derangement of the myocardial contractile process, to such extrinsic problems as cardiotoxins, the electrolyte environment, or neurogenic control. Although the data are not conclusive, they are meaningful because they imply that the myogenic failure of profound shock is reversible.

Summary. Left ventricular papillary muscle from rats in profound hemorrhagic shock and from control animals were compared with respect to tension development and negative treppe. In both muscles tension development increased as resting fibre length increased and as frequency of stimulation decreased. No difference in the performance of muscles from the two types of animals was detected; the contractile element of cardiac muscle appears not to be irreversibly altered by hemorrhagic hypotension.

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Received June 1, 1965. P.S.E.B.M., 1965, v120.

Regulation of Cholesterol Catabolism by Bile Salts and Glycyrrhetic Acid *in vivo*. (30427)

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Bergström and Danielsson(1) found that continuous intraduodenal infusion of sodium taurochenodeoxycholate (5 to 10 mg/hr) into rats reduced the biliary excretion of taurocholate by these animals compared with that in bile-fistula rats not receiving such an infusion. They concluded that the concentration of bile salts supplied to the liver *via* the portal blood influenced the rate of hepatic synthesis of bile salts from cholesterol. Further evidence for this apparent negative feedback control of cholesterol catabolism was provided by observations that added bile salts depressed the ATP-dependent oxidation of cholesterol by fortified rat-liver mitochondrial preparations *in vitro*(2,3). However, different bile salts inhibited this *in vitro* cholesterol catabolism to different degrees, taurocholate being much less potent than taurochenodeoxycholate at the same concentration. Furthermore, bile salts have been observed to inhibit ATP biosynthesis in liver mitochondria by uncoupling oxidative phosphorylation and by inhibiting NADH oxidation(4). In both respects taurochenodeoxycholate is a more potent inhibitor than taurocholate. It was, therefore, important to discover whether or not taurocholate and taurochenodeoxycholate differed in their effects on cholesterol catabolism *in vivo* in rats. Since they are both normal constituents of rat bile and present in portal blood (due to the enterohepatic circulation), they might both be expected to regulate cholesterol catabolism to a similar degree if a "true" negative feedback control is operative. This was not found to be so.

Methods. Bile ducts of female albino rats (200 to 300 g) were ligated at the mid-point under anesthesia (intravenous hexobarbital). A plastic cannula was inserted in the upper half of the duct to receive bile, and a second cannula was inserted into the lower half for intraduodenal infusion. Animals were kept in restraining cages with access to Oxoid rat diet and drinking water containing 5% glucose, 0.05% KCl and 1% NaCl. Ten μc of

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TABLE I. Effects of Intraduodenal Bile Salt Infusion on Biliary Secretion of Bile Salt-H^3 by Rats Injected with Cholesterol-U-H^3.

Animal	Exp No.	*Bile salt-H^3 0-6 hr	6-12 hr	12-18 hr	18-24 hr	18-24 hr output as % of 6-12 hr output
		%				
Control	1	100	97	93	69	71
	2	100	60	61	39	65
Taurocholate-infused	1	100	96	67	78	81
	2	100	66	63	63	95
Taurochenodeoxy-cholate-infused	1	100	66	20	15	23
	2	100	90	8	8	9

* Time intervals are from the time of cholesterol-U-H^3 injection. Bile salt-H^3 is expressed as a percentage of outputs during 0-6 hr period. Bile salt infusions commenced at end of this period.

cholesterol-U-H^3 (Radiochemical Centre, Amersham, England), emulsified with 30 times its weight of Tween 20 in 1 ml of isotonic saline, was injected intraperitoneally 18 hours after operation. Bile salts were continuously administered intraduodenally at 10 mg/hr (0.8 ml/hr) from syringes driven by a Palmer slow-injection apparatus, commencing 6 hours after radioactive cholesterol injection.

For radioactive assays, bile samples (0.5 ml) were extracted twice with 0.5 ml petroleum ether (b.p. 60-80°) (after dilution with 0.5 ml ethanol and 0.3 ml aqueous ammonia (sp. gr. 0.88)) to remove neutral sterols. Protein in the aqueous layer was precipitated by addition of 9 ml ethanol and heating to 80°. Bile pigment was removed by addition of 15 mg charcoal and heating to boiling. The almost colorless supernatant after centrifugation, containing bile salts, was evaporated to dryness and the residue was dissolved in 60% aqueous ethanol for counting. Radioactivity was measured by scintillation counting using an I.D.L. Tritomat 6020. Aliquots of the original bile samples and of the petroleum ether extracts were analyzed spectrophotometrically for di- and tri-hydroxycholanates(5) and cholesterol(6) respectively.

