Nuclear and Cytosolic Distribution of Conjugated Cholic Acid and Radiolabelled Glycocholic Acid in Rat Liver

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(Received 4 May 1978)

1. Normally fed and cholestyramine-treated rats were injected through the superior mesenteric vein with different amounts of radiolabelled glycocholic acid and the appearance of radioactivity in bile was measured. 2. In normally fed rats radioactivity appeared in bile within 30 s of injection and reached a maximum after $2\frac{1}{2}$ min; in the cholestyramine-treated animals the appearance of radioactivity was slower and less of the injected material was excreted into bile. 3. At 10 min after injection, livers were removed and the amounts of radioactive glycocholic acid and endogenous cholic acid conjugates in nuclei and cytosol were determined; most of the bile acid was found in the cytosol, only small amounts being found in nuclei. 4. Nuclear preparations from both normally fed and cholestyramine-fed rats were extracted with KCl (0.4 M) in an attempt to identify a putative bile acid receptor, but no such receptor was found. 5. Regulation of bile acid synthesis does not involve nuclear binding of bile acids.

The synthesis of bile acids from cholesterol occurs only in the liver and is regulated by the microsomal enzyme cholesterol 7 α -hydroxylase (EC 1.14.–.) (Danielsson *et al.*, 1967; Myant & Mitropoulos, 1977). The newly synthesized bile acids are also conjugated in the liver with either taurine or glycine, and then actively transported across the canalicular membrane into bile. After promoting the intestinal digestion and absorption of fat, the bile acids are actively transported across the mucosa of the terminal ileum (Lack & Weiner, 1961) and returned to the liver in the portal vein. After carrier-mediated uptake across the liver plasma membrane (Glasinovic *et al.*, 1975; Reichen & Paumgartner, 1975; Schwarz *et al.*, 1975; Accatino & Simon, 1976) the bile acids pass across the hepatocyte to be returned to the bile. The passage of bile acids from liver into bile and small intestine and their return to the liver is referred to as the enterohepatic circulation (Small *et al.*, 1972).

The rate of bile acid synthesis appears to be linked to this circulation, since the formation of an external biliary fistula, or feeding anion-exchange resins such as cholestyramine, leads to induction of cholesterol 7 α -hydroxylase (Danielsson *et al.*, 1967; Boyd *et al.*, 1969; Shefer *et al.*, 1970) and a concomitant increase in the rate of synthesis of bile acids from cholesterol (Eriksson, 1957). This increase in cholesterol 7 α -hydroxylase activity can be prevented by treatment with actinomycin (Einarsson & Johansson, 1968) or by feeding bile acids (Shefer *et al.*, 1970, 1973). The agent responsible for regulating the rate of synthesis of cholesterol 7 α -hydroxylase has not been un-

equivocally identified, but it has been largely accepted that it is the concentration of bile acids in the enterohepatic circulation; in particular, the amounts of bile acids returning to the liver in the portal vein (Shefer *et al.*, 1969, 1970; Danielsson & Sjovall, 1975) control the synthesis of the enzyme system or at least a component of this system.

The mechanism whereby the bile acids exert this effect is unknown, but the similarity between their structure and that of many different steroid hormones suggests that the steroid-cytosol receptor-protein model, described for many of these hormones (Yamamoto & Alberts, 1976), might operate. The initial event in steroid-induced changes in the rate of target-enzyme synthesis is the avid binding (dissociation constant ≈ 0.1 nM) of the steroid by specific cytosolic receptor proteins followed by the interaction of this complex with nuclear receptors. Values for the number of nuclear receptors/cell vary between 1×10^3 and 15×10^3 (Buller & O'Malley, 1976). The steroid-receptor-nuclear interaction results in the production of mRNA and changes in the rate of synthesis of the target enzyme. A prominent feature of this mechanism is the ability of target-cell nuclei to concentrate and retain the steroid hormone (Stumpf, 1968; Williams & Gorski, 1972).

