Water Solubility of Cholesterol

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Four different procedures showed the solubility of cholesterol in water at 30.0° to be: 2.6×10^{-8}, 2.6×10^{-8}, $2.7\text{–}2.9 \times 10^{-8}$, and 2.5×10^{-8} Gm./ml.

As PART of the authors' work on the nucleation and growth of cholesterol particles in aqueous media (1), it was necessary to determine the solubility of cholesterol in water and in 0.90% aqueous NaCl. It was indeed surprising to find that no definitive work could be found in the literature on the water solubility of this important compound. Handbooks (2, 3) give a value of about 2 mg./ml. Lange and Amundson (4) reported a value of 52 mcg./ml. based on a gravimetric determination. Gemant (5) reported a value of 1.5 mcg./ml. Our preliminary studies indicated that the value was less than 1 mcg./ml.

EXPERIMENTAL

Purification and Characterization of Cholesterol Monohydrate.—Fisher certified reagent grade cholesterol was purified by a method similar to the one described by Fieser (6). Cholesterol was dissolved in boiling glacial acetic acid, and the solution was rapidly cooled in an ice bath. The crystals separating out were filtered and washed once with acetone. This recrystallization step was repeated three times. Then the material was dried in an oven at 90°. The sample was then recrystallized eight times from a 70% alcohol–water mixture and then dried at 30° in a vacuum desiccator.

To verify that the crystals were the monohydrate phase, water loss determinations were carried out gravimetrically by heating samples at 105° for 48 hr. Also the Karl Fischer method for water determination was employed. In both studies it was found that within the precision of the results the compound was the monohydrate. A melting point determination with a Kofler hot stage microscope gave a value of 148.5° in good agreement with the literature value.

Analytical Chemical Considerations.—Several methods of analysis were considered, but only one was found suitable for this work. The fluorimetric method of Albers and Lowry was first attempted employing the Aminco-Bowman spectrofluorophotometer. However, the repeatability of the results at the low cholesterol levels was unsatisfactory. A direct U.V. method ($\lambda = 205$ mμ) was also explored. This method also proved to be unsatisfactory when the aqueous solutions were filtered through Millipore filters (Millipore Filter Corp., Bedford, Mass.). It appeared that a significant amount of U.V. absorbing impurities was extracted from the filters.

The method of analysis finally selected for this work was a modification of the one described by Brown (7). It is described under *Procedures for Solubility Determination.*

Procedures for Solubility Determination.—Cholesterol concentrations in water were determined for three different situations in which excess solids were present. Also a Coulter Counter method, independent of the chemical analysis methods, was used.

Method A.—One hundred milliliters of water (or 0.90% aqueous NaCl) and varying amounts of cholesterol (1 to 30 mg.) were placed in 125-ml. glass vials that were previously cleaned ultrasonically and dried. The vials were flame sealed and placed on the wheel of a 60 r.p.m. Ferris-wheel type constant-temperature water bath at 30.0°. At the end of 30 days the suspensions were filtered into 500-ml. round-bottom flasks through filters (Millipore) of 0.10, 0.22, and 0.45-μ pore sizes. The filtrates were then evaporated in these flasks using a Buchler flash evaporator. Then the reagent mixture of perchloric acid, acetyl chloride, and ethylene dichloride was added to the dry residue. To facilitate dissolution of the residue, the flasks and their contents were subjected to about 1 min. of ultrasonic irradiation. The solutions were then poured into test tubes with ground glass stoppers and placed in an oven at 50° for 15 min. Then absorbance readings were taken at $\lambda = 523$ mμ employing the Beckman DU spectrophotometer. Water (or saline) blank solutions were carried through all of the steps, including the 30-day equilibration in the glass vials.

Standard solutions of cholesterol in alcohol were used to construct the Beer's law curve in the following manner. One milliliter of an alcohol cholesterol solution of the desired concentration and 75 ml. of water (or saline) were mixed, and the procedure as in the aqueous case was followed exactly.

Method B.—In another type of experiment, approximately 100 mg. of the cholesterol sample was placed in 1 L. of water. The flask was magnetically stirred and kept in a water bath at 30.0°. Aliquots of 100 ml. were taken over a 2- to 3-week period. These were filtered through the 0.22-μ pore size filters and analyzed for cholesterol as described under *Method A.*

Method C.—Supersaturated solutions of cholesterol were prepared by boiling an aqueous suspension for about 10 hr. and then cooling the supernatant to 30° and by mixing concentrated alcohol solutions with large amounts of water. These were aged for various periods, filtered, and analyzed.

