Dilley, R. A. (1964), *Biochem. Biophys. Res. Commun. 18*, 716.
Dilley, R. A. (1966), *Brookhaven Symp. Biol. 19*, 258.
Dilley, R. A., and Rothstein, A. (1967), *Biochim. Biophys. Acta 135*, 427.
Dilley, R. A., and Vernon, L. P. (1964), *Biochemistry 3*, 817.
Dilley, R. A., and Vernon, L. P. (1965), *Arch. Biochem. Biophys. 111*, 365.
Dilley, R. A., and Vernon, L. P. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 395.
Fishman, M., and Moyer, S. J. (1942), *J. Gen. Physiol. 25*, 755.
Fiske, C. H., and Subbarow, Y. (1929), *J. Biol. Chem 81*, 629.
Gross, J. A., Becker, M. J., and Shefner, A. M. (1964), *Experientia 20*, 261.
Hind, G., and Jagendorf, A. T. (1965), *J. Biol. Chem. 240*, 3195.
Izawa, S., and Hind, G. (1967), *Biochim. Biophys. Acta 143*, 377.
Jagendorf, A. T., and Hind, J. (1963), *Natl. Acad. Sci. Natl. Res. Council Publ. 1145*, 599.
Jagendorf, A. T., and Neumann, J. (1965), *J. Biol. Chem. 240*, 3210.
Jagendorf, A. T., and Uride, E. (1966), *Brookhaven Symp. Biol. 19*, 215.
Johnson, C. L., Mauritzen, C. M., Starbuck, W. C., and Schwartz, A. (1967), *Biochemistry 6*, 1121.
Machinist, J. M., and Crane, F. L. (1965), *J. Biol. Chem. 240*, 1788.
Mitchell, P. (1966), *Biol. Rev. 41*, 445.
Neumann, J., and Jagendorf, A. T. (1964), *Arch. Biochem. Biophys. 107*, 109.
Nishimura, M., Ito, T., and Chance, B. (1962), *Biochim. Biophys. Acta 59*, 177.
Nobel, P. S., and Mel, H. C. (1966), *Arch. Biochem. Biophys. 113*, 695.
Schwartz, A. (1965), *J. Biol. Chem. 240*, 939.
Tanford, C. (1961) *in* Physical Chemistry of Macromolecules, New York, N. Y., Wiley.
Thomas, J. R., Minnaert, K., and Elbers, P. F. (1956), *Acta Botan. Neerl. 5*, 315.
Tolberg, A., and Macey, R. I. (1965), *Biochim. Biophys. Acta 109*, 424.
Vambutas, V. K., and Racker, E. (1965), *J. Biol. Chem. 240*, 2660.
Vignais, P. V., Vignais, P. M., and Stanislas, E. (1962), *Biochim. Biophys. Acta 60*, 284.

The Metabolism of Bile Acids in the Developing Rat Liver*

Henry Danielsson† and William J. Rutter

ABSTRACT: The metabolism of cholic acid, deoxycholic acid, 5β-cholestane-3α,7α,12α-triol, and 7α-hydroxycholest-4-en-3-one was studied in homogenates of liver from rat embryos and suckling rats. The specific activities of the enzymes concerned with conjugation of bile acids were found to increase 30- to 40-fold between 15 days after fertilization and 5 days after birth. A similar increase in activity was observed for the enzymes catalyzing the oxidation of 5β-cholestane-3α,7α,12α-triol and the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one. The results suggest that the activities of the enzymes concerned with the conversion of cholesterol to bile acids increase synchronously in the developing rat liver.

The main pathways for the formation and interconversion of the bile acids (*cf.* Figure 1) have now been elucidated (Bergström *et al.*, 1960; Danielsson, 1963; Mendelsohn and Staple, 1963; Mendelsohn *et al.*, 1965; Danielsson and Einarsson, 1966). As far as is known, the liver is the sole source of these acids. The pattern of accumulation in embryological development of these enzymes has not been defined. Whitehouse *et al.* (1962) demonstrated that the livers from 13-day-old rat embryos catalyzed the formation of labeled carbon dioxide from [26-^{14}C]cholesterol (I) and [26-^{14}C]5β-cholestane-3α,7α,12α-triol (V). Thus at least some of the activities in the pathway are present at a relatively early stage in development. In the present studies, the metabolism of 7-hydroxycholest-4-en-3-one (III), 5β-cholestane-3α,7α,12α-triol (V), cholic acid,

* From the Department of Biochemistry, University of Washington, School of Medicine, Seattle, Washington 98105. *Received September 11, 1967.* Supported by Grant HD-02126 from the U. S. Public Health Service and Grant 13X218 from the Swedish Medical Research Council.

