with sarcomas the largest and in some cases the only tumor mass was attached to connective tissue coating around the chamber.

Microscopically the plasma-cell neoplasms were composed of large cells with plasma-cell characteristics. Metastases were frequent. They were found in ovary, pituitary, spleen and lymph nodes. One tumor line caused a myeloma type change in kidney and another line produced osteolytic lesions.

Either whole mounts or sections were made of the porous membrane with adjacent cell layers from some diffusion chambers (Fig. 2). Viable appearing mammary tumor tissue was found in 4 of 5 chambers from mice that developed plasma-cell tumors. Histologically recognizable mammary tumor tissue was present in 3 of the 4 chambers taken from mice with sarcomas. The cells appeared necrotic in one chamber but the tissue surrounding the chamber was also necrotic.

Discussion. Induction of plasma-cell tumors was an unexpected result of these experiments originally set up to study passage of mammary tumor agent through pores of various sizes. The findings on mammary tumors will be published separately.

Unpublished observations show that rings, each with 1 membrane attached, induce sarcomas when implanted subcutaneously, but further experiments are needed to elucidate the role of diffusion chamber parts, the tissue inside the chamber and the stilbestrol in induction of plasma cell tumors.

Summary. Seven plasma-cell neoplasms and 6 fibrosarcomas developed in strain BALB/c mice that carried diffusion chambers in the peritoneal cavity. The diffusion chambers contained mammary tumor tissue obtained from strain C3H mice with the milk agent. Five plasma-cell tumors and all sarcomas were successfully transplanted to strain BALB/c mice. The 2 plasma-cell tumors and the 2 sarcomas transplanted to C3H mice failed to grow.

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Regulation of Cholesterol Oxidation by Liver *in vitro*.*† (24971)

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Careful balance studies of absorption and excretion of bile acids and cholesterol in rats (1) indicated that administration of bile acids in the diet depresses mobilization of liver cholesterol. Further analysis of this phenomenon in both rats(2,3) and mice(4) has shown that both rates of synthesis and mobilization of liver and adrenal cholesterol are reduced upon feeding bile acids. Bile acids are major products of cholesterol catabolism in the liver (5). Daily production of bile acids in the rat is greatly enhanced when enterohepatic circulation of acids is interrupted by cannulation of bile duct(6,7). Bergstrom and Danielsson(8) showed that it is the concentration of bile acid conjugates in portal blood which actually influences rate of further synthesis of bile acids in the liver. These findings indicate that *in vivo*, the level of recirculating bile salts controls cholesterol catabolism in liver and further production of bile acids. We have now observed this "feedback effect" in an *in vitro* system prepared from rat liver, which oxidizes cholesterol to CO_2 and bile acids (5,9).

Methods. Cholesterol-27-C^{14} was synthesized from 3β-hydroxy-5-norcholestene-25-one(10-11) and purified *via* the digitonide

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TABLE I. Oxidation of Cholesterol-27-C^{14} and Sodium Pyruvate-2-C^{14} by Liver Mitochondria of "Bile-fistula" and Control Rats.

	Animal	Specific activity $Ba^{14}CO_3$ (cpm/mg)* from oxidation of Pyruvate (P)	Cholesterol (C)	C/P
Exp. 1	"Fistula"	6.2	25.9	4.2
	Control	8.4	10.7	1.3
Exp. 2	"Fistula"	2.4	10.2	4.2
	Control	2.1	7.3	3.6

* Corrected for equivalent amounts of mitochondria (mg N).

Exp. 1—Bile was drained 4 days following operation before animal was sacrificed.

Exp. 2—Bile was recirculated 17 days following operation, then drained 3 days before sacrifice.

and final passage through an alumina column. Sodium pyruvate-2-C^{14} was obtained from Atomic Energy Research Establishment, Amersham, England. 3α, 7α, 12α-Trihydroxycoprostane-27-C^{14} was synthesized from triformylchloride and di-isopropyl-1,3-C^{14}-cadmium(12,13). Glycocholic and taurocholic acids were synthesized from cholic acid by method of Norman(14) and recrystallized to constant melting point. These synthetic conjugates contained less than 5% cholic acid as determined by chromatographic separation on paper and paper ionopheresis(15). Bile cannulae, which allowed recirculation of bile into the duct, were inserted in male Wistar rats weighing 150-180 g. The bile was allowed to drain into balloons (a) directly after operation or (b) after recirculation for several days before drainage. Animals were fed *ad lib.* and given 0.5% saline as drinking fluid, and after at least 3 days drainage of bile, were sacrificed and their livers removed and weighed. Livers were taken only from those animals which had given a consistent bile flow through the cannula, for at least 3 days. Washed liver mitochondria and a boiled supernatant fraction were isolated from liver homogenates prepared in 10% (weight/volume) aqueous sucrose and incubated at pH 8.5 with cholesterol-27-C^{14} or sodium pyruvate-2-C^{14}, as previously described(5,9). The carbon dioxide-C^{14} evolved was trapped in 2 N sodium hydroxide, precipitated with barium chloride and counted in liquid scintillation counter as suspension of barium carbonate in a Thixin gel. Nitrogen determinations were carried out by micro-Kjeldahl method and the ammonia determined by direct Nesslerization and by titration.

