# The *in Vitro* Catabolism of Cholesterol. A Comparison of the Formation of 26-Hydroxycholesterol and Chenodeoxycholic Acid from Cholesterol in Rat Liver*

D. Mendelsohn and L. Mendelsohn

ABSTRACT: The conversion of cholesterol into 26-hydroxycholesterol and chenodeoxycholic acid has been studied using a rat liver preparation *in vitro*. The results show that cholesterol can be enzymatically converted into chenodeoxycholic acid by this system. However, no definite evidence for the enzymatic formation of 26-hydroxycholesterol from cholesterol was obtained. In all instances where radioactive 26-hydroxycholesterol was isolated, further purification of this compound revealed that most of the radioactivity was due to an autoxidation contaminant of cholesterol, most probably 25-hydroxycholesterol. Since the enzyme system employed in this study is able to transform cholesterol into both cholic and chenodeoxycholic acid, the fact that no enzymatic conversion of the former compound into 26-hydroxycholesterol was observed indicates that 26-hydroxycholesterol is probably not an important intermediate in bile acid genesis.

A tentative hypothesis has recently been put forward that cholic and chenodeoxycholic acids are formed from cholesterol in mammalian liver initially *via* different pathways (Danielsson, 1963). According to this scheme, hydroxylation of the cholesterol side chain in the 26 position is an early or perhaps even the first stage in the formation of chenodeoxycholic acid. This implies that 26-hydroxycholesterol[1] might lie on the cholesterol to bile acid pathway. The findings of Mitropoulos and Myant (1967) have also led these authors to suggest that 26-hydroxycholesterol could be an intermediate in the biogenesis of chenodeoxycholic acid from cholesterol. We have recently postulated a sequence of the early stages of bile acid formation which does not include 26-hydroxycholesterol as an intermediate (Mendelsohn *et al.*, 1966a). It was therefore thought necessary to investigate the *in vitro* metabolism of 26-hydroxycholesterol and chenodeoxycholic acid from cholesterol in greater detail. Our results show that whereas cholesterol can be converted into chenodeoxycholic acid by rat liver *in vitro*, the formation of 26-hydroxycholesterol could not be definitely detected. A preliminary report of this work has already appeared (Mendelsohn and Mendelsohn, 1966).

## Experimental Section

26-Hydroxycholesterol was synthesized from kryptogenin according to Sheer *et al.* (1956). The product melted at 177–178° (lit. (Sheer *et al.*, 1956) mp 177–178°) and gave a single spot when subjected to thin layer chromatography in three different solvent systems. Commercial chenodeoxycholic acid was purified as follows. The crude product was first chromatographed (isooctane–isopropyl ether–acetic acid, 50:25:25) using the preparative thin-layer technique (1-mm thick silica gel; Kieselgel DS-5, Camag, Switzerland). After elution of the main band from the gel with methanol and evaporation of the solvent, the residue was recrystallized a number of times from ethyl acetate–petroleum ether (bp 40–60°). The chenodeoxycholic acid melted at 140–142° (lit. (Fieser and Rajagopalan, 1950) mp 140–142°) and gave a single spot when chromatographed on thin-layer chromatography in three different solvent systems. Immediately prior to use the radioactive [4-$^{14}$C]cholesterol was purified by elution with benzene through a neutral alumina column (activity grade III).

*Conversion of Cholesterol into Chenodeoxycholic Acid.* Incubation conditions and extraction procedure were the same as described previously for cholic acid (Mendelsohn *et al.*, 1966b). The chenodeoxycholic acid isolated from the incubations was recrystallized to constant specific activity (Table I). After the fourth crystallization, the behavior of the product on thin-layer chromatography in three different solvent systems (benzene–isopropyl alcohol–acetic acid, 30:10:1; benzene–ethyl acetate, 2:1; isooctane–isopropyl ether–acetic acid, 50:25:25) was compared with that of authentic chenodeoxycholic acid. In all cases, the substance isolated from the incubations behaved like the reference compound and, furthermore, radioactivity was observed

* From the Department of Chemical Pathology, Witwatersrand University Medical School, Johannesburg, South Africa. *Received July 29, 1968.* Due acknowledgement is made to the Atomic Energy Board and the Council for Scientific and Industrial Research for the assistance and support enabling the research concerned to be undertaken, and for permission to publish the results of the work.