† Permanent address: Department of Chemistry, Karolinska Institutet, Stockholm, Sweden.

and deoxycholic acid[1] was examined in homogenates of liver from rat embryos and suckling rats. The developmental profile of all substrates tested was similar; a prominent increase in activity was observed between 15 days after fertilization and 5 days after birth.

Materials

[24-^{14}C]Deoxycholic acid (3 μg/mg) was synthesized as described by Bergström *et al.* (1953). Tritium-labeled deoxycholic and cholic acids were prepared by exposure of 1 g of each compound to 2 c of tritium gas (Radiochemical Centre, Amersham, England) for 4 weeks at room temperature as described by Wilzbach (1957), and Bergström and Lindstedt (1957). The material was then treated with 1 M KOH in 50% aqueous ethanol for 24 hr at room temperature. After acidification of the alkaline solution, the bile acid was extracted with ether and the ether extract was washed with water until neutral. The specific activity of the crude cholic acid was about 1 mc/mg and for crude deoxycholic acid about 0.4 mc/mg. Parts of the crude tritium-labeled bile acids were diluted with unlabeled material and were purified by repeated reversed-phase partition chromatography (Norman and Sjövall, 1958) using phase system C1 for cholic acid, and phase system F1 for deoxycholic acid. The chromatographically pure deoxycholic and cholic acids had specific activities of 0.2 and 0.3 mc/mg, respectively.

Tritium-labeled 7α-hydroxycholest-4-en-3-one (24 μc/mg) was synthesized from tritium-labeled chenodeoxycholic acid and unlabeled isovaleric acid as described recently (Björkhem *et al.*, 1965). Tritium-labeled 5β-cholestane-3α,7α,12α-triol was synthesized from tritium-labeled cholic acid and unlabeled isovaleric acid (Berséus *et al.*, 1965). The material used had a specific activity of 0.3 mc/mg. ATP, coenzyme, NADPH, and taurine were purchased from the Sigma Chemical Co., St. Louis, Mo.

Methods

Preparation of Homogenates. Rats of the Sprague–Dawley strain were employed in these experiments. The livers were excised rapidly, washed in a modified Bucher medium (Bergström and Gloor, 1955) (pH 7.4), blotted dry on a piece of filter paper, and weighed. For all substrates except 7α-hydroxycholest-4-en-3-one, 10% homogenates (liver wet weight per volume) were prepared in the same medium with a Potter-Elvehjem homogenizer equipped with a loosely fitting pestle. The homogenate was centrifuged for 10 min at 1000*g*,