An important difference between the metabolism of the steroid hormones and the bile acids is the relative concentration of each in serum and target tissues. The concentration of bile acids is normally approx. $1 \mu\text{M}$ (Okishio & Nair, 1966), whereas the concentration of the steroid hormones is normally

approx. 1 nM. The relatively large concentration of bile acids in the liver apparently means therefore that any high-affinity receptors would be consistently saturated. Furthermore, in experiments using equilibrium dialysis at 37°C, we were unable to detect high-affinity binding of bile acids by rat liver 100000g supernatants (Strange *et al.*, 1976, 1977). This suggests that, if bile acids regulate enzyme synthesis, they do so by a mechanism fundamentally different from that of other steroids. These experiments are not conclusive, however, since it is possible that a receptor was not detected in our experiments because it is unusually labile. Further, in animals with an intact enterohepatic circulation there is only a low rate of bile acid synthesis and presumably therefore little induction of cholesterol 7 α -hydroxylase. In the cholestyramine-fed or biliary-drained animals, however, both these processes are greatly enhanced. The concentration of bile acids in livers from these animals is unknown, but it is under precisely these conditions that the hepatic concentration of bile acids and, in particular, the nuclear concentration may be critical.

We now describe experiments to investigate further the possibility that bile acids regulate their own synthesis by a mechanism involving nuclear receptors. Three types of experiment were performed in rats given either a normal diet or one containing cholestyramine; these were (1) measurement of the distribution between nucleus and cytosol of a bolus of radioactive glycocholic acid, (2) measurement of the distribution of endogenous cholic acid conjugates, and (3) attempts made to isolate a bile acid receptor from nuclei by extraction with KCl.

Materials and Methods

Chemicals

[24-¹⁴C]Glycocholic acid (sp. radioactivity 50 Ci/mol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and [G-³H]-glycocholic acid (sp. radioactivity 2 Ci/mmol) from New England Nuclear, Boston, MA, U.S.A. AristaR sucrose and DNA (calf thymus) were from BDH, Poole, Dorset, U.K. Bio-Gel A-0.5m (200–400 mesh) was purchased from Bio-Rad Laboratories, Bromley, Kent, U.K. Cholestyramine (polystyrene trimethylbenzylammonium chloride) was purchased as Cuemid from Merck, Sharp and Dohme, West Point, PA, U.S.A. Soluene-100 was from Packard Instrument Co., Downers Grove, IL, U.S.A.

Animals

Male Wistar rats (300–330g) fed *ad libitum* were used. In cholestyramine-feeding experiments, animals were maintained for 18 or 40h on a similar

diet containing 4% (w/w) cholestyramine (Strange *et al.*, 1976).

Determination of subcellular distribution of glycocholic acid

Rats were anaesthetized with diethyl ether and the bile duct was cannulated by using fine polyethylene tubing. The mesentery of the small intestine was displayed and a solution (400 μ l) of 20mM-Tris/HCl buffer, pH 7.45, containing 3mM-MgCl₂ and radio-labelled glycocholic acid ([¹⁴C]glycocholic acid, 20nmol, 1 μ Ci; or [³H]glycocholic acid, 5nmol, 10 μ Ci) was injected into a branch of the superior mesenteric vein. Immediately after this injection bile samples were collected directly into scintillation vials for 30s periods over 10min. During this time the animal's abdomen was covered with cotton swabs soaked in NaCl (150mM; 35°C) to conserve body heat and to prevent dehydration. Immediately after the last bile collection, the liver was perfused via the portal vein with approx. 20ml of ice-cold 20mM-Tris/HCl buffer, pH 7.45, containing 3mM-MgCl₂ and 0.32M-sucrose until free from blood.

The liver was removed, cut into small pieces and homogenized in 30ml of ice-cold 20mM-Tris/HCl buffer (pH 7.45)/3mM-MgCl₂/0.32M-sucrose. The homogenate was filtered through two layers of gauze and portions (13ml) were diluted to 20ml with a solution containing sucrose (0.25M) and MgCl₂ (1mM). After mixing, 20ml of 20mM-Tris/HCl buffer, pH 7.45, containing 3mM-MgCl₂ and 0.32M-sucrose was gently layered under this mixture to give two distinct layers. After centrifugation (10min, 2°C, 700g) the pellet was retained, the supernatant was decanted, re-centrifuged (120min, 2°C, 100000g) and the clear supernatant harvested after removal of the lipid layer.

