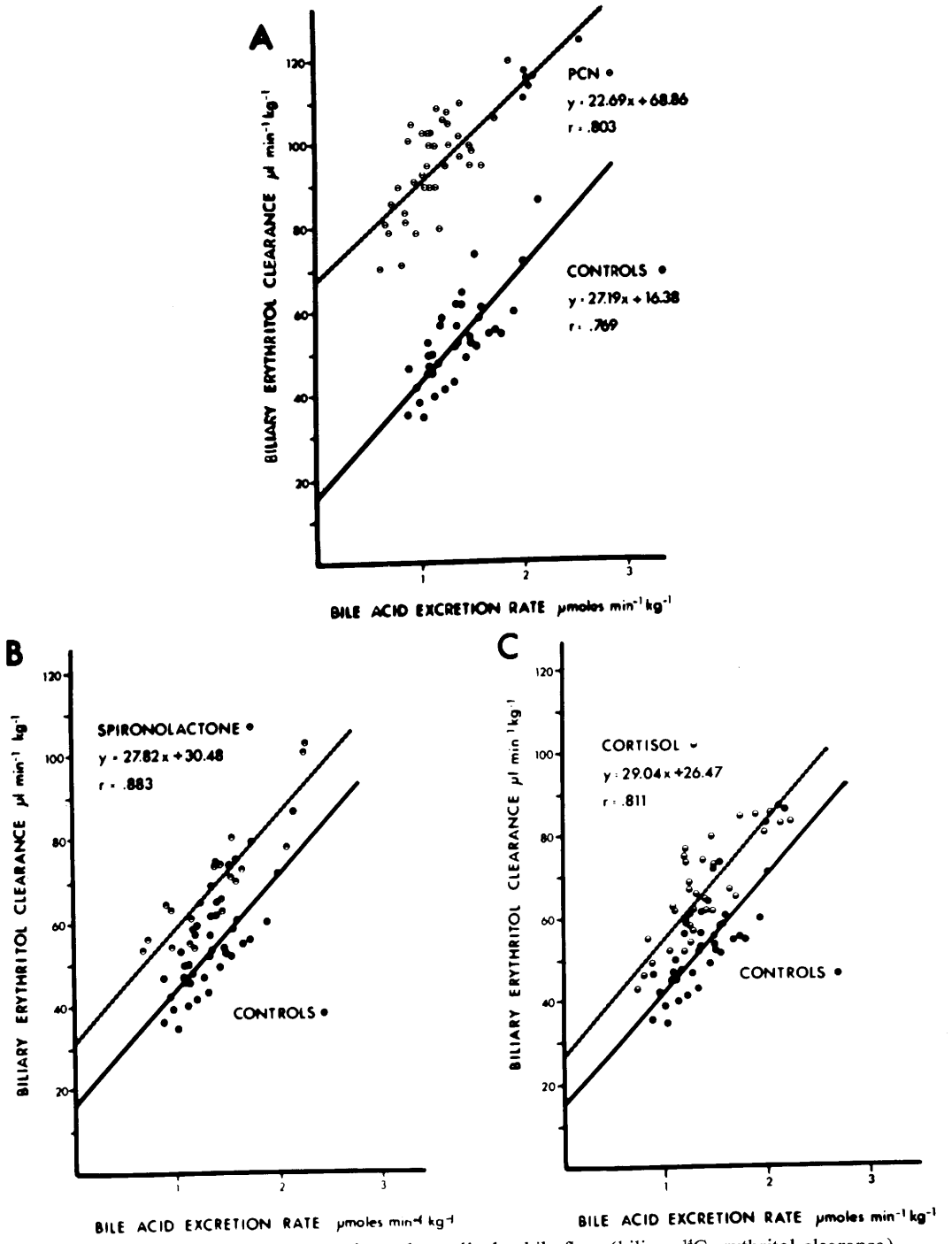

FIG. 1. Correlation between estimated canalicular bile flow (biliary ^{14}C-erythritol clearance) and biliary bile-salt excretion in control and steroid-treated rats. The regression equation ($y = bx + a$) was calculated according to the method of least squares (19); r indicates the correlation coefficient. The number of determinations is shown in Table III.

dependent ATPase of the canalicular membrane. However, further studies are needed to clarify this point.

Although the mechanisms through which PCN and spironolactone influence biliary secretion have not yet been fully elucidated, several of our earlier data underline the biological importance of such changes in canalicular function. Increased bile flow induced by these two steroids plays a decisive role in enhancing the biliary elimination of several substances. The biliary transport maximum of bromsulfophthalein, phenol-3,6-dibromophthalein disulfonate, and bilirubin is significantly higher in steroid-treated rats than in controls; this change occurs despite the lower concentrations of the dyes in the bile of animals given PCN or spironolactone (2–4).

The steroids probably influence the disposition of many other drugs and natural compounds—which are mainly excreted into the bile—at least partly through this mechanism. The toxicity and pharmacological actions of digitoxin and progesterone are significantly reduced in rats treated with PCN or spironolactone (1, 5). In rats, PCN prevents lithocholic acid-induced damage and biliary concrement formation (22). Such steroids may also exert choleretic actions in certain cholestatic states, and PCN seems to be especially promising in this respect. However, its choleretic effect remains to be determined in experimental and human cholestatic conditions.

Summary. In the rat, pregnenolone-16α-carbonitrile (PCN), spironolactone, and cortisol increased bile flow by 88, 49, and 41%, respectively. The total biliary bile salt excretion rates ran parallel in control and steroid-treated animals, indicating that enhanced biliary secretion is not due to osmotic choleresis caused by augmented bile salt elimination. ^{14}C-Erythritol clearance studies demonstrate that the steroid-induced increase of bile flow is of canalicular origin. The correlation between estimated canalicular bile flow and biliary bile salt excretion reveals that PCN accelerates biliary flow by significantly enhancing the bile-salt independent fraction of canalicular bile. The effect of spironolactone and cortisol is similar but less pronounced than that of PCN.

This work was supported in part by grants awarded to the Institut de Médecine et de Chirurgie Expérimentales by the Medical Research Council of Canada (Block Term Grant MT-1829), Succession J. A. DeSève, and the Colonial Research Institute, Freeport, Bahamas. The authors thank the Companies listed in the *Methods* section for the compounds used in this study.

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Received May 10, 1973. P.S.E.B.M., 1974, Vol. 145.