Results. Table I compares oxidation of cholesterol and of sodium pyruvate by fortified preparations of washed liver mitochondria, obtained from livers of controls and from "fistula" rats which had been drained of bile through open cannulae. There was enhanced oxidation of cholesterol, relative to pyruvate oxidation, by liver mitochondrial preparations from bile-fistula rats, compared with oxidation of cholesterol by mitochondrial preparations from control animals. This is indicated by the ratio C/P, computed as ratio of specific radioactivities of CO_2 (as barium carbonate) isolated from incubations with cholesterol-27-C^{14} and sodium pyruvate-2-C^{14} respectively. This ratio was significantly greater for oxidation by liver mitochondrial preparations from bile-fistula animals than for oxidation by similar preparations from control animals.

When taurocholic and glycocholic acids were added to incubations with mitochondria prepared from normal rat livers, a selective inhibition of cholesterol oxidation was observed. Oxidation of sodium pyruvate and of 3α, 7α, 12α-trihydroxycoprostane (THC) was depressed to a much lesser extent (Table II). Unconjugated bile acids (cholic, deoxycholic, lithocholic acids) and tauro- and glycolithocholic acids inhibited oxidation of pyruvate as well as cholesterol oxidation.

TABLE II. Effect of Added Bile Acids upon Oxidation of Sodium Pyruvate-2-C^{14}, Cholesterol-27-C^{14} and 3α, 7α, 12α-trihydroxycoprostane-27-C^{14} (THC) by Rat Liver Mitochondria.

	Addition	% inhibition observed in oxidation of Pyruvate	Cholesterol	THC
		%		
Exp. 1	Cholic acid	44.5	67.7	
	Taurocholic acid	3.0	60.6	
	Glycocholic "	0.0	47.8	
Exp. 2	Cholic acid	63.5	81.5	65.7
	Taurocholic acid	18.8	45.5	27.0
	Glycocholic "	26.5	49.7	28.0

5 mg bile acids, neutralized with Tris, added to final incubation vol of 12.5 ml in 0.1 M Tris hydrochloride buffer, pH 8.5.

Discussion. Cholesterol is oxidized to hydroxycholanic acids by liver mitochondria *in vitro* with loss of terminal methyl groups of the side chain(5). Thus, formation of CO_2 from these carbon atoms serves as index of cholesterol oxidation and bile acid formation.

These experiments indicate that cholic acid conjugates, but not the unconjugated acid, regulate oxidation of cholesterol *in vitro* as well as *in vivo*(6-8). Oxidation of cholesterol *in vitro* was significantly increased when liver tissue was depleted of endogenous bile salts by drainage of bile and interruption of the enterohepatic circulation. Conversely, when bile acid conjugates were added back to the oxidative system, cholesterol oxidation was decreased. That these were specific effects upon cholesterol oxidation and not just activation or inhibition of all mitochondrial oxidases, was indicated by comparative studies upon oxidation of sodium pyruvate by the same mitochondrial preparations under identical conditions.

These studies of cholesterol oxidation in a cell-free system *in vitro* confirm the *in vivo* findings of a feedback regulation of the transformation of cholesterol to bile acids. The mechanism of this regulatory control cannot therefore be ascribed to alterations in permeability of liver cells to circulating cholesterol, under the influence of conjugated bile salts in portal blood.

3α, 7α, 12α-Trihydroxycoprostane (THC), which has the side chain structure of cholesterol and nuclear structure of cholic acid, is readily oxidized by the rat liver to cholic acid and CO_2(12,16) and has been implicated as intermediate in metabolic conversion of cholesterol to cholic acid. In these experiments, bile acid conjugates hardly depressed oxidation of THC, although oxidation of cholesterol was much inhibited. Evidently, the conjugates do not control cholesterol oxidation by competitively inhibiting enzymes which would metabolize any trihydroxycoprostanes, formed as intermediates in transformation of cholesterol to the bile acids. This would appear to support Bergstrom's hypothesis(17) that the rate-determining step in cholesterol catabolism and bile acid formation is associated with nuclear hydroxylation, especially 7α-hydroxylation; and it is this step which is sensitive to the conjugated bile acids in the enterohepatic circulation.

Summary. A feedback mechanism, whereby conjugated bile acids control oxidation of cholesterol in liver, has been discerned *in vitro*. Addition of cholic acid conjugates depressed oxidation of cholesterol, but not that of sodium pyruvate or 3α, 7α, 12α-trihydrocoprostane, by fortified rat liver mitochondrial preparations. Preparations from livers of rats, deprived of bile salts by interruption of enterohepatic circulation, oxidized cholesterol to a greater extent than controls. The site of action of the feedback effect is discussed briefly.

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