[1] Systematic names of the sterols and bile acids referred to are: 26-hydroxycholesterol, cholest-5-ene-3$\beta$,26-diol; chenodeoxycholic acid, 3$\alpha$,7$\alpha$-dihydroxy-5$\beta$-cholanoic acid; cholic acid, 3$\alpha$,7$\alpha$,12$\alpha$-trihydroxy-5$\beta$-cholanoic acid.

TABLE I: Melting Points and Specific Activities of Chenodeoxycholic Acid Isolated from the Incubation Mixtures.[a]

| Recrystallization | Wt (mg) | Mp (°C) | Sp Act. (cpm/mmole × $10^4$) |
|---|---|---|---|
| 1. Ethyl acetate–petroleum ether | 8.4 | 141–142 | 4.85 |
| 2. Ethyl acetate | 5.1 | 141–142 | 4.80 |
| 3. Ethyl acetate[b] | 8.6 | 142–143 | 2.39 |
| 4. Ethyl acetate–petroleum ether | 5.8 | 142–143 | 2.43 |

[a] See text for details. [b] The 5.1 mg of chenodeoxycholic acid from system 2 was diluted with 5 mg of nonradioactive chenodeoxycholic acid to give a total weight of 10.1 mg which was then recrystallized from ethyl acetate. [4-$^{14}$C]Cholesterol, 250,000 cpm/flask. Ten flasks were used for each incubation. A significant number of counts were not detected in chenodeoxycholic acid isolated from the control flasks.

TABLE II: Melting Points and Specific Activities of 26-Hydroxycholesterol Isolated from the Incubation Mixtures.[a]

| Recrystallization | Wt (mg) | Mp (°C) | Sp Act. (cpm/mmole × $10^4$) |
|---|---|---|---|
| C[b] | 8.2 | 173–175 | 0.60 |
| 1. Methanol–water | | | |
| E | 8.6 | 174–176 | 0.61 |
| C | 5.1 | 176–177 | 0.98 |
| 2. Ethyl acetate | | | |
| E | 5.3 | 176–177 | 1.0 |
| C | 3.4 | 176.5–177.5 | 0.89 |
| 3. Acetone–water | | | |
| E | 3.7 | 176.5–177.5 | 0.97 |

[a] See text for details. [b] C = control; E = experiment. [4-$^{14}$C]Cholesterol, 50,000 cpm/flask. Ten flasks were used for each incubation.

only in the area occupied by the material and nowhere else on the chromatogram.

*Conversion of Cholesterol into 26-Hydroxycholesterol.* The following were the differences in incubation conditions and extraction procedure when the incorporation of labeled cholesterol into 26-hydroxycholesterol was studied. Tween 80 (6–8 mg) (L. Light & Co. Ltd., England) was required per flask for complete emulsification of the 26-hydroxycholesterol which was added as trapping agent (250 μg/flask). At these concentrations of Tween 80 and 26-hydroxycholesterol, respectively, no inhibition of the enzyme system was observed. Each flask contained 50,000 cpm of [4-$^{14}$C]cholesterol as substrate. At the end of 1 hr, the incubations were terminated by the addition of 40 ml of ethanol to each flask. The precipitate was removed by centrifugation and washed once with an equal volume of warm ethanol, and the combined supernatant solutions were evaporated to dryness *in vacuo* at 37°. 26-Hydroxycholesterol (5 mg) was added as internal standard to the dry residue followed by 50 ml of 5% KOH in methanol. The mixture was refluxed for 30 min; after cooling, 50 ml of water was added and the solution was extracted exhaustively with petroleum ether. On evaporation of the combined petroleum extracts, the residue was dissolved in a small volume of warm ethanol and subjected to thin-layer chromatography (benzene–ethyl acetate, 2:1). The plate was run in the same solvent a number of times to effect a separation from unreacted radioactive cholesterol. After elution of the 26-hydroxycholesterol band with warm methanol–chloroform (1:1) the procedure of chromatography and elution was repeated twice, first in isooctane–isopropyl ether–acetic acid (50:25:25) and finally in benzene–ethyl acetate (2:1). 26-Hydroxycholesterol (5 mg) was then added to the dry residue of the diol band from the last chromatographic separation which was recrystallized from a number of solvents (Table II).