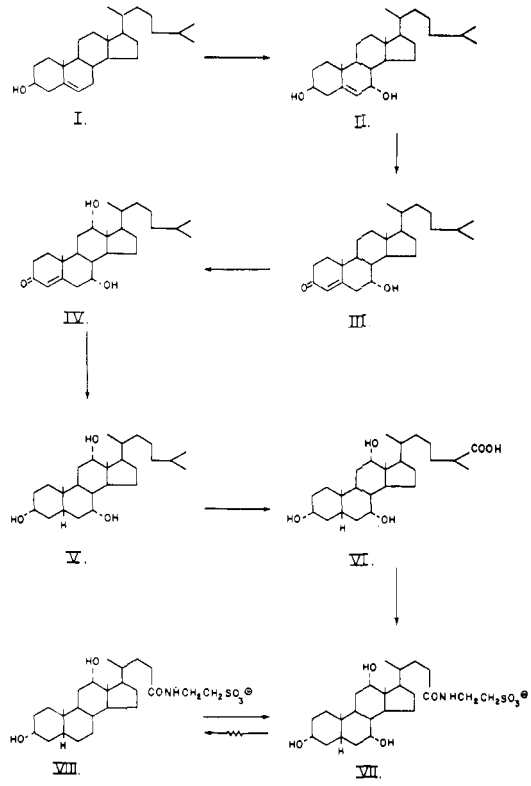

FIGURE 1: Some reactions in the biosynthesis of taurocholic acid. (I) Cholesterol; (II) cholest-5-en-3β,7α-diol; (III) 7α-hydroxycholest-4-en-3-one; (IV) 7α,12-dihydroxycholest-4-en-3-one; (V) 5α-cholestane-3α,7α,12α-triol; (VI) 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; (VII) taurocholic acid; (VIII) taurodeoxycholic acid. (→) Reaction catalyzed by liver enzymes; (←∿∿—) reaction catalyzed by microbial enzymes.

and the supernatant fluid obtained was used as source of enzyme. In the experiments with 7α-hydroxycholest-4-en-3-one, 20% homogenates, prepared as above, were centrifuged for 15 min at 20,000*g*; the supernatant fluid was then centrifuged for 2 hr at 150,000*g* to obtain the microsomal fraction which was suspended in a volume of homogenizing medium equal to that of the 20,000*g* supernatant fluid.

Incubation Conditions. For measurements of conjugation with taurine, 20–320 μg of cholic or deoxycholic acids (20 μg of labeled material and the remainder unlabeled material) was added to the incubation tubes in ethanol solution. The ethanol was then removed by evaporation. The 1000*g* supernatant fluid (1 ml) was added, followed by 0.1 ml of the homogenizing medium containing 3 μmoles of ATP, 0.5 μmole of coenzyme A, and 1 μmole of NADPH, and when appropriate, 1 μmole of taurine. The incubations, unless otherwise stated, were conducted for 2 hr at 37°.

Tritium-labeled 5β-cholestane-3α,7α,12α-triol (5 μg) and 5 μl of ethanol were added to 1 ml of the enzyme solution prepared by diluting 0.2 ml of 10% homogenate (embryonic liver), or 0.1 ml of a 10% homogenate

[1] The following systematic names are given bile acids referred to in this report by trivial names: cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid. Abbreviations used: ATP, adenosine triphosphatase; NADPH, reduced nicotinamide–adenine dinucleotide phosphate; TPNH, reduced triphosphopyridine nucleotide.

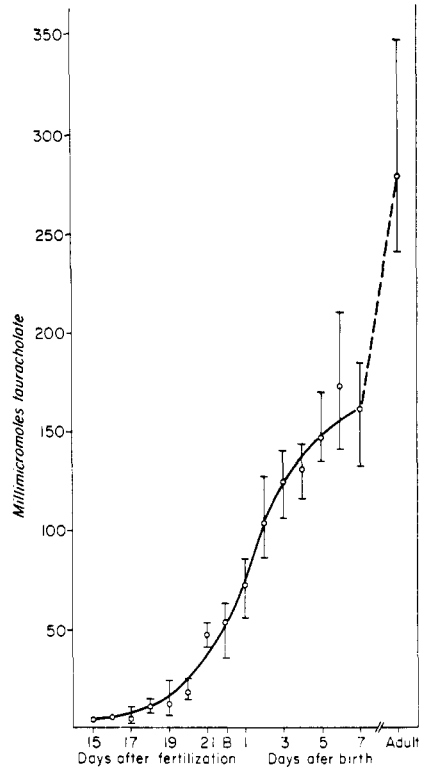

FIGURE 2: Conjugation of cholic acid in homogenates of liver from rat embryos and suckling rats. Ranges represent determinations with homogenates from three to five animals.

