[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF DENVER]

THE SOLUBILITY OF NON-RADIATED CHOLESTEROL IN LIQUID AMMONIA

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Franklin and Kraus have pointed out the marked similarity of water and liquid ammonia as solvents, and determined the solubility in liquid ammonia—generally at atmospheric pressure (—33.35°)—of about two hundred inorganic and two hundred and fifty organic compounds.[1]

This work was a continuation of the work of Gore.[2]

The work reported was largely qualitative. Franklin and Kraus report cholesterol as insoluble in liquid ammonia.[3]

Liquid ammonia has recently been used in this Laboratory to separate the female sex hormone from cholesterol.[4]

Advantage was taken of the insolubility of cholesterol in liquid ammonia by Koch, Cahan and Gustavson in the concentration of the antirachitic factor in irradiated cholesterol.[5]

The above work has made a quantitative study of the solubility of cholesterol in liquid ammonia highly desirable.

Method of Procedure

The cholesterol used was purified by several crystallizations from acetone and melted sharply at 147°. After preparation, the cholesterol was shielded from all light rays to preclude the possibility of radiating any considerable portion of it. The reaction tubes used in the experiments were of "T" shape, very similar to the type used by Franklin in his earlier experiments. Their use and manipulation can be best shown by the description of a typical determination.

A weighed amount of cholesterol was placed in a small filter paper. The whole was rolled up in the form of a small capsule and tied with cotton thread. Both thread and filter paper were previously extracted with liquid ammonia in a closed Soxhlet system. The capsule was placed in one leg of the reaction tube and covered with 3–5 cc. of anhydrous liquid ammonia. The tube was then sealed off. The study of the temperature effect on solubility was accomplished by immersing the "T" tube in a water thermostat accurately maintained at the required temperature. The tube was frequently shaken to facilitate the dispersion of the solute throughout the solvent. Tubes were allowed to remain in the thermostat for 3,

[1] Franklin and Kraus, *Amer. Chem. J.*, **20**, 820 (1898).

[2] Gore, *Proc. Roy. Soc. (London)*, **20**, 441 (1872).

[3] Ref. 1, p. 834.

[4] Frank and Gustavson, *J. Am. Med. Assoc.*, **84**, 1715 (1925).

[5] Koch, Cahan and Gustavson, *J. Biol. Chem.*, **67**, No. 2, lii (1926).

4, 5, 6 and 7-day periods. No increase in the quantity of cholesterol dissolved was found after 72 hours, showing that equilibrium had been established. The temperature at —38° was maintained by immersing the tube in liquid ammonia contained in a Dewar flask open to the atmosphere (630 mm. barometric pressure). The cholesterol-saturated liquid ammonia was poured over into the other leg of the tube and the volume of the solution taken at 20°. The ammonia was then distilled back by carefully warming the solution and cooling the tube containing the capsule. Care had to be exercised at this point to avoid an error due to foaming. Since the quantity of cholesterol soluble in 3–5 cc. of liquid ammonia at low temperatures is small, the error introduced in weighing would be correspondingly larger.

The solubility at —38° was checked by the following method, which insured obtaining a saturated solution.

A "T" tube was prepared with a constriction in the side arm and a piece of cotton was placed in this constricted portion of the tube. A capsule containing cholesterol was placed in the other leg of the tube, covered with liquid ammonia and sealed off in the usual manner. The tube was placed in a water-bath at 45° and shaken frequently. On cooling to room temperature the liquid became turbid due to precipitation of the cholesterol. The tube was then placed in a Dewar flask containing liquid ammonia and allowed to stand for 24 hours. The ammonia with some suspended cholesterol was filtered through the cotton into the other leg of the tube. The whole tube was immersed in liquid ammonia during this part of the operation. The ammonia was then distilled back in the usual manner. No weighable residue remained. The Liebermann-Burchardt test was negative.

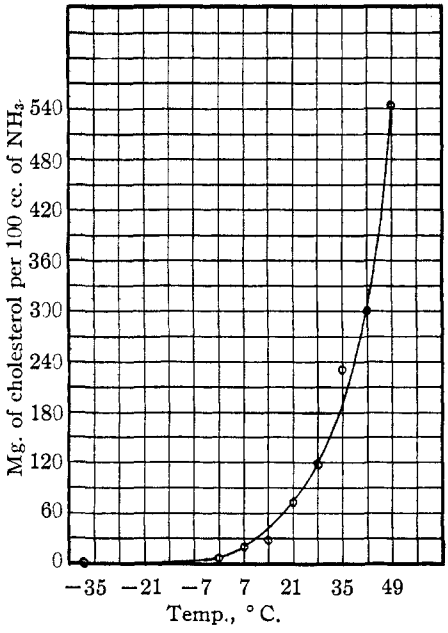

Fig. 1.

The temperature range above 49° was not investigated because of the high pressures developed in the tubes at elevated temperatures.

The results in Fig. 1 are shown in milligrams of cholesterol per 100 cc. of liquid ammonia. The results in each case are the average of four determinations. The tabulation below gives the average weight of choles-

terol extracted, the average volume of liquid ammonia and the average solubility of the cholesterol in milligrams per 100 cc. of liquid ammonia.

TABLE I

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Temperature, °C.	Av. vol. of ammonia, Cc.	Av. wt., mg.	Av. solubility, mg. per 100 cc.
—38	4.00	0.00	0.00
0	4.05	0.25	6.16
7	4.13	0.83	20.2
14	4.05	1.10	27.2
21	3.30	2.40	72.6
28	3.20	3.75	117.2
35	3.70	8.60	232.2
42	3.25	9.80	301.2
49	3.95	21.55	545.0

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Summary

1. The solubility of cholesterol in liquid ammonia at temperatures ranging from —38 to +49° has been determined.

2. The solubility at —38° is beyond the sensitiveness of the Liebermann-Burchardt test.

3. Liquid ammonia can be used to separate certain preparations from cholesterol.

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THE REARRANGEMENT OF ACID AZIDES AND HYDROXAMIC ACIDS OF GEOMETRICAL ISOMERS

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In an article by Jones and Wallis[2] an account of the rearrangements of compounds with optically active radicals was presented. It was shown that *d*-benzylmethylacetazide, dissolved in benzene, rearranged at room temperatures (10–35°) and that an optically active product, *d*-benzylmethyl methyl isocyanate, $(C_7H_7)(CH_3)CH.N{=}C{=}O$, and nitrogen (97%) were formed. These results suggested the experiments described in the following article.

[1] This paper is based upon a thesis submitted by J. Philip Mason to the Faculty of the Graduate School of Princeton University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Jones and Wallis, THIS JOURNAL, **48**, 169 (1926).