

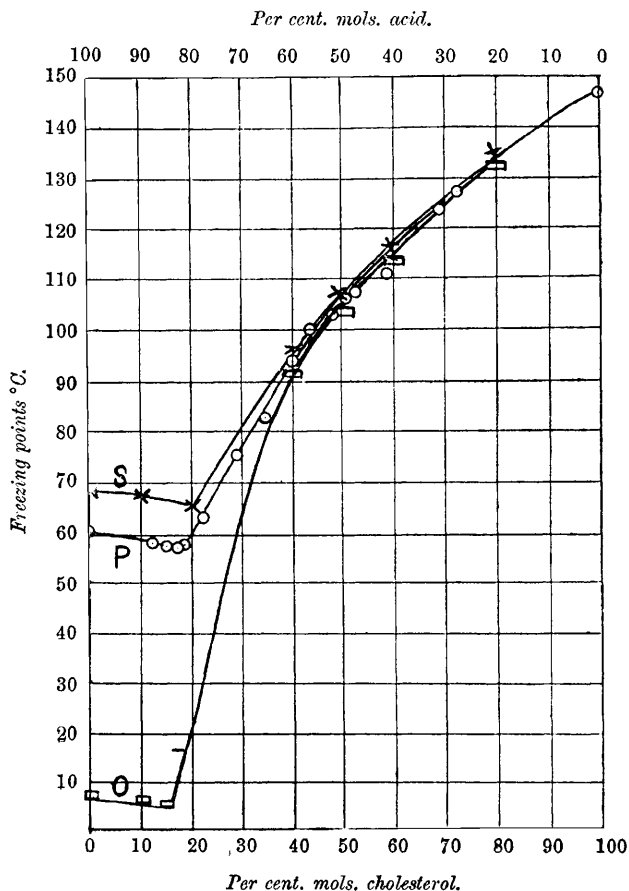
XXXIX.—*Cholesterol and Fatty Acids.*

By JAMES RIDDICK PARTINGTON.

BENEKE (*Ann. Chem. Pharm.*, 1863, **127**, 105) observed that cholesterol which had been crystallised from hot acetic acid "differs from ordinary cholesterol." Lindenmeyer (*J. pr. Chem.*, 1863, **90**, 321, 331) states that he had been informed by Dr. Zalesky that cholesterol crystallises in thin needles from acetic acid, but in plates from other solvents. He prepared crystals in this way, pressed them between filter-paper, and dried "at the ordinary temperature." They melted at 110°, and in two experiments the losses at

120° were 12·7 and 13·7 per cent. The calculated loss for $C_{26}H_{44}O, C_2H_4O_2$ was 13·9 per cent., and Lindenmeyer concluded that the substance was an equimolecular compound, the defect in the loss being put down to difficulties in removing the whole of the acetic acid.

F. C. Moore (*Med. Chron.*, 1908, p. 204) quotes the above experi-



ments as evidence for the existence of a compound of cholesterol and acetic acid, and repeated some of the tests. The evidence was :

(1) The different melting points of pure cholesterol and the "compound."

(2) The change of melting point after treating with water, alcohol, or alkalis.

(3) The change of crystalline form after complete dissolution in alcohol, and crystallisation.

Moore and C. P. White (*Med. Chron.*, 1908; *J. Path. Bact.*, 1908, 13, 5) then extended this conclusion (it appears to the author without any satisfactory experimental proof) to other fatty acids, such as palmitic and stearic; the latter author stating that "cholesterol has the remarkable character of being able to take up fatty acids to form loose combinations which are not the esters. Presumably in these combinations the acids exist as acids of crystallisation."

Since the evidence in the case of acetic acid is not conclusive, and in the cases of stearic and palmitic acids is wanting, I decided, on the suggestion of Dr. A. Lapworth, to make further experiments on the matter. The first cases investigated were the mixtures of cholesterol with palmitic, stearic, and oleic acids.

The method used was to determine the temperatures at which the first portion of solid separated from fluid mixtures of the two components. If any definite solid compound is produced at these temperatures, the curve representing temperature of separation as a function of the fractional molecular composition of the mixture will either exhibit a maximum with two minima corresponding with the so-called eutectics, or mixtures of the solid components, or else will show a sharp break in one branch of the two systems of curves descending to a single eutectic point. If, on the contrary, the substance separating is invariably one of the pure components, and, at a single point, both pure components side by side, the system will consist of two unbroken curves meeting in a eutectic point. The existence of solid solutions, that is, of homogeneous crystals containing both components in proportions which are continuous functions of the proportions existing in the fused mass, has not been suggested, and the work here described has not given any indication that this is to be inferred.

EXPERIMENTAL.

Freezing-point Experiments.

Apparatus.—The inner tube of a Beckmann freezing-point apparatus was surrounded by a wide test-tube to serve as an air-jacket. The substances were weighed directly into this tube, and fused by careful heating with a flame. The cork, through which passed a thermometer and a platinum stirrer, was then fitted on, the whole placed in the air-jacket, and the temperature at which solid began to separate was determined.

In no experiment was there any indication of a fluid crystal phase; the mixture remained perfectly clear until freezing com-

menced, when the separating substance was distinctly seen to consist of small solid crystals dispersed through the still liquid mass. No moisture was at any time evolved, and the colour, even at the high temperatures, was never darker than a pale amber-yellow, indicating an absence of decomposition.