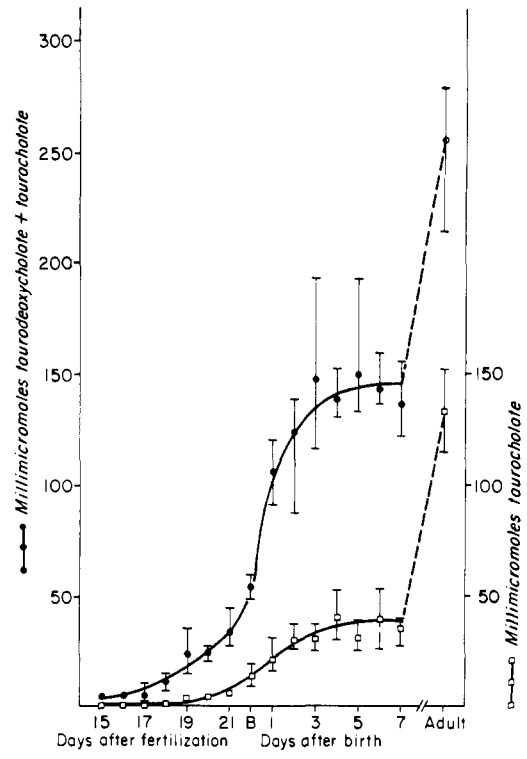

FIGURE 3: Conjugation and hydroxylation of deoxycholic acid in the developing rat liver. Ranges represent determinations with homogenates from three to five animals.

(postnatal liver) with 0.8 and 0.9 ml of the homogenizing medium, respectively. ATP (3 μmoles), 0.5 μmole of coenzyme A, and 1 μmole of NADPH, dissolved in 0.1 ml of homogenizing medium, were then added. The incubations were carried out for 30 min at 37°.

Tritium-labeled 7α-hydroxycholest-4-en-3-one (10 μg), dissolved in 20 μl of acetone, was added to a 1-ml aliquot containing the microsomes from 1 ml of 20,000*g* supernatant fluid obtained from a 20% homogenate as described earlier. NADPH (1 μmole) in 0.1 ml of homogenizing medium was added, and the mixture was incubated for 30 min at 37°.

Analysis of Incubations. Incubations were stopped by addition of five volumes of ethanol. The precipitate was removed by centrifugation, and 50–500-μl aliquots of the supernatant, together with appropriate reference compounds, were subjected to thin-layer chromatography on Kieselgel G (Merck, Darmstadt, Germany). The following solvent systems were employed: toluene–acetic acid–water (10:10:1), for analyses of incubations with bile acids (Gänshirt *et al.*, 1960); acetone–ethyl acetate–isovaleric acid (50:50:0.4), for 5β-cholestane-3α,7α,12α-triol; and benzene–ethyl acetate (3:7), for 7α-hydroxycholest-4-en-3-one (Danielsson and Einarsson, 1966). The reference compounds were located by exposing the chromatoplate to iodine vapor at room temperature. The iodine was then evaporated at room temperature or by heating the plate to about 70°. To test whether various labeled compounds in the extracts of the incubation mixtures had the same chromatographic properties as the reference compounds, the chromatograms were frequently run with internal standards. For analysis of incubations with 7α-hydroxycholest-4-en-3-one, the chromatograms were always run with internal standards. The appropriate zones of the chromatograms were scraped off the chromatoplate with a razor blade and transferred into counting vials. Kinard's (1957) scintillation solution (10 ml) was added, and the samples were counted in a Packard TriCarb scintillation spectrometer. The conversion of the different substrates was calculated from the amount of radioactivity in the appropriate zones of the chromatograms. Taurocholic acid was further identified as the major metabolite formed from labeled deoxycholic acid by the following procedure. One extract from each of the sixteen different incubations (in these cases with [24-^{14}C]deoxycholic acid) was hydrolyzed with 1 M KOH in 50% aqueous ethanol in a closed steel tube for 12 hr at 110°. The saponification mixture was acidified and extracted twice with ether. The combined ether extracts were washed with water until neutral and the solvent was evaporated. The residue together with added unlabeled cholic and deoxycholic acids was chromatographed on a column of 4.5 g of hydrophobic Hyflo Super-Cel using phase

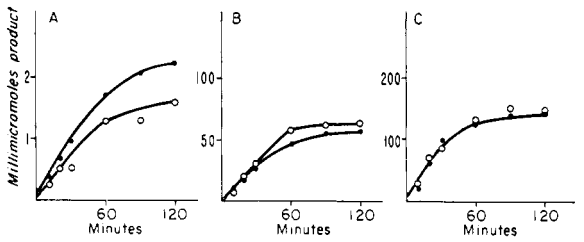

FIGURE 4: Time course of conversion of cholic acid into taurocholic acid (○–○–○) and of deoxycholic acid into taurodeoxycholic and taruocholic acids (●–●–●). (A) Homogenate from 16-day-old embryos; (B) homogenate from newborn rat; (C) homogenate from 5-day-old rat.

