Boyd has made a further test of Bloor's hypothesis on one of the least active of the body tissues. Determination (9) of the lipid content of the jelly of Wharton from the umbilical cord showed the actual percentages of all lipids, particularly phospholipid and free cholesterol, to be much lower than that of any other tissue of the body. The fact that there was a uniform absence of cholesterol esters led to the conclusion that the presence of large amounts of this lipid in a tissue was indicative of degeneration rather than inactivity.

According to a recent report, Ludewig and Chanutin (10) found no increase in the percentage concentration of the lipid phosphorus in the hypertrophied heart and kidney of rats. These workers claim their data do not support Bloor's hypothesis regarding the relationship between phospholipids and physiological activity. However, they did observe that the lipid phosphorus content per unit of surface area parallels the degree of hypertrophy in the heart and kidney. It may be more difficult to explain the results on heart tissue, but as Bloor specifically stated, the term physiological activity includes all cellular activity and not merely energy-producing activity. The results on the kidney would, therefore, seem to favor the hypothesis, inasmuch as increased work on the kidney increased the number of cells and proportionally the lipid phosphorus content. Since the heart responded in a manner similar to the tissue of organs rather than as muscle tissue, it would appear that the metabolic activities are as different in heart and skeletal muscle as these types of muscle differ structurally and that their responses to increased demands of work are dissimilar. There is one other point evident in this work which may not appear to be significant, but which we think is worth mentioning, especially since all lipid phosphorus values do not agree with directly determined phospholipid values. The work of Bloor and Boyd is based on the direct determination of the phospholipid fatty acids, whereas the work of Ludewig and Chanutin is based on the phospholipid calculated from lipid phosphorus, or, rather, inferred from the lipid phosphorus values.

The preponderance of evidence favors the viewpoint that as histological appearance of a tissue varies with its state of physiological activity, in like manner the lipid composition of tissue varies. When a tissue becomes active for any period of time, its phospholipid content increases and usually also its free cholesterol. Degeneration, retrogression or inactivity is found to be associated with decreasing or low values of these two lipids and in addition usually increasing or high values for cholesterol esters and neutral fat, both of which latter lipids are at low levels in active tissues.

REFERENCES

(1) Bloor, W. R., Okey, R., and Corner, G. W., J. Biol. Chem., 86:291 (1930).
(2) Bloor, W. R., J. Biol. Chem., 80:443 (1928).
(3) Bloor, W. R., Snider, R. H., J. Biol. Chem., 107:459 (1934).
(4) Boyd, E. M., Surg. Gynecol. and Obstet., 59:744 (1934).
(5) Boyd, E. M., Canadian Med. Assoc. J., 31:626 (1934).
(6) Boyd, E. M., Surg. Gynecol. and Obstet., 60:205 (1935).
(7) Boyd, E. M., J. Biol. Chem., 108:607 (1935).
(8) Boyd, E. M., J. Biol. Chem., 112:591 (1936).
(9) Boyd, E. M., J. Biol. Chem., 111:667 (1935).
(10) Ludewig, S., and Chanutin, A., J. Biol. Chem., 115:327 (1936).

VARIATIONS OF CERTAIN CHEMICAL AND PHYSICAL PROPERTIES OF BUTTER FAT AS REVEALED BY MELTING TIME

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ALTHOUGH the physical and chemical properties of butter fat have been studied by many investigators, the relationships which exist between the properties themselves have received little attention. The iodine number, Reichert-Meissl number, saponification number, hardness index, and melting point are the most important constants usually determined in studying the characteristics of butter fat.

The efforts which have been made to correlate the various constants have not produced uniformly successful results. Hunziker, Mills, and Spitzer (5), while studying the influence of ration on the composition of butter fat, found no regular relationship existing between hardness index and melting point or between iodine number and melting point. Two groups of investigators, however, did find correlation between certain of the properties. Haglund, Wode, and Olsson (4), found that the hardness of butter is inversely proportional to the iodine number of butter fat, while Coulter and Hill (2), determining the hardness index of butter fat rather than that of butter, pointed out a somewhat similar curvilinear relationship existing between the hardness index of butter fat and the iodine number of butter fat.