*Conversion of Cholesterol into 26-Hydroxycholesterol and Chenodeoxycholic Acid Using the Cell-Free Preparation Incubation and Extraction Conditions Described by Mitropoulos and Myant (1967).* On completion of the incubation and after evaporation of the total butanol extract to a low volume, the 26-hydroxycholesterol and chenodeoxycholic acid were purified, respectively, as follows.

*26-Hydroxycholesterol* (5 mg) was added as carrier to half the total butanol extract before subjecting this material to the series of chromatographic separations outlined in Table III. The following thin-layer chromatography system was employed to separate 25- and 26-hydroxycholesterol. Silica gel plates (0.25 mm thick, Kieselgel DS-5, Camag, Switzerland) were prepared in the usual manner and dried at 100° for 1 hr. The cooled plates were coated with propylene glycol by carefully immersing them in a 10% solution of the glycol in acetone for 5 min. After air drying, the diols were spotted on and the plate was subjected to multiple runs (45 min each) in benzene saturated with propylene glycol. A satisfactory separation of the diols was achieved after the third or fourth run.

*Chenodeoxycholic Acid.* The second half of the total butanol extract was plated and chromatographed in chloroform–methanol (9:1). The band at the origin was eluted off the silica gel with methanol, the eluate was evaporated to dryness, and the residue was hydrolyzed with 5 ml of 5% aqueous KOH at 15 psi for 3 hr. After cooling, 5 ml of water was added followed by a few drops of concentrated HCl to bring the pH to below 2. This solution was extracted three times with ether, the combined extracts were evaporated, 4 mg of carrier chenodeoxycholic acid was added, and the whole was subjected to the series of chromatographic separations outlined in Table IV.

TABLE III: Total Radioactivity Recovered in 26-Hydroxycholesterol Isolated from the Incubation Mixtures When Subjected to Thin-Layer Chromatography in a Number of Systems.[a]

| Solvent System | Total cpm Recov from the 26-Hydroxycholesterol Area |
|---|---|
| 1. Benzene–ethyl acetate (2:1) | 890 |
| 2. Isooctane–isopropyl alcohol–acetic acid (50:25:25) | 260 |
| 3. Benzene–ethyl acetate (2:1) | 96 |
| 4. Benzene–isopropyl ether–acetic acid (30:10:1) | 88 |
| 5. Reversed-phase thin-layer chromatography in benzene saturated with propylene glycol[b] | 3 |

[a] See text for details. [b] After running in system 5 the majority of the counts was recovered in the area corresponding to 25-hydroxycholesterol.

*Conversion of Tritium-Labeled 26-Hydroxycholesterol into Chenodeoxycholic Acid.* Tritium-labeled diol (specific activity 3 mCi/mg), prepared by the method of Wilzbach (1957) with excess labile tritium removed, was obtained through the Radiochemical Centre, Amersham, England. After dilution of the crude tritium-labeled material with unlabeled diol, it was purified by repeated thin-layer chromatography until the specific activity remained constant ($1.2 \times 10^6$ cpm/$\mu$mole). Incubation conditions and extraction procedure were the same as described above for the conversion of cholesterol into chenodeoxycholic acid, with the exception that tritium-labeled 26-hydroxycholesterol ($1.2 \times 10^5$ cpm/flask) was employed as substrate. In these experiments, Tween 80 was not used since the substrate was added to each flask in 50 $\mu$l of methanol and furthermore no trapping agent was employed. Control experiments indicated that the small amount of methanol did not inhibit the enzyme system. The crude chenodeoxycholic acid isolated from the incubations was diluted with 6 mg of carrier chenodeoxycholic acid and purified by chromatographing the material on thin layers of silica gel as outlined in Table V.

TABLE IV: Total Radioactivity Recovered in Chenodeoxycholic acid Isolated from the Incubation Mixtures When Subjected to Thin-Layer Chromatography in a Number of Systems.[a]

| Solvent System | Total cpm Recov from the Chenodeoxycholic Acid Area |
|---|---|
| 1. Isooctane–isopropyl alcohol–acetic acid (50:25:25) | 328 |
| 2. Benzene–isopropyl ether–acetic acid (30:10:1) | 140 |
| 3. Benzene–ethyl acetate (5:1) | 45 |
| 4. Ethyl acetate–isooctane–acetic acid (45:45:9) | 20 |

[a] See text for details.

