nisms of membrane fusions has grown, it has become clearer that explanations of membrane fusions can start from different models of membrane architecture (*12*). One of the fruitful results of such model building is that ideas will be generated that can be tested experimentally, and this may help to explain the observations reported here.

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Porphyrin-Heme Pathway: Regulation by Intermediates in Bile Acid Synthesis

Abstract. *Dihydroxycoprostane and trihydroxycoprostane, intermediates in normal bile acid synthesis in the liver, enhanced the rate of porphyrin synthesis in cultured liver cells by induction of δ-aminolevulinate synthetase, the rate-limiting enzyme for this pathway. Other 5β-cholestane derivatives and cholest-5-ene derivatives were ineffective. The selectivity of the induction may indicate that the above-mentioned coprostanes have a physiological role in porphyrin synthesis.*

We have found that two normal intermediates in bile acid synthesis, dihydroxycoprostane (*1*) and trihydroxycoprostane stimulate porphyrin formation in cultured liver cells by induction of δ-aminolevulinic acid synthetase (ALAS), the rate limiting enzyme for this biosynthetic pathway. These compounds are precursors in the formation of bile acids from cholesterol (*2*), and their rate of production in man is estimated to be 200 to 400 mg/day. Their accumulation in liver cells can therefore be a potent endogenous stimulus for porphyrin overproduction in liver disease.

Dihydroxycoprostane and trihydroxycoprostane were prepared by electrolytic reduction and condensation of isovaleric acid with either chenodeoxycholic acid or cholic acid (*3*). The reaction mixture was fractionated by alumina chromatography and the final product was recrystallized from aqueous acetone (*3*). Other intermediates in bile acid synthesis such as 7α-hydroxycholesterol and 26-hydroxycholesterol were prepared as described (*4*, *5*). Related compounds were obtained from commercial sources and were either recrystallized or purified by thin-layer chromatography prior to use.

Chick embryo liver cells were grown in culture according to the method described by Granick (*6*). Briefly, livers from 14- to 15-day chick embryos were minced, trypsinized, and suspended in Earle's basic salt solution. Aliquots of 5×10^5 cells were added to glass vials, each of which contained a 16-mm cover slip. To each vial, 1 ml of Eagle's basal medium supplemented with amino acids and antibiotics was added, and the cells were grown at 37°C in 5 percent CO_2 in air for 24 hours. The medium was then changed, and additions were made to the vials. The compounds to be tested were added to the cultures in 5 μl of propylene glycol, with at least four replicate vials at each dose. In each assay, controls included 10 to 15 vials to which 5 μl of propylene glycol and 5 to 14 vials to which 20 μg of allylisopropylacetamide (AIA), a compound known to be a potent inducer of porphyrin formation, were added. After incubation for an additional 20 to 24 hours, the vials were frozen, and their contents were lyophilized. Porphyrins were extracted and quantified as described (*7*) in a spectrophotofluorimeter (Hitachi model MPF 2A), at an excitation wavelength of 400 nm and an emission wavelength of 650 nm. Coproporphyrin III was used as a standard (Fig. 1).

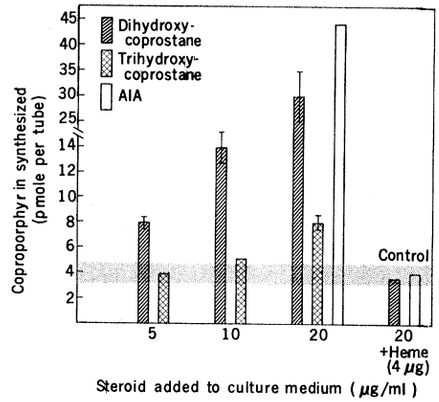

Fig. 1. Induction of porphyrin synthesis in liver cell culture by di- and trihydroxycoprostanes (see text).

Dihydroxycoprostane and trihydroxycoprostane caused significant stimulation of porphyrin synthesis (Fig. 1). The effect was dose-related and consistent throughout the dose ranges studied. Dihydroxycoprostane always induced significantly more porphyrin synthesis than trihydroxycoprostane. Further delineation of the mechanism of coprostane induction of porphyrin synthesis was obtained by studying the effect of hemin on this process (Fig. 1) and by directly determining the activity of mitochondrial ALAS in coprostane-treated 17-day-old chick embryos (*8*). Hemin completely suppressed the porphyrinogenesis induced by dihydroxycoprostane in cultures (Fig. 1), while a 20-fold increase in hepatic ALAS activity was produced in vivo in the chick embryo. These findings indicate that the coprostane compounds act on the porphyrin-heme pathway in a manner analogous to that of drugs, foreign chemicals, and neutral 5β-steroid hormone metabolites (*6*, *8*, *9*).

In contrast 7α-hydroxycholesterol and 26-hydroxycholesterol, bile acid intermediates that do not have a 5β nucleus, failed to stimulate porphyrin production. Similarly, the dihydroxy- and trihydroxy-5β-cholanoic acids and their taurine conjugates were also inactive in cell culture. Although the monohydroxy