The procedure used to prepare nuclei was essentially that described by Widnell & Tata (1964). The pellet from the first low-speed centrifugation was resuspended in 12ml of sucrose (2.4M) containing MgCl₂ (1mM) by gentle hand homogenization with an all-glass homogenizer and centrifuged (60min, 2°C, 50000g). The plug of contaminating mitochondria and whole cells, which formed at the top of the tube, was removed. The nuclear pellet, which formed at the base of the tube, was resuspended in 2ml of sucrose (0.25M) containing MgCl₂ (1mM). The mean ratio of concentrations of protein to DNA of 19 preparations of these purified nuclei was 6.4. The value originally reported by Widnell & Tata (1964) was 4.8.

The purity and integrity of this nuclear preparation was assessed by transmission electron microscopy after fixation in glutaraldehyde. Viewed at a magnification of 4900 the nuclear preparation consisted almost entirely of nuclei with only very slight micro-

somal contamination; no whole cells or erythrocytes were seen. The nuclei were intact, with a defined double membrane.

Extraction of nuclear receptors

Certain steroids can be extracted from nuclei still bound to their receptor proteins by using buffer solutions containing high concentrations of KCl (approx. 0.4M) (O'Malley *et al.*, 1971; Lawson & Wilson, 1974). To isolate the putative glycocholic acid-receptor complex from liver nuclei, rats were injected with [^3H]glycocholic acid (10nmol; 20 μCi) and the nuclei prepared as described. The nuclear pellet was gently homogenized by hand in 5ml of either 20mM-sodium phosphate/20mM-potassium phosphate buffer (pH 7.4) containing KCl (300mM) [prepared by adjusting a solution of Na_2HPO_4 (20mM) containing KCl (300mM) to pH 7.4 with KH_2PO_4 (20mM) containing KCl (300mM)], or 20mM-Tris/HCl buffer (pH 7.5) containing EDTA (5mM) and KCl (400mM). The latter buffer was used with and without 2-mercaptoethanol (100mM). Various buffer systems were used, since a receptor might be unstable in a particular buffer.

After homogenization (at 0°C) the solutions were kept on ice for 30min, centrifuged (30min, 2°C, 100000g) and the supernatant was added to an agarose column (2.2cm $\times$ 15cm) to separate [^3H]glycocholic acid that might be bound to receptor protein from unbound [^3H]glycocholic acid. The columns were equilibrated and eluted (4°C) with the respective homogenization buffer containing a lower concentration of KCl (100mM). The flow rate was 15ml/h, the void volume (determined with Blue Dextran) was 22ml and the salt volume (determined with Na^+ and [^3H]glycocholic acid) was 54ml. Fraction volume was 2ml.

The possibility that the postulated cytosolic bile acid receptor was denatured during the preparation of nuclei in the high-strength sucrose solution was examined by preparing a less-pure preparation of liver nuclei in a lower molarity of sucrose. After injection of [^3H]glycocholic acid, livers were removed and homogenized as before. The pellet from the first low-speed centrifugation was washed twice with 20ml of 20mM-Tris/HCl buffer, pH 7.5, containing 3mM- MgCl_2 and 0.32 M-sucrose and then homogenized by hand with 10ml of ice-cold 20mM-Tris/HCl buffer, pH 7.5, containing EDTA (5mM) and KCl (400mM), left in ice for 30min and centrifuged (30min, 2°C, 100000g). Portions of the supernatant (5ml) were chromatographed on the agarose column as described above.

In all experiments portions of each column fraction were taken for radioactivity counting (0.5ml) and for the measurement of cholic acid conjugates by radioimmunoassay (0.1ml). The minimum amounts of

radiolabelled glycocholic acid and cholic acid conjugates that could be detected in each fraction were 0.2pmol and 60pmol respectively.