TABLE V: Total Radioactivity and Specific Activity of Chenodeoxycholic Acid Isolated from the Incubation Mixtures Using Tritium-Labeled 26-Hydroxycholesterol as Substrate.[a]

| Solvent System | Total cpm Recov from the Chenodeoxycholic Acid Area |
|---|---|
| C[b] | 11,270 |
| 1. Benzene–ethyl acetate (2:1) | |
| E | 21,600 |
| C | 8,990 |
| 2. Benzene–ethyl acetate (2:1) | |
| E | 9,450 |
| C | 4,800 |
| 3. Benzene–isopropyl ether–acetic acid (30:10:1) | |
| E | 4,700 |
| C | 4,900 |
| 3. Benzene–ethyl acetate (2:1) | |
| E[c] | 4,800 |

| Recrystallization | Wt (mg) | Mp (°C) | Sp Act. (cpm/mmole $\times 10^4$) |
|---|---|---|---|
| C | 6.3 | 141–142 | 4.71 |
| 1. Ethyl acetate | | | |
| E | 5.9 | 141–142 | 4.69 |
| C | 3.1 | 141–142 | 4.70 |
| 2. Ethyl acetate–petroleum ether | | | |
| E | 2.8 | 141–142 | 4.68 |

[a] See text for details. [b] C = control; E = experiment. [c] Nonradioactive chenodeoxycholic acid (5 mg) was added to the dry residue from the last chromatographic separation and recrystallized as outlined above.

*Experiment to Show the Effect of the Extraction Procedure on the Exchange of Tritium between 26-Hydroxycholesterol and Unlabeled Chenodeoxycholic Acid.* Chenodeoxycholic acid (10 mg) and tritiated 26-hydroxycholesterol ($1.2 \times 10^6$ cpm) were dissolved in 1 ml of ethanol. After evaporation of the solvent, the residue was subjected to the extraction and purification procedure as described above for the isolation of chenodeoxy-

TABLE VI: Total Radioactivity and Specific Activity of Chenodeoxycholic Acid after Subjecting It to the Extraction Procedure in the Presence of Tritium-Labeled 26-Hydroxycholesterol.[a]

| Solvent System | Total cpm Recovd from the Chenodeoxycholic Acid Area |
|---|---|
| 1. Benzene–ethyl acetate (2:1) | $6.37 \times 10^5$ |
| 2. Benzene–isopropyl ether–acetic acid (30:10:1) | $1.37 \times 10^5$ |
| 3. Benzene–ethyl acetate (2:1)[b] | $6.04 \times 10^4$ |

| Recrystallization | Wt (mg) | Mp (°C) | Sp Act. (cpm/mmole $\times 10^5$) |
|---|---|---|---|
| 1. Ethyl acetate | 12.3 | 140–142 | 4.09 |
| 2. Ethyl acetate–petroleum ether | 8.2 | 141–142 | 4.20 |

[a] See text for details. [b] Nonradioactive chenodeoxycholic acid (18.6 mg) was added to the dry residue from the last chromatographic separation and recrystallized as outlined above.

cholic acid from the incubation solutions. The results of this experiment are presented in Table VI.

In all incubations, flasks prepared in an identical manner, except that they contained enzyme that had been boiled for 20 min, acted as controls.

After developing the plates chromatographically a small area of each plate (including the area occupied by the marker) was sprayed lightly with anisaldehyde reagent (Neher and Wettstein, 1951) in order to locate the position of the required compound. During the latter operation care was taken to protect the rest of the plate from the spray.

Radioactive tritium was assayed in a Packard Tri-Carb scintillation spectrometer while a Nuclear-Chicago gas-flow counter was used to measure radioactive carbon. Counting was continued until the error was less than $\pm 3\%$.

## Results

The results outlined in Table I demonstrate the ability of the *in vitro* system employed in this study to enzymatically convert cholesterol into the primary bile acid chenodeoxycholic acid. The percentage conversion of cholesterol into chenodeoxycholic acid varied from 0.6 to 0.75%.