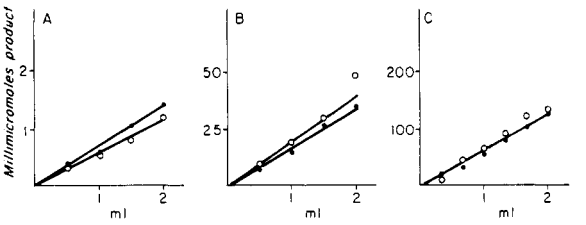

FIGURE 5: Effect of enzyme concentration on conjugation of cholic acid (○–○–○) and deoxycholic acid (●–●–●). Incubations were carried out for 20 min with indicated amounts of homogenate from 16-day-old embryos (A), newborn rat (B), and 5-day-old rat (C).

system F1 (Norman and Sjövall, 1958), which gives complete resolution of these acids. The fractions containing cholic acid were combined and the solvent was evaporated. Sufficient unlabeled cholic acid was added to the residue to bring the total amount of cholic acid to 25 mg. The mixture was then crystallized three times from methanol–water, ethyl acetate, and methanol–water. Constant specific radioactivity was reached after one crystallization. In each case at least 90% radioactive material was accounted for as cholic acid.

Results

Metabolism of Cholic and Deoxycholic Acids. Figure 2 records the relative rates of conversion of cholic acid to taurocholic acid by extracts of liver of various ages. In all of these experiments, taurocholic acid accounted for at least 90% of cholic acid metabolized (see Methods). As judged from its chromatographic properties, glycocholic acid was the other main metabolite, but the accumulation of this compound was not quantitatively measured. The addition of taurine was required for maximum conjugation of cholic acid only with adult liver homogenates; in all other cases, inclusion of this compound in the incubation medium did not influence the extent of conjugation by the extracts.

As shown in Figure 2, there was an approximate 40-fold increase in specific conjugation activity between 15 days after fertilization and 7 days after birth. The largest increment (four-fold) occurred between 20 days after fertilization and 2 days after birth. A further increase in specific activity occurred sometime between 7 and 10 days of age and adulthood. As shown in Figure 3, a similar developmental pattern for the conjugation of deoxycholic acid was found. In these experiments, both the conjugation of deoxycholate and its hydroxylation were determined. The latter reaction could be detected in livers older than 20 days after fertilization. As detailed in Methods, taurocholic acid formation from deoxycholic acid was measured not only by chromatographic means, but also by isotope dilution involving crystallization to constant specific radioactivity.

As shown in Figure 4, the conjugation reaction with cholic acid and deoxycholic acids was not linear during the 2-hr incubations employed in these experiments; thus, the validity of the assay for making quantitative comparisons could be questioned. However, the kinetic profile of the conjugation reaction obtained from liver homogenates from 16-day-old embryos, from newborn, and 5-day-old rats was of the same form. Furthermore, as shown in Figure 5, a linear relationship exists between the extent of conjugation of the acids and the amount of liver extract present in the reaction mixture. Under these conditions, a valid assay of conjugation was achieved. The values obtained in the 2-hr incubations (Figures 2 and 3) corresponded to a reaction time of about 1 hr at the initial reaction rate. The specific activity determined under initial reaction rate conditions for taurocholic formation (mμmoles of taurocholate formed/min per 100 mg of tissue wet weight) are 0.03, 1.0, and 2.8 for the livers of 16-day-old embryos, newborn, and 5-day-old rats, respectively. From these studies it is concluded that the developmental pattern shown in Figures 2 and 3 reflects the enzymatic capability to convert cholic and deoxycholic acids into taurocholic and taurodeoxycholic acids.

Metabolism of 5β-Cholestane-3α,7α,12α-triol. The first step in the degradation of the C_{27} side chain is a hydroxylation at position C-26. The ability of liver extracts from animals of various ages to metabolize 5β-cholestane-3α,7α,12α-triol, the substrate for the 26-hydroxylase, is summarized in Figure 6. The main products formed during incubation had chromatographic properties identical with those of 5β-cholestane-3α,7α,12α,26-tetrol, the product of the 26-hydroxylase, and 3α,7α,12α-trihydroxy-5β-cholestane-26-oic acid, one of its further metabolites. These two compounds accumulated in about equal quantities and together accounted for 70–80% of the substrate which disappeared during the reaction. The remaining metabolites were more polar than these compounds and included cholic acid. Essentially the same quantitative relationship among the metabolites existed during all stages of development, thus it can be presumed that the relative activities of the responsible enzymes remained essentially constant during this period.