Method D.—Because it was desirable to have a method of solubility determination that would be completely independent of the chemical analysis methods, the use of the Coulter Counter (Coulter Electronics, Chicago, Ill.) for this purpose was explored. Different amounts of 10 mcg./ml. of cholesterol solution in 95% alcohol were added to 150 ml. of 0.90% aqueous NaCl solutions in water-jacketed beakers maintained at 30.0°. Crystallite particle counts were taken with the instrument periodically for several days. After 2 to 3 days, the counts when they occurred reached a steady value as expected from other studies (8). From the size distribution-time data, it was possible to make an upper limit estimate for the cholesterol solubility.

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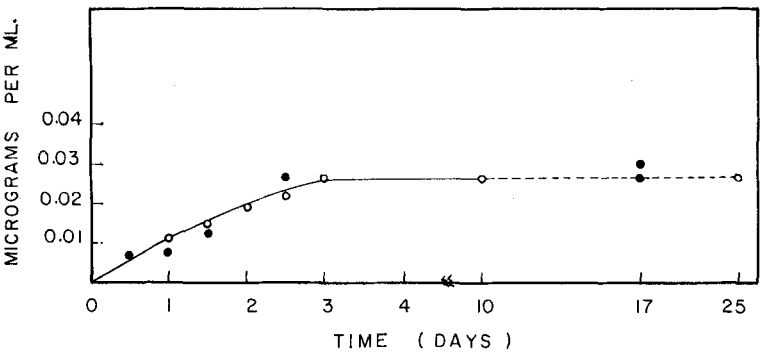

Fig. 1.—Time-dissolution plots for cholesterol in water at 30.0°C.

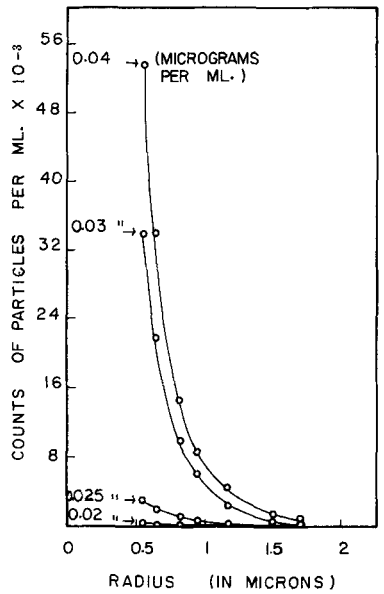

Fig. 2.—Final particle size distribution curves obtained with Coulter Counter for different initial concentrations of cholesterol in saline at 30.0°C. Data show that cholesterol solubility is about 2.5 × 10^{-8} Gm./ml.

RESULTS AND DISCUSSION

Method A.—Of nine vials containing amounts of total cholesterol varying from 1 to 30 mg., eight of these gave a solution concentration of 2.6 × 10^{-8} Gm./ml. ± 20% after the 30-day period. Of these eight, two were run in 0.90% aqueous NaCl which showed no significant effect. Seven of these eight were filtered through the 0.22-μ pore size filters, and one was filtered through a 0.10-μ size filter. Two additional vials filtered through the 0.45-μ size filters appeared to show significantly higher results (1.5 and 2 times greater). Water and saline from vials containing no cholesterol gave background readings corresponding to 0 to 0.2 × 10^{-8} and 0.3 × 10^{-8} Gm./ml., respectively.

Method B.—Figure 1 gives results of two time dependent runs. The final value of about 2.6 × 10^{-8} Gm./ml. is in good agreement with the results of *Method A*.

Method C.—This method gave values ranging from 2.7 to 2.9 × 10^{-8} Gm./ml. after 2 to 7 days of aging.

Method D.—In Fig. 2, the final particle size distribution curves for different initial cholesterol concentrations are shown. From these data one may conclude that the solubility of cholesterol is about 2.5 × 10^{-8} Gm./ml., the curve for which particle growth was just measurable. These results were reproducible and suggest that the Coulter Counter may be used to estimate solubilities of very sparingly soluble materials when the growth characteristics are known.

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