Utilizing the same enzyme system a small amount of radioactivity was consistently observed in the isolated 26-hydroxycholesterol. However, the fact that within experimental error no difference was noted in the specific activity of 26-hydroxycholesterol from both control and experimental flasks suggested that one might be dealing here not with enzymatic action but rather with an autoxidation product of cholesterol (see Table II). This point will be discussed further below.

While our findings indicated that 26-hydroxycholesterol might not be formed enzymatically from cholesterol *in vitro*, those of Mitropoulos and Myant (1967) suggested the opposite. A further series of experiments were thus performed to determine whether the nature of the *in vitro* system employed could influence the results. As shown in Table III, a small quantity of radioactivity was still observed in 26-hydroxycholesterol after purification in four solvent systems when using the *in vitro* conditions described by Mitropoulos and Myant (1967). However, since one would have expected a much greater incorporation of label from cholesterol into 26-hydroxycholesterol if this reaction was enzyme assisted, the possibility of an autoxidation product similar to 26-hydroxycholesterol being responsible for the low counts in the latter compound was further pursued.

It is well known that several different steroids can be produced during the autoxidation of cholesterol. One of these is 25-hydroxycholesterol (Fieser *et al.*, 1957). Because of the chemical similarity between 26- and 25-hydroxycholesterol the latter was considered as a possible autoxidation contaminant which could be isolated from the incubation mixtures together with 26-hydroxycholesterol. Indeed, it proved rather difficult to find a suitable chromatographic system which would separate the two diols. Eventually, the reversed-phase thin-layer chromatography system described above was successfully employed to separate 25- and 26-hydroxycholesterol. It is of interest (see Table III) that in each case the majority of counts was recovered in that area of the plate corresponding to the position occupied by 25-hydroxycholesterol.

With respect to chenodeoxycholic acid and using the *in vitro* conditions described by Mitropoulos and Myant (1967) we were unable to detect significant incorporation of labeled cholesterol into this bile acid (Table IV).

In order to provide further evidence for the role of 26-hydroxycholesterol in bile acid genesis *in vitro*, experiments were performed to determine whether labeled 26-hydroxycholesterol could be converted to chenodeoxycholic acid. As can be seen from Table V, the specific activity of chenodeoxycholic acid isolated from both control and experiment showed no significant difference. This indicated that 26-hydroxycholesterol was not enzymatically converted into chenodeoxycholic acid under our experimental conditions.

However, a puzzling feature of the results presented in Table V was the high specific activity of the chenodeoxycholic acid found both in control and experiment. Since in these experiments appropriate controls had been carried out, it was therefore decided to check the effect of the extraction procedure as a possible cause of the radioactivity in the chenodeoxycholic acid. Surprisingly, after adding tritiated 26-hydroxycholesterol to chenodeoxycholic acid and subjecting the mixture to the above-mentioned extraction procedure followed by the usual purification procedure, we were able to isolate chenodeoxycholic acid with a high specific activity (Table VI).

## Discussion

Fredrickson and Ono (1956) first reported the enzymatic conversion of cholesterol to 25- and 26-hydroxycholesterol by a mouse liver mitochondrial system *in vitro*. However, when both the 25- and 26-hydroxylated derivatives of cholesterol were injected into bile fistula rats, neither was converted into cholic acid. Using a similar mitochondrial preparation from mouse liver, Danielsson (1961) confirmed 26-hydroxycholesterol as a metabolite of cholesterol but failed to demonstrate the formation of 25-hydroxycholesterol. When the latter author injected 26-hydroxycholesterol into a bile fistula rat, he found that the diol was converted predominantly into chenodeoxycholic acid. Possibly as a result of his earlier findings, Danielsson (1963) put forward the suggestion that 26-hydroxycholesterol might be an early intermediate during the conversion of cholesterol to chenodeoxycholic acid in mammalian liver. Based solely on the findings that radioactivity was present in the area corresponding to 26-hydroxycholesterol on thin-layer chromatograms, Mitropoulos and Myant (1967) considered that their results were consistent with Danielsson's postulate on the role of 26-hydroxycholesterol in bile acid genesis. These workers used an enzyme system from rat liver composed of mitochondria and a boiled supernatant fraction.