As seen from Figure 6, the temporal pattern of

TABLE I: Metabolism of 7α-Hydroxycholest-4-en-3-one in the Microsomal Fraction Fortified by Addition of 1 μmole of NADPH.

Age	Amt of Substrate Metabolized (%)	7α,12α-Dihydroxycholest-4-en-3-one	
		% of Substrate (init)	% of Metabolites Formed
17 days after fertilization	4.4	0.5	11
18 days after fertilization	6.6	0.6	9
19 days after fertilization	2.9	0.5	17
21 days after fertilization	6.7	3.9	55
Newborn	10.4	5.2	50
1 day after birth	11.4	6.1	54
2 days after birth	17.0	9.5	56
3 days after birth	14.7	9.1	62
4 days after birth	25.6	15.7	61
Adult	30.3	17.5	58

development of the several enzymes catalyzing the oxidation of 5β-cholestane-3α,7α,12α-triol is similar to that observed for the conjugation of cholic and deoxycholic acids. It is difficult to ascertain, however, whether there was strict quantitative correspondence in the accumulation of these enzymes because a linear relationship at the incubation periods employed between the enzyme concentration and the extent of oxidation of 5β-cholestane-3α,7α,12α-triol was obtained only with dilute enzyme solutions obtained in a 1–2% homogenate. Good reproducibility between homogenates from different animals was obtained, but the extent of metabolism of the substrate dropped off sharply at high levels of homogenate. The values reported here, therefore, were obtained at the lowest levels of homogenate which would yield significant conversions of substrate. With this expedient, it is believed that acceptable comparative data were obtained. There was an increased specific activity of approximately 15-fold in the metabolism of this substrate during the developmental period studied and an additional 2-fold between this and the adult.

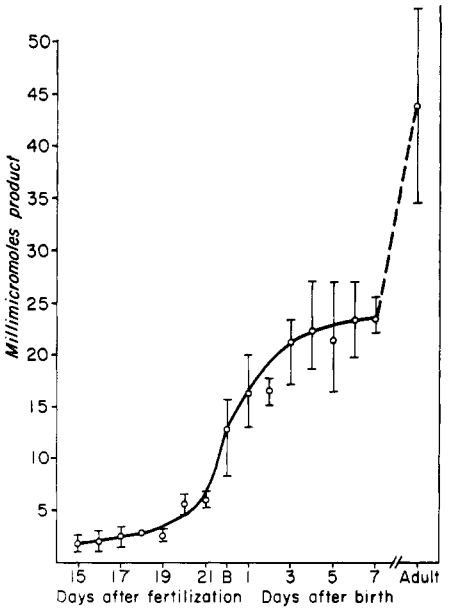

FIGURE 6: Oxidation of 5β-cholestane-3α,7α,12α-triol in the developing rat liver. The values represent total amount of products formed. Ranges represent determinations with homogenates from four animals.

Metabolism of 7α-Hydroxycholest-4-en-3-one. 7α-Hydroxycholest-4-en-3-one is the substrate for the 12α-hydroxylase, an enzyme requiring NADPH (Danielsson and Einarsson, 1966) and localized in the microsomal fraction of the liver homogenate. The disappearance of this compound and the appearance of 7α,12α-dihydroxy-cholest-4-en-3-one by microsomal fractions of liver homogenates from embryos and young rats is reported in Table I. The relative ability of the microsomal fraction to metabolize the substrate increases about sevenfold between 17 days after fertilization and 4 days after birth. During this period, the accumulation of the product of the 12α-hydroxylase increases almost 30-fold! The 7α,12α-dihydroxycholest-4-en-3-one represents about 10% of the total products present in the incubation mixtures from late prenatal and postnatal livers of the embryos. It represents more than 50% of the metabolites formed from the substrate.