Analytical

DNA was measured by the method of Burton (1956). Samples were prepared for analysis as described by Widnell & Tata (1964). Portions of homogenate (0.5ml) and nuclei (0.1ml) were mixed with ice-cold 0.2M- HClO_4 (10ml) and the precipitate was washed three times with this solution. DNA was extracted from the precipitate by treatment with 2 $\times$ 2ml of 0.5M- HClO_4 at 75°C for 10min. The recoveries of known amounts of DNA added to preparations of liver homogenate and nuclei were 108 and 109% respectively. The calf thymus DNA used to prepare each standard curve was first standardized against pure DNA prepared from *Escherichia coli* by the method of Marmur (1961).

Total protein concentrations were measured by the Lowry method as described by Herbert *et al.* (1971) with bovine albumin (Armour Pharmaceutical Co., Chicago, IL, U.S.A.) as the standard.

Radioactivity counting was performed in a toluene-based scintillant [2,5-diphenyloxazole, 4g; 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 0.2g; toluene, 667ml; Triton X-100, 333ml]. Samples of homogenate (200 μl), nuclei (500 μl) and 100000g supernatant (500 μl) were added to scintillation vials containing 700 μl of Soluene and left at room temperature (20°C) with occasional mixing until dissolution was complete (approx. 3h). Scintillant (15ml) was then added, and after mixing the samples were kept overnight in the dark at 4°C and then counted for radioactivity. Quenching of samples was corrected for by using an internal standard. The smallest count rates were obtained in experiments to determine the [^{14}C]glycocholic acid content of the purified nuclear preparation; typical count rates were 3.5d.p.s., which is about 10 times the background value.

To confirm the integrity of injected [^{14}C]glycocholic acid and [^3H]glycocholic acid in subcellular fractions, samples of homogenate (5ml), cytosol (5ml) and nuclei (2ml) were extracted with boiling methanol (10ml) and after cooling and centrifugation (10min, 20°C, 1000g) the methanolic supernatant was evaporated to dryness under a stream of N_2 , redissolved in 500 μl of methanol and examined by t.l.c. (Kelly & Doisy, 1964). In each case, all the radioactivity was found to have the same mobility as a glycocholic acid standard.

The concentration of cholic acid conjugates (glycine and taurine) in subcellular preparations and fractions from the agarose column were measured by radioimmunoassay. The assay used a rabbit antiserum to a bovine serum albumin-cholic acid con-

jugate prepared by the method of Nars & Hunter (1973). Cross-reactivity studies with a variety of conjugated and unconjugated bile acids showed that only glycocholic acid and taurocholic acid were significantly and equally bound by the antiserum. The assay uses a ^{125}I -labelled histamine-glycocholic acid conjugate as tracer (Spenny *et al.*, 1977) and has been described by Beckett *et al.* (1978).

Portions of homogenate (100 μl) and 100000g supernatant (200 μl) were added to 1M-NaOH (1ml), thoroughly mixed and the pH adjusted to approx. 10 by adding 85 μl of 11.6M-HCl. This mixture (20 μl) was used in the radioimmunoassay. Portions of the nuclear preparation (200 μl) were added to 2M-NaOH (100 μl) and the pH was adjusted to approx. 10 by addition of 11.6M-HCl (15 μl). The recovery of known amounts of glycocholic acid added to homogenate was 119%, to nuclei, 98% and to 100000g supernatant, 103%.

Results

Appearance of [^{14}C]glycocholic acid in bile

The radioactive bile acid appeared in bile within 1 min of the injection of [^{14}C]glycocholic acid (20 nmol; 1 μCi) in three normally fed animals and reached a maximum after 2½ min (Fig. 1). A total of 32857 ± 940 (mean $\pm$ S.E.M.) d.p.s. (89%) of the injected radioactivity was excreted into bile during the 10 min period.

In four animals given a smaller mass of [^3H]glycocholic acid (5 nmol; 10 μCi) the time course of excretion into bile was similar.

Nuclear and cytosolic distribution of radiolabelled glycocholic acid in normally fed rats

Whole liver homogenates from four normally fed rats, 10 min after injection of [^{14}C]glycocholic acid

Fig. 1. Time course of appearance of radiolabelled glycocholic acid in bile

Radiolabelled glycocholic acid was injected into the superior mesenteric vein of anaesthetized rats and bile was collected through a cannula for 30 s periods over 10 min. Radioactivity in bile from: ●, three normally fed animals; □, three cholestyramine-fed (18 h) animals; ▲, three cholestyramine-fed (40 h) animals. Results shown are means $\pm$ S.E.M.