Results. The Table gives results from two similar experiments, each employing 3 animals, one as a control (with no infusion of bile salts), one receiving taurocholate, and one receiving taurochenodeoxycholate. Radioactivities of bile salts in bile collected during consecutive 6-hour periods are shown as percentages of the radioactivity of the bile salts secreted by the same animal during the 6-hour initial period between injection of cholesterol-U-H^3 and commencing bile-salt infusion, when each experimental animal was serving as its own control for subsequent observations. For different animals, initial radioactivity secretion (before bile salt infusion) varied from 1000 to 2000 counts/sec per 6 hours in bile salt fractions, and from 20 to 40 counts/sec per 6 hours in neutral lipid fractions (petroleum ether extracts).

The results show that whereas taurochenodeoxycholate infusion depressed radioactive bile-salt production, taurocholate infusion had no such effect.

Infusion of either of the bile salts increased outputs of radioactive neutral lipid, which was assumed to be unmetabolized cholesterol-U-H^3, as spectrophotometric analyses indicated concomitant increases in secretion of biliary cholesterol during bile-salt infusion. Biliary cholesterol concentrations fell rapidly to near the initial levels when infusion was discontinued.

Spectrophotometric analyses for di- and tri-hydroxycholanates showed that the infused bile salts were excreted in the bile within 2 hours. Infusion of taurocholate for a prolonged period (36 hours) caused a 6-fold increase in output of a taurodihydroxycholanate. This gave, on alkaline hydrolysis, an acid which was not deoxycholic, but closely resembled chenodeoxycholic in chromatographic mobility(7), in the colors given by a diagnostic spray reagent(8), and visible

absorption spectrum on heating with 88% (w/w) phosphoric acid containing 0.25% (v/v) anisaldehyde (4 minutes at 100°). Further study of this acid is necessary for positive identification.

Intraduodenal infusion of aqueous sodium β-glycyrrhetate (125 μg/hr) had no significant effect on the secretion of either radioactive bile salts or cholesterol.

Discussion. If hepatic synthesis of bile salts from cholesterol were subject to regulation by a negative feedback mechanism, taurocholate should inhibit hepatic cholesterol catabolism in the same manner as taurochenodeoxycholate. However, this is not evident from the experimental results obtained: intraduodenal infusion of taurocholate had no effect on the hepatic oxidation of cholesterol-U-H^3 to radioactive bile salts, whereas taurochenodeoxycholate strongly inhibited this process.

If, on the other hand, the rate of bile salt synthesis is dependent on the availability of ATP in the liver, then the different effects of these two taurine conjugates on cholesterol catabolism might be explained on the basis that taurochenodeoxycholate is a much more potent inhibitor of ATP synthesis than taurocholate(4).

The results of the *in vivo* experiments described here are also compatible with the finding that taurochenodeoxycholate is a more potent inhibitor of mitochondrial cholesterol oxidation *in vitro* than taurocholate.

Intraduodenal infusion of small amounts of β-glycyrrhetate (the aglycone of glycyrrhizin, a saponin found in liquorice root) had no significant effect on biliary levels of radioactive bile acids or cholesterol; this contrasts with Shibata's finding(9) that intramuscular injection of very small quantities of glycyrrhizin into rabbits (1 mg/kg) apparently increased rates of bile acid secretion, as measured by Murakami's colorimetric method (10). However, it has been shown(11) that metabolites of orally-administered glycyrrhetic acid are largely excreted *via* the bile, and also that glycyrrhetic acid interferes with the Murakami method of estimating bile acids (*cf.* 12). Shibata's finding is, therefore, open to some doubt.

Summary. Tritiated cholesterol was injected intraperitoneally into rats having 2 bile-duct cannulae, one for bile collection and the other for intraduodenal infusion of bile-salt solutions. Intraduodenal infusion of taurocholate (10 mg/hr) had no effect on rates of radioactive bile-salt formation from cholesterol-H^3. Intraduodenal infusion of taurochenodeoxycholate (10 mg/hr) inhibited radioactive bile salt formation. Taurocholate infusion also resulted in a 6-fold increased secretion of a dihydroxycholanate, apparently taurochenodeoxycholate. Sodium β-glycyrrhetate infusion (125 μg/hr) had no significant effect on secretion of either radioactive cholesterol or radioactive bile salts.

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Received June 1, 1965. P.S.E.B.M., 1965, v120.