Discussion

In the present paper we have attempted to define the pattern of accumulation of several enzymes involved in the formation of taurocholic acid from cholesterol. The choice of the enzymes for study depended somewhat on the availability of appropriate substrates and assay procedures as well as localization within the metabolic sequence. The conjugation of taurine with cholic acid,

the terminal reaction in the sequence, was particularly suitable since the cofactors were defined and a satisfactory quantitative assay was available. In the other cases, the high initial concentrations of the immediate substrate of a reaction was relied upon to assay a single, or at most two, enzymes. In each instance analysis of the products checked the validity of this assumption. In the case of incubations with 5β-cholestane-3α,7α,12α-triol, the initial product of the 26-hydroxylase, 5β-cholestane-3α,7α,12α,26-tetrol, and the next major exodative product, 3α,7α,12α-trihydroxy-5β-cholestane-26-oic acid, was formed in about equal amounts and accounted for about 70–80% of the 26-hydroxylase and the subsequent oxidation to the cholestan-26-oic acid was reflected in these experiments. In addition, we believe that the hydroxylation of 7α-hydroxycholest-4-en-3-one, an early intermediate in the pathway, is accurately reflected in the proportion of 7α,12α-dihydroxycholest-4-en-3-one accumulating since this product represents a substantial fraction of the total metabolites derived from this substrate even in the livers of early embryos.[2] Even though deoxycholic acid is not an intermediate in the conversion of cholesterol to cholic acid, the metabolism of this compound was studied because of its general relationship to bile acid metabolism. The reactions involved 7α-hydroxylation and conjugation with taurine which were readily measured. It seemed possible that one or both of these enzymes might be induced by deoxycholic acid. This compound is formed from cholic acid by the action of intestinal microorganisms during the enterohepatic circulation of bile (Bergström *et al.*, 1960); therefore, the appropriate enzymes for its metabolism might not arise until the establishment of an appropriate intestinal flora; that is, after birth. The present experiments, however, indicate significant 7-hydroxylation from 20-day embryos with subsequent increases more or less following conjugation activity. Thus, it cannot have been "induced" by deoxycholic acid formed from cholic acid by the intestinal microorganisms. It seems also unlikely that the enzyme could be induced by deoxycholic acid present in maternal blood (Grunday and Sjövall, 1961) since the embryonic liver would presumably be subject to this stimulus at much earlier periods in development. The fact that the developmental pattern of the 7α-hydroxylase resembles that from the other enzymes suggests that it is regulated by a similar mechanism.

One of the main features of the results is the similarity in the developmental profile of the systems measured. Prior to 19 days after fertilization, embryonic livers exhibit a low and rather constant activity; subsequently, there is a sharp increase in specific activity and a new steady-state level of activity is reached about 3 days after birth and maintained until the animals are at least 1-week old. The specific activities also are higher in the livers of adults compared to those of early postnatal animals. The general similarity in the developmental profile of the enzymes suggests a common coordinating mechanism. However, the activities of some of the enzymes at least do not appear to change in constant proportion. In Figure 3, for example, the profiles of the 7α-hydroxylase and the conjugation enzyme are not congruent. In the early developmental period, the augmentation of the activity of the conjugation enzyme appears greater than that of the 7α-hydroxylase. Conversely, there appears a larger relative increase in the 7α-hydroxylase over the conjugating enzyme in adult livers compared to those from 1-week-old animals. The question whether there is coordinate regulation of synthesis of the proteins is beyond the scope of the present experiments. The quantitative relationships among the various enzyme activities still remain in some doubt, in part because of the difficulties encountered in measuring activities present in particulate fractions, but even if the assays were valid, they might not reflect the relative rates of synthesis of the enzymatic entities since the turnover of individual entities might differ during the developmental period.

It is of considerable interest that the accretion of the enzymes associated with bile acid formation occurs rather late in the development of liver function. Rutter and Weber (1965), for example, have shown that the transition from the synthesis of aldolase A to aldolase B is already noticeable in the livers of the 12-day embryo. The accretion of the activities of a number of the enzymes involved in gluconeogenesis occurs more or less haphazardly during the period from 14 days to birth. There is a marked change in the proportion of free and bound ribosomes between 14 and 18 days, presumably reflecting the development of "rough" endoplasmic reticulum (Hosler and Rutter, unpublished). On the other hand, a number of "microsomally bound" enzymes (*e.g.*, glucose-6-phosphatase, TPNH, and cytochrome C reductase) have developmental profiles superficially resembling those of the enzymes studied here (Dallner *et al.*, 1965). Thus, it seems possible that the accumulation of the enzyme activities studied here with other membrane-bound enzymes may be coordinated in some manner.