Table 1. Amounts of radiolabelled glycocholic acid in subcellular fractions of livers from normally fed rats [^{14}C]Glycocholic acid (20 nmol) or [^3H]glycocholic acid (5 nmol) was injected into the superior mesenteric vein of anaesthetized rats and after 10 min the livers were removed, homogenized and nuclei and 100000g supernatants were prepared as described in the Materials and Methods section. Results are each from four animals injected with either (a) 20 nmol of [^{14}C]glycocholic acid or (b) 5 nmol of [^3H]glycocholic acid and are means $\pm$ S.E.M.

	Glycocholic acid			
	(d.p.s./mg of protein)		(d.p.s./mg of DNA)	
	(a)	(b)	(a)	(b)
Homogenate	0.72 ± 0.11	15.4 ± 3.67	44.7 ± 1.87	1261 ± 322.7
Nuclear preparation	0.24 ± 0.04	0.88 ± 0.44	2.89 ± 0.84	4.40 ± 2.20
100000g supernatant	0.83 ± 0.06	20.5 ± 0.40	—	—

(20 nmol; 36667 d.p.s.), retained 1109 d.p.s. After injection of [^3H]glycocholic acid (5 nmol; 366670 d.p.s., four animals), 18326 d.p.s. of the injected radioactivity was retained by the liver homogenate. The distribution of the radiolabelled bile acid between nuclei and 100000g supernatant is shown in Table 1. Since the specific radioactivity of the [^3H]glycocholic acid was 40 times that of the [^{14}C]glycocholic acid, higher count rates were obtained by using this radioisotope, even though the mass of injected [^{14}C]glycocholic acid was 4 times greater.

The amount of radioactivity in liver nuclei from animals given 20 nmol of [^{14}C]glycocholic acid was 14.7 d.p.s.; this corresponds to 9500 molecules of injected [^{14}C]glycocholic acid being associated with each nucleus. In nuclei from animals given the lower dose the average amount of [^3H]glycocholic acid was 41.07 d.p.s., which corresponds to 360 molecules per nucleus (assuming 10 pg of DNA/nucleus; Davidson, 1972). In three of the four animals given 5 nmol of [^3H]glycocholic acid, however, no radioactivity was found in the nuclei even though substantial amounts of [^3H]glycocholic acid were present in the whole-liver homogenates. This suggests that liver nuclei have a low ability to bind injected glycocholic acid and that much of the nuclear radioactivity found in animals given 20 nmol of [^{14}C]glycocholic acid is due to non-specific binding.

The 100000g supernatant preparations from the two groups of animals contained 1.5% of the larger mass of injected radioactivity (20 nmol) and 2% of the smaller one (5 nmol).

Appearance of [^{14}C]glycocholic acid in bile of cholestyramine-fed rats

The time course of appearance of radioactivity in the bile of the two groups of animals given cholestyramine (18 and 40 h) are shown in Fig. 1. Both groups were injected with 20 nmol of [^{14}C]glycocholic acid and bile was collected for 10 min. The appearance of radioactivity was slower in both these groups com-

pared with the corresponding group of normally fed animals, and significantly less ($P < 0.005$ in each case) of the injected [^{14}C]glycocholic acid was excreted into bile. In the animals given cholestyramine for 18 h, 22650 ± 1881 (mean $\pm$ S.E.M.) d.p.s. of the injected radioactivity was excreted, whereas after 40 h of cholestyramine feeding 22762 ± 376 (mean $\pm$ S.E.M.) d.p.s. of the injected material was excreted.

Nuclear and cytosolic distribution of [^{14}C]glycocholic acid in cholestyramine-fed rats

As expected from the finding that less radioactivity was excreted into bile in the cholestyramine-fed rats, significantly more [^{14}C]glycocholic acid was found in liver homogenates both from the four animals given cholestyramine for 18 h (4538 d.p.s.; $P < 0.05$) and the four rats given cholestyramine for 40 h (7865 d.p.s.; $P < 0.05$) (Table 2) compared with normally fed animals.