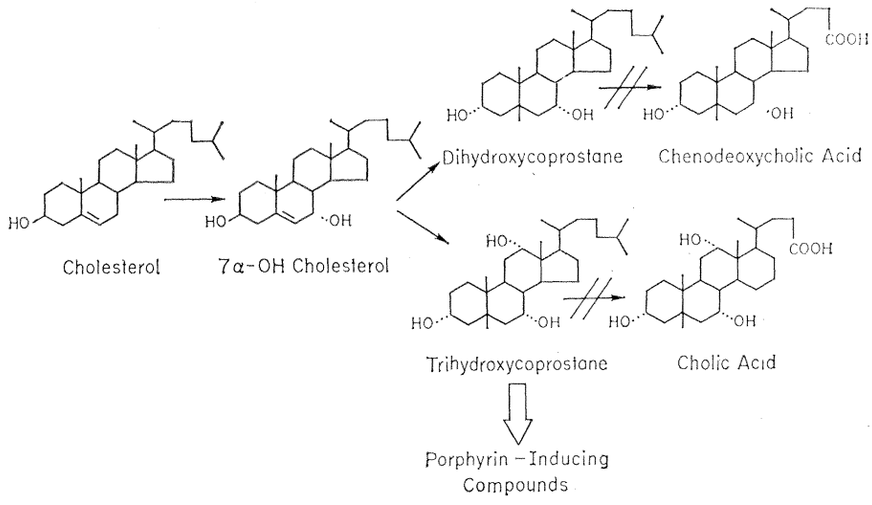

Fig. 2. Pathways for conversion of cholesterol to bile acids in man. The double bars indicate sites at which impairment of enzymatic activities would lead to accumulation of intermediates that induce hepatic porphyrin synthesis.

bile acid lithocholic acid was slightly active in high concentration, no increase in porphyrin formation was induced by 3β-hydroxy-5-cholenoic acid, a derivative of cholesterol found in rat and hamster bile (*10*) but not yet identified in human bile (*5*). Thus the stimulation of prophyrin synthesis by compounds in the metabolic pathway for bile acid synthesis from cholesterol appears confined to relatively few 5β neutral intermediates, such as the coprostane compounds described.

The rates of hepatic synthesis of dihydroxycoprostane and trihydroxycoprostane from cholesterol exceed greatly the production of 5β-steroid metablites derived from adrenal and gonadal hormones. However, in normal man both bile acid intermediates rapidly undergo side chain oxidation to form chenodeoxycholic and cholic acids (Fig. 2). The normal rate of production of intermediates can be estimated to be 0.17 μg per gram of liver per minute. This value is obtained by assuming a constant rate of bile acid synthesis of 400 mg per 24 hours per 1500 g of liver in a 70-kg man. Actually, greater rates probably occur at certain times during the day since there is evidence for a diurnal variation in bile acid production (*2*). In our studies a concentration of 5 μg of dihydroxycoprostane per milliliter of culture fluid was found to induce porphyrin synthesis. Under abnormal circumstances that cause a block in intermediate metabolism in man, this amount of coprostane could accumulate per gram of liver within a very short time.

It is speculative to extrapolate these findings with avian embryonic hepatocytes to liver disease in man. However, the phenomenon of porphyrin overproduction in acquired liver disorders such as cirrhosis (*11*) is well recognized, although unexplained. It is possible that the mechanism of this phenomenon is related to the alterations known to occur in bile acid metabolism. Thus the bile acid pool has been found to be decreased in size, attributable mostly to a reduction in cholic acid synthesis (*12*). It is also known that the predominant bile acid retained in serum in cirrhosis is chenodeoxycholic acid (*13*). The mechanism for the reduction in the bile acid pool size is not known, but is related to a change in synthesis rate rather than to an increase in fecal loss (*12*). A reduction in the rate of bile acid synthesis can occur either because less cholesterol enters the metabolic pathway, or because intermediates in bile acid synthesis accumulate as a result of a reduction in enzymatic activity necessary for primary bile acid synthesis. In some instances this enzymatic block may occur during side chain oxidation (Fig. 2) and cause increases of dihydroxycoprostane and trihydroxycoprostane in the tissue. Unless these intermediates undergo an unidentified alternate pathway of metabolism and excretion, their accumulation in liver cells could lead to induction of ALAS and overproduction of porphyrins. The close relation of porphyrin-heme biosynthesis to the cytochrome P-450–mixed function oxidase system also raises the possibility that cholesterol derivatives such as the coprostanes significantly influence this critical detoxification mechanism for drugs, carcinogens, and other chemicals in the liver.

Table 1. Metabolites of cholesterol found to have no effect on porphyrin synthesis in liver cell culture. Each compound was added to culture tubes in four different concentrations: 1, 5, 10, and 20 μg. Only the value for the highest concentration is given. Numbers in parentheses show the number of tubes to which compound was added at each concentration.

Compound	Coproporphyrin (pmole/vial)
Control	4.01 ± 0.22 (13)
5β-Cholestane derivatives	
Lithocholic acid	5.42 ± 0.43 (5)*
Deoxycholic acid	4.37 ± 0.23 (4)
Chenodeoxycholic acid	3.88 ± 0.32 (5)
Taurochenodeoxycholic acid	4.32 ± 0.46 (4)
Cholic acid	3.93 ± 0.30 (5)
Cholest-5-ene derivatives	
7α-Hydroxycholesterol	3.96 ± 0.36 (4)
26-Hydroxycholesterol	3.56 ± 0.46 (5)
3β-Hydroxy-5-cholenoic acid	4.36 ± 0.50 (5)
3β-Hydroxy-5-cholenyl taurine	3.92 ± 0.10 (4)

* A statistically significant increase ($P < .05$) was found only in tubes containing the highest concentrations.

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References and Notes

1. Systematic names for the compousds used in this study are: dihydroxycoprostane, 5β-cholestane-3α,7α-diol; trihydroxycoprostane, 5β-cholestane-3α,7α,12α-triol; 26-hydroxycholesterol, cholest-5-ene-3β,26-diol; 7α-hydroxycholesterol, cholest-5-ene-3β,7α-diol; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid.
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