The data presented in Tables I and II indicate that whereas the *in vitro* system employed in this study was able to convert cholesterol into chenodeoxycholic acid, no definite evidence for the formation of 26-hydroxycholesterol was obtained. The fact that the specific activities of the isolated 26-hydroxycholesterol were similar in both the test and control suggested the presence of an autoxidation product of cholesterol rather than one formed enzymatically. Furthermore, if in fact 26-hydroxycholesterol was an early intermediate on the cholesterol to chenodeoxycholic acid pathway, one would have expected the isolated 26-hydroxycholesterol to have had a higher, or at least a similar, specific activity to that of chenodeoxycholic acid.

A possible explanation for the difference between our results and those of the other workers mentioned above could have been due to the fact that whereas the latter employed a mitochondrial preparation, we worked with a system derived essentially from the microsomal plus supernatant fractions of rat liver. It was therefore decided to repeat the experiment using the conditions described by Mitropoulos and Myant (1967). Under these circumstances we were unable to confirm the findings of the latter that chenodeoxycholic acid could be formed from cholesterol *in vitro* (Table IV). With regard to 26-hydroxycholesterol, a small amount of radioactivity was found accompanying this compound. Again, the low counts suggested the presence of an autoxidation product of cholesterol. Of the number of steroids that can be formed by the autoxidation of cholesterol, 25-hydroxycholesterol (Fieser *et al.*, 1957) is the one which could most reasonably be expected to accompany 26-hydroxycholesterol during its isolation from the incubation mixtures. Efforts were, therefore, directed toward achieving a chromatographic separation of the two diols. When this was accomplished, it was indeed found that most of the radioactivity in the 26-hydroxycholesterol fraction resided in that area of the chromatogram occupied by 25-hydroxycholesterol (Table III).

Finally, a series of experiments were undertaken to determine whether tritiated 26-hydroxycholesterol as substrate could be converted into chenodeoxycholic acid *in vitro*. The fact that chenodeoxycholic acid with essentially the same specific activity was isolated from both control and experiment (Table V) indicated that under our experimental conditions 26-hydroxycholesterol was not enzymatically converted into chenodeoxycholic acid. These results are in agreement with our other findings in which no enzymatic conversion of cholesterol to 26-hydroxycholesterol was found. A peculiar feature of this experiment was the rather high specific activity of the isolated chenodeoxycholic acid from both test and control flasks. Since appropriate controls had been used, it was decided to check the extraction procedure as a possible cause for the relatively large amount of radioactivity found in chenodeoxycholic acid. After adding nonradioactive chenodeoxycholic acid to tritiated 26-hydroxycholesterol and subjecting the mixture to the usual extraction and purification procedure, chenodeoxycholic acid with a high specific activity could in fact be obtained (Table VI). While no attempt was made here to ascertain the reason for this observation, the data do illustrate the need for running an appropriate control in this type of experiment. The findings presented here that an *in vitro* system composed of mitochondria plus boiled supernatant was unable to enzymatically convert cholesterol into chenodeoxycholic acid (Table IV) are in agreement with those of Fredrickson (1956). It was previously suggested (Mendelsohn *et al.*, 1966b) that both mitochondrial and microsomal enzymes were necessary for the transformation of cholesterol to primary bile acids *in vitro*. We have since obtained evidence (unpublished observations) indicating that during the preparation of our microsomal system, in all probability the mitochondria are sufficiently damaged to allow "solubilization" of certain enzymes necessary for some of the stages in bile acid biosynthesis from cholesterol.

Studies by Bergström and coworkers (1960) have shown that during the conversion of cholesterol to bile acids in mammalian liver, alterations in the steroid nucleus precede those reactions concerned with the degradation of the side chain. Once the nuclear changes are completed, probably the initial step in side-chain oxidation is the introduction of a hydroxyl group at one of the terminal atoms (Danielsson, 1960; Suld *et al.*, 1962). These results do not support the theory that 26-hydroxylation of cholesterol is an early stage in bile acid genesis. It is of interest that in a recent summary of bile acid metabolism (Bergström and Danielsson, 1967) no mention was made of 26-hydroxycholesterol as an intermediate in these series of reactions. Since the *in vitro* system employed in this study is capable of converting cholesterol into the primary bile acids cholic (Mendelsohn *et al.*, 1966b) and chenodeoxycholic acid (present studies), the fact that no enzymatic conversion

of the former compound to 26-hydroxycholesterol was observed indicates that 26-hydroxycholesterol is probably not an intermediate in bile acid metabolism.