There was no significant difference in the amount of [^{14}C]glycocholic acid found in nuclei from livers taken from normally fed animals and from livers of the two groups of cholestyramine-fed animals. In cholestyramine-fed rats (40 h) given a smaller mass (5 nmol) of [^3H]glycocholic acid, the amount of nuclear radioactivity was also similar to that found in nuclei from normally fed animals given the same dose.

Supernatants from the cholestyramine-fed rats (18 and 40 h) contained 9.6 and 6.2% respectively of the injected radioactivity. These were significantly ($P < 0.01$ in each case) more than the corresponding amount of radioactivity in supernatants from normally fed rats.

Endogenous cholic acid conjugates in liver homogenate, nuclei and supernatant

The concentration of endogenous cholic acid conjugates in liver subcellular preparations are shown in Table 3. A total of about $1.8 \mu\text{mol}$ of cholic acid conjugates was found in homogenates

Table 2. Amounts of radiolabelled glycocholic acid in subcellular fractions of livers from cholestyramine-fed rats [^{14}C]Glycocholic acid (20 nmol; $1 \mu\text{Ci}$) was injected into the portal vein of anaesthetized, cholestyramine-fed rats and after 10 min the livers were removed, homogenized and nuclei and 100000g supernatants were prepared as described in the Materials and Methods section. Results show the mean $\pm$ S.E.M. for the two groups of rats fed cholestyramine for 18 and 40 h.

	Glycocholic acid			
	Cholestyramine-fed for 18 h		Cholestyramine-fed for 40 h	
	(d.p.s./mg of protein)	(d.p.s./mg of DNA)	(d.p.s./mg of protein)	(d.p.s./mg of DNA)
Homogenate	2.75 ± 0.94	320.5 ± 106.7	4.77 ± 2.38	361.9 ± 150.3
Nuclear preparation	0.42 ± 0.04	3.98 ± 0.83	0.33 ± 0.09	3.32 ± 1.01
100000g supernatant	6.42 ± 1.94	—	4.85 ± 1.94	—

Table 3. *Endogenous taurine and glycine conjugates in liver subcellular fractions*

The amounts of taurine and glycine conjugates of cholic acid in liver homogenate, nuclei and 100000g supernatant were measured by radioimmunoassay (Beckett *et al.*, 1978). Subcellular fractions were prepared from animals given a normal diet (four rats) or a diet containing 4% cholestyramine for either 18 h (four rats) or 40 h (four rats). Results show means $\pm$ S.E.M.

	Taurine and glycine conjugates of cholic acid					
	Normal diet		Cholestyramine (18h)		Cholestyramine (40h)	
	(nmol/mg of protein)	(nmol/mg of DNA)	(nmol/mg of protein)	(nmol/mg of DNA)	(nmol/mg of protein)	(nmol/mg of DNA)
Homogenate	1.17 $\pm$ 0.24	88.7 $\pm$ 8.50	0.40 $\pm$ 0.13	45.3 $\pm$ 15.4	0.76 $\pm$ 0.10	60.0 $\pm$ 6.90
Nuclear preparation	0.18 $\pm$ 0.01	1.70 $\pm$ 0.46	0.11 $\pm$ 0.06	1.02 $\pm$ 0.51	0.04 $\pm$ 0.01	0.43 $\pm$ 0.10
Supernatant	0.81 $\pm$ 0.21	—	0.31 $\pm$ 0.23	—	0.45 $\pm$ 0.22	—

from normally fed animals; assuming a liver volume of 10ml the concentration of cholic acid conjugates is therefore about 180 μ mol/litre. Only a small part (0.7%) of the homogenate cholic acid conjugates was found in the nuclear preparation; the amount of endogenous bile acid in this fraction was 12.6nmol, which corresponds to 10.2×10^6 molecules/nucleus. About 30% of the total cholic acid conjugates in the liver homogenates were found in cytosol.