[2] Since 7α,12α-dihydroxycholest-4-en-3-one is an intermediate in the metabolic pathway, a change in the accumulation could theoretically come from a change in the rate of its formation or the rate of its disappearance. Thus it is, in principle, possible to account for the increased accumulation of 7α,12α-dihydroxycholest-4-en-3-one on the basis of an increased metabolism of substrate (sixfold) and a drastically decreased rate of metabolism of this compound. This possibility appears unlikely since it requires that the enzymes metabolizing the 7α,12α-dihydroxycholest-4-en-3-one are decreasing at the same time that all enzymes measured in the formation of the bile salts are increasing. A more likely possibility is that there are two pathways for the metabolism of 7α-hydroxycholest-4-en-3-one, only one of which involves the 12-hydroxylation and subsequent conversion of the bile acid products. The other pathway could be responsible for the majority of substrate metabolized in the early embryonic livers (17, 18, and 19 days) when the 7α,12α-dihydroxycholest-4-en-3-one represents only 10–15% of the total products formed. If the accumulation of the 7α,12α-dihydroxycholest-4-en-3-one quantitatively reflects the 12-hydroxylase, then there is an increase in specific activity of approximately 30-fold from 17-day embryonic livers to those obtained from 4-day-old animals. The largest increment in specific activity occurs between the 19-day-old embryo and the 3-day-old animal and, therefore, corresponds to the pattern of the other activities studied here.

Acknowledgments

The authors wish to acknowledge the contribution of Dr. Carl S. Weber in the early phase of this work. The capable technical assistance of Mr. David A. Walsh is gratefully acknowledged.

References

Bergström, S. Danielsson, H., and Samuelsson, B. (1960), *in* Lipide Metabolism, Bloch, K., Ed., New York, N. Y., Wiley, p 291.

Bergström, S., and Gloor, U. (1955), *Acta Chem. Scand. 9*, 34.

Bergström, S., and Lindstedt, S. (1957), *Acta Chem. Scand. 11*, 1275.

Bergström, S., Rottenberg, M., and Voltz, J. (1953), *Acta Chem. Scand. 7*, 481.

Berséus, O., Danielsson, H., and Kallner, A. (1965), *J. Biol. Chem. 240*, 2396.

Björkhem, I., Danielsson, H., Issidorides, C., and Kallner, A. (1965), *Acta Chem. Scand. 19*, 2151.

Dallner, G., Siekevitz, P., and Palade, G. E. (1965), *Biochem. Biophys. Res. Commun. 20*, 135, 142.

Danielsson, H. (1963), *Advan. Lipid Res. 1*, 335.

Danielsson, H., and Einarsson, K. (1966), *J. Biol. Chem. 241*, 1449.

Gänshirt, H., Koss, F. W., and Morianz, K. (1960), *Arzneimittelforsch. 10*, 943.

Grundy, S. M., and Sjövall, J. (1961), *Proc. Soc. Exptl. Biol. Med. 107*, 306.

Kinard, F. E. (1957), *Rev. Sci. Instrum. 28*, 239.

Mendelsohn, D., Mandelsohn, L., and Staple, E. (1965), *Biochim. Biophys. Acta 97*, 379.

Mendelsohn, D., and Staple, E. (1963), *Biochemistry 2*, 577.

Norman, A., and Sjövall, J. (1958), *J. Biol. Chem. 233*, 872.

Rutter, W. J., and Weber, C. S. (1965), *in* Developmental and Metabolic Control Mechanisms in Neoplasia, Ward, D. N., Ed., Baltimore, Md., Williams & Wilkins, pp 195–218.

Whitehouse, M. W., Cottrell, M. C., Briggs, T., and Staple, E. (1962), *Arch. Biochem. Biophys. 98*, 305.

Wilzbach, K. E. (1957), *J. Am. Chem. Soc. 79*, 1013.