Acknowledgment

The authors thank Dr. M. Tonkin of the Schering Corp. for the generous donation of 25-hydroxycholesterol.

References

Bergström, S., and Danielsson, H. (1967), *VIIth Intern. Congr. Biochem., Tokyo.*

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

Danielsson, H. (1960), *Acta Chem. Scand. 14*, 348.

Danielsson, H. (1961), *Arkiv Kemi 17*, 373.

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

Fieser, L. F., Huang, W. Y., and Bhattacharyya, B. K. (1957), *J. Org. Chem. 22*, 1380.

Fieser, L. F., and Rajagopalan, S. (1950), *J. Am. Chem. Soc. 72*, 5530.

Fredrickson, D. S. (1956), *J. Biol. Chem. 222*, 109.

Fredrickson, D. S., and Ono, K. (1956), *Biochim. Biophys. Acta 22*, 183.

Mendelsohn, D., and Mendelsohn, L. (1966), *S. Afr. J. Med. Sci. 31*, 121.

Mendelsohn, D., Mendelsohn, L., and Staple, E. (1966a), *Biochemistry 5*, 1286.

Mendelsohn, D., Mendelsohn, L., and Staple, E. (1966b), *Biochemistry 5*, 3194.

Mitropoulos, K. A., and Myant, N. B. (1967), *Biochem. J. 103*, 472.

Neher, R., and Wettstein, A. (1951), *Helv. Chim. Acta 34*, 2278.

Sheer, I., Thompson, M. J., and Mosettig, E. (1956), *J. Am. Chem. Soc. 78*, 4733.

Suld, H. M., Staple, E., and Gurin, S. (1962), *J. Biol. Chem. 237*, 338.

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

# Interaction of Egg Lecithin with Cholesterol in the Solid State*

J. E. Zull, Susan Greanoff,† and Hugh K. Adam

ABSTRACT: The attenuated total reflectance technique of infrared spectroscopy has been used to obtain spectra of solid films of egg lecithin–cholesterol mixtures. The presence of lecithin produces a low frequency shift of $\sim$150 $cm^{-1}$ in the $^{-}OH$ stretching absorption of cholesterol. A maximum interaction was observed at a stoichiometry of 2 sterol:1 phospholipid molecules. The interaction is not produced by other lipids containing polar groups similar to those found in lecithin.

There is considerable evidence that cholesterol–lecithin interactions may be important in certain biological and biomedical systems. In particular, it has been recognized that these two compounds are major components of the lipid fraction in cell membranes (O'Brien and Sampson, 1965; Ways and Hanahan, 1964), and speculative models of cellular membranes often include a lecithin–cholesterol complex. For example, Finean (1966), Vandenheuvel (1963), and O'Brien (1965) have all discussed a model for the myelin sheath membrane which involves van der Waals interaction between hydrocarbon portions and coulombic forces between the positively charged choline nitrogen of lecithin and the $^{-}OH$ group of cholesterol in a bimolecular leaflet structure. The ability of lecithin to solubilize cholesterol in bile has also been recognized, and this interaction may be important in understanding the processes of gall stone formation (Niederhiser *et al.*, 1966).

By studying mixed monolayers of phospholipids and cholesterol, Leathes (1925) demonstrated a "condensing effect" which was thought to be due to interaction between the sterol and the phospholipids. Chapman and Penkett (1966) have published nuclear magnetic resonance data which show that the freedom of motion of lecithin side chains is greatly reduced in the presence of cholesterol, suggesting extensive interaction of the hydrocarbon moieties. On the other hand, Shah and Schulman (1967) have concluded that cholesterol actually reduces the hydrophobic interaction of the lecithin side chains in a monolayer, producing a liquid film. Furthermore, surface potential measurements led these authors to conclude that there was no significant interaction between the polar group of lecithin and cho-

* From the Department of Biology and the Materials Science Laboratories, Case Western Reserve University, Cleveland, Ohio 44106. *Recieved June 4, 1968.* This work was supported by a grant from the Office of Saline Water (No. 14-01-0001-1285).

† Participant in the undergraduate research program at Case Western Reserve University, summer of 1967.