Feeding animals with cholestyramine for 18 or 40h resulted in a significant decrease in the amount of cholic acid conjugates found in the liver homogenates (640nmol, $P < 0.05$; 1020nmol, $P < 0.05$ respectively) compared with normally fed rats. The nuclear content of cholic acid conjugates did not change after cholestyramine feeding for 18h but was significantly

decreased (3.14nmol; $P < 0.005$) after 40h. The amount of bile acid remaining in the nuclei after 40h of cholestyramine still corresponded to 2.6×10^6 molecules/nucleus. There was no significant difference in the amount of cholic acid conjugates in the cytosol from the different groups of rats.

Attempts to extract a glycocholic acid receptor from nuclei

These attempts were made from both partially purified nuclei and from nuclei purified by preparation in 2.4M-sucrose. The majority of the radioactive glycocholic acid (90%) in both nuclear preparations was solubilized by treatment with KCl (0.4M). Fig. 2 shows the elution pattern when the

Fig. 2. *Elution pattern of KCl nuclear extract*

Radiolabelled glycocholic acid was injected into the superior mesenteric vein of anaesthetized rats and the livers were removed. Partially purified nuclei were prepared as described in the Material and Methods section and extracted with 20mM-Tris/HCl buffer, pH 7.5, containing 5mM-EDTA and 400mM-KCl. After centrifugation (100000g, 30min, 2°C) the supernatant was applied to an agarose column (2.2cm $\times$ 15cm) and eluted with 20mM-Tris/HCl buffer, pH 7.5, containing 5mM-EDTA, and 100mM-KCl. Fractions (2ml) were collected and the A_{280} (●), radioactivity (□) and concentration of cholic acid conjugates (▲) were measured.

KCl extract from partially purified nuclei from a cholestyramine-treated (40h) animal was chromatographed on an agarose column. A considerable amount of 280nm-absorbing material was eluted after the column void volume (22ml), but neither [^3H]-glycocholic acid nor endogenous conjugates of cholic acid were found in these fractions. Similar elution patterns were obtained when KCl extracts of partially purified nuclei from normally fed rats were chromatographed and when extracts from purified nuclei from both groups of animals were eluted from the agarose column.

Discussion

The purpose of the present study was to determine whether bile acids regulated the rate of their own synthesis by a mechanism involving nuclear binding of bile acid-receptor proteins. Such receptors are involved in the actions of several steroid hormones, including oestrogens (Jensen & DeSombre, 1972), progesterone (Buller & O'Malley, 1976) and dexamethasone (Rousseau *et al.*, 1973).

In initial experiments, the time course of the passage of bile acids from the superior mesenteric vein, across the liver, into the bile was measured so as to determine the time after injection after which most of the injected bile acid had been excreted. By administering the bile acids into a branch of the superior mesenteric vein the bolus was mixed with blood before reaching the liver and the portal venous blood supply to the liver remained intact. Our results are in agreement with those of Smallwood *et al.* (1975) and show that after 10min only 3% of the injected dose remained in the liver homogenate. The transport of glycocholic acid by the cholestyramine-treated rats was significantly slower. In our experiments between 77 and 91% of the administered radiolabelled glycocholic acid was found in the liver homogenate and bile. Ng & Hofmann (1977) have described the fate of [^{14}C]-glycocholic acid injected into the femoral vein of the rat; the liver and the bile contained about 70% of the injected material, and a further 10% was found in extrahepatic tissues such as muscle, intestine and kidney.

A common feature of the high-affinity receptor model is that most (approx. 85%) of the administered steroid is found in target-cell nuclei irrespective of the mass of hormone injected (Stumpf, 1968). Thus, radiolabelled oestrogen is mainly found in nuclei from the chicken uterus, little being found in the cytosol (Jensen & DeSombre, 1972). By comparison, Brown *et al.* (1964) have shown that about 60% of an intravenously administered dose of [^3H]-bilirubin is found in the cytosol. The distribution of radioactivity is related therefore to the action of the particular compound; bilirubin is efficiently excreted by the liver into bile and has no known regulatory role at