Materials.—The cholesterol and the fatty acids were pure specimens supplied by Kahlbaum. The oleic acid, which was quite colourless, was frozen out and kept on a porous tile in the ice-chest for twelve hours. It was then allowed to melt in a vacuum desiccator over calcium chloride. The purified acid melted at 6.5°; higher melting points (14° according to Gottlieb, *Annalen*, 1846, 57, 38) have been recorded, but there is no reason to believe that the sample used was impure. The cholesterol was dried by heating in an air-oven to 110° for some time, and was kept in a desiccator. The palmitic and stearic acids were used directly.

Results.—The tables and accompanying curves show that, when liquid mixtures of cholesterol with palmitic, stearic, and oleic acids, in any proportions, are cooled, the solids separating are invariably one of the pure components, or a mechanical mixture of both pure components. Within the whole range of concentrations and temperatures investigated, there is no evidence of the existence of a solid compound of cholesterol with any one of the acids.

The experiments do not exclude the possibility that in some cases solid compounds may exist at the ordinary temperature, but no substance of this type has been detected. The existence of these, or of solid solutions, could be decided by an examination of the cooling curves over a large range of temperature. Solids of approximately constant composition may doubtless be obtained on cooling solutions of cholesterol and fatty acids saturated with both components, or by other means, but the conclusion that the masses are chemical compounds requires stringent proof, which does not appear yet to have been adduced.

TABLE I.

Palmitic Acid + Cholesterol.

Palmitic acid, per cent.	Cholesterol, per cent.	Molecules of acid, per cent.	Freezing point.
100.0	0.0	100.0	60.0°
83.3	16.7	88.2	58.5
80.0	20.0	85.9	57.5
76.9	23.1	83.3	57.0
74.0	26.0	81.1	57.25
68.9	31.1	74.0	61.1
64.5	35.5	73.2	65.0
62.0	38.0	71.1	73.25
58.8	41.2	65.4	82.5

TABLE I (continued).

Palmitic Acid + Cholesterol.

Palmitic acid, per cent.	Cholesterol, per cent.	Molecules of acid, per cent.	Freezing point.
50.0	50.0	60.0	91.0
48.5	51.5	58.5	97.0
46.1	53.9	56.2	100.0
43.8	56.2	53.9	100.5
42.3	57.7	52.5	103.0
41.5	58.5	51.5	106.0
39.1	60.9	49.2	106.5
37.9	62.1	47.9	107.0

Second Series.

50.0	107.0
40.8	111.0
31.7	124.0
27.8	127.0
0	147.0

TABLE II.

Stearic Acid + Cholesterol.

Molecules of cholesterol, per cent.	Molecules of acid, per cent.	Freezing point.
0	100	68.5°
10	90	67.0
20	80	65.0
40	60	91.4
50	50	104.7
60	40	115.0
80	20	134.0
100	0	147.0

TABLE III.

Oleic Acid + Cholesterol.

Molecules of cholesterol, per cent.	Molecules of acid, per cent.	Freezing point.
0	100	6.5°
10	90	6.0
15	85	5.0
20	80	64.0
40	60	95.9
50	50	107.1
60	40	116.4
80	20	134.0

(The mixture 15 : 85 solidified completely at 5.0°, and was therefore the eutectic.)

Cholesterol and Acetic Acid; Cholesterol and Propionic Acid.

Some experiments have been made with these systems, but the results have proved inconclusive, since the melting points of the supposed "compounds" corresponded with the points on the freezing curves for mixtures, yet the loss of weight on heating and the acidity were in each case approximately those required by the formulæ: $C_{27}H_{46}O, C_2H_4O_2$, $C_{27}H_{46}O, C_3H_6O_2$.

This case can be decided by an examination of the vapour-pressure curves during continual withdrawal of the volatile component, and some experiments, the results of which will shortly be communicated, are in progress to settle this point.

Colloidal Cholesterol.

During the course of the work it was observed that a solution of cholesterol in alcohol gave a colloidal solution when added in small quantities to water, with stirring. The alcohol was removed by dialysis, and the colloidal solution has been kept for three months without very much deposition of cholesterol. It was immediately precipitated by mineral acids and alkalis (but not by ammonia), and by ferric, platinic, barium, and calcium chlorides; by zinc, copper, magnesium, and ferrous sulphates; cadmium nitrate, and oxalic acid. Acetic acid and silver nitrate did not precipitate it, but increased the size of the particles. Potassium iodide rendered the solution less opalescent.

O. Porges and E. Neubauer (*Biochem. Zeitsch.*, 1908, **7**, 152) prepared a colloidal solution of cholesterol by adding a solution of cholesterol in acetone to water and dialysing. They examined in detail its precipitation by salts, and their results are in agreement with the above. They also concluded that it was an anodic suspensoid.

The solution described above is also an anodic colloid; it was found that cholesterol wandered to the anode in a cataphoresis experiment. Further, the precipitates with ferric chloride and calcium chloride, after washing with hot water, gave, on heating with dilute nitric acid, solutions which contained the cations, but no trace of anion. The precipitate with hydrochloric acid, after washing, did not contain a trace of anion, and on prolonged washing passed again into colloidal solution.

In conclusion, I desire to thank Dr. Lapworth for the interest he has displayed during the course of the work.

SCHUNCK LABORATORY, CHEMICAL DEPARTMENT,
UNIVERSITY OF MANCHESTER.