the nucleus, whereas oestradiol is known to affect the synthesis of enzymes in chicken uterus after interaction with the nucleus. The distribution of radiolabelled glycocholic acid between nuclei and cytosol would therefore be an indication of the action of the bile acid in the hepatocyte. The distribution of radiolabelled glycocholic acid was similar to that of bilirubin; only a very small percentage of the injected material was found in the purified nuclear preparation. A large part of binding of bile acid to nuclei appears to be non-specific, since decreasing the mass of injected material resulted in little nuclear binding of glycocholic acid even though significant quantities of [^3H]-glycocholic acid were present in the liver homogenate, indicating that liver nuclei are unable to concentrate glycocholic acid. Further, the amounts and the distribution of radiolabelled glycocholic acid between nuclei and cytosol in normally fed animals and in those given cholestyramine in their diet was similar, even though the rate of bile acid synthesis in cholestyramine-fed animals (40h) is increased about 5-fold (Danielsson *et al.*, 1967; Boyd & Percy-Robb, 1971). In animals given cholestyramine for 18h the rate of formation of 7 α -hydroxycholesterol (cholest-5-ene-3 β ,7 α -diol) (Danielsson *et al.*, 1967) is not increased, although presumably the induction of cholesterol 7 α -hydroxylase has been initiated.

We found the concentration of endogenous cholic acid conjugates in the liver (approx. 180 μM) to be similar to the value reported by Okishio & Nair (1966) for the whole homogenate (250 μM), using g.l.c. Both these values are probably overestimates, since the homogenate is contaminated with bile. Since radioactivity is found in bile almost immediately after injection, the amount of bile in the liver must be no larger than the volume of bile excreted in 30s. Assuming a bile flow rate of 1ml/h and a biliary bile acid concentration of 10mM the volume of biliary contamination would be no more than 10 μl and equivalent to 100nmol of bile acid. Before removal, livers were perfused *in situ* with buffer until free from blood so that contamination by radiolabelled or endogenous bile acids in blood was negligible. About 0.7% of the endogenous conjugates of cholic acid were found in the nuclear preparation. Okishio & Nair (1966) found about 10% of the total liver bile acid in nuclei, although their nuclear preparation was only partially purified and was certainly contaminated with other subcellular fractions. Feeding cholestyramine resulted in a decrease in the homogenate content of cholic acid conjugates, and although there was a decrease in the amount found in nuclei from animals given cholestyramine for 40h, significant amounts of bile acid were still found in this preparation. It is not known, however, how much of this nuclear-bound bile acid is the result of non-specific binding of homogenate bile acid to nuclei, possibly during the homogenization procedure.

Attempts to extract a glycocholic acid receptor from both partially purified and pure nuclei by using KCl were unsuccessful. The extraction procedure has been used by different workers to release a steroid-receptor protein complex from nuclei. Although measurable amounts of radiolabelled glycocholic acid and conjugates of cholic acid were released from partially purified nuclei, none was eluted from the agarose column bound to protein. If a high-affinity glycocholic acid receptor similar to that described for other steroids were present in liver nuclei, then the receptor-bile acid complex should be eluted intact from this column (O'Malley *et al.*, 1971). The endogenous bile acid does not appear to be associated with nuclear protein, further supporting the concept that much of it may be non-specifically bound.

The mechanism whereby bile acids might regulate the rate of cholesterol 7 α -hydroxylase synthesis remains unclear; they do not appear to act via a high-affinity receptor protein. In view of the large amounts of bile acid ($\approx 200 \mu\text{M}$) in liver homogenates of even the cholestyramine-fed animals, it appears likely that a bile acid receptor would be consistently saturated unless the hepatocyte could compartmentalize most of the bile acid extracted from the portal vein for excretion into bile, leaving a small amount ($\approx 0.1 \text{ nM}$) for interaction with the nucleus. A further suggestion that bile acid synthesis is regulated by a hormone produced by the intestinal mucosa as a result of interaction with bile acids is not supported by another study (Hoffman *et al.*, 1977).

We thank Dr. H. Cameron and Dr. E. McGooghan for carrying out the electron microscopy, Mr. J. Hayes for preparing DNA from *E. coli* and Dr. H. Cruft for advice on the preparation of nuclei.

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