The constants which have been studied in this laboratory are: melting time, melting point, hardness index, and iodine number. The melting time determination measures the time required for a 25 cc. sample of butter fat, initially at 0° C., to melt completely when placed in a constant-temperature bath held at 45° C., the procedure was devised by Gallup for the purpose of studying the "standing up" qualities of butter fat, but also has proved of value in showing the relationship between all four of the constants studied.

Procedure For the Determination of Melting Time

25 cc. samples of butter fat in 50 cc. lipped tubes* are brought to 65° C. and held at this temperature for 10 minutes in order to expel air. The tubes are then immediately immersed in ice water at a level ⅛ inch below the level of the samples and held upright as the bath is agitated. When the fat is cooled in this way it usually solidifies without occlusion of air and without forming a small crater in the center. The tubes are packed in ice and kept at 0° C. for at least six hours before the contents are tested. At the time of testing the tubes are

attached to the lead plate, as shown in the diagram of apparatus, placed in the constant-temperature bath, which is held at 45° C. and the initial time recorded. Small glass rods are inserted through the stoppers and allowed to rest on the solid plugs of fat until liquid layers form above the solid fat; the rods are then removed. The time required for the samples to melt completely, that is, for the last trace of cloudiness to disappear, is recorded as the melting time. If the plug floats, owing to occlusion of air, the determination must be repeated because the time required for melting in this case is greater than the normal time. The plugs may be kept from floating by removing the air bubbles with a sharp splinter or needle.

A check determination is made by repeating the procedure given above on each original sample; allowable variation between the two values obtained is 30 seconds.

* Each tube is standardized by measuring the time required for a 25 cc. sample of mineral oil contained in the tube to rise from 0° C. to 40° C. when placed in the constant-temperature bath held at 45° C. Tubes uniform in this respect are selected.

Melting Time Apparatus

The apparatus is shown in Figure 1. The bath consists of an inverted bell jar (G) supported in a wooden frame (I). A constant level is maintained by the device (F) and the bath may be drained by unclamping the tube (L). The mercury-toluene thermoregulator (K) operates a relay which is connected to the heating unit (B) and may be adjusted for various temperatures by means of the screw shown. The thermometer (J), the electric stirrer (A), and the other regulatory devices shown are placed near the back of the jar, in order to leave room for the semi-circular removable lead plate (D). Four circularly grooved rubber stoppers, only two of which are shown, are forced into holes in the lead plate. At the beginning of a test, the lead plate is removed, four 50 cc. tubes (H) are placed on the stoppers, and the plate is again placed on the lip of the jar, thus immersing the tubes. The short glass rods (C), 3 mm. in diameter and flattened at

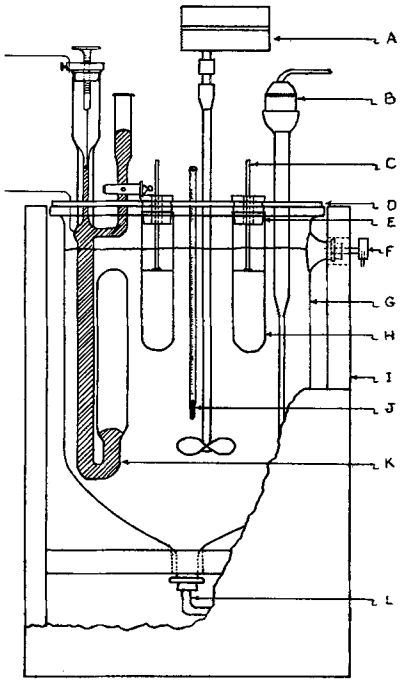

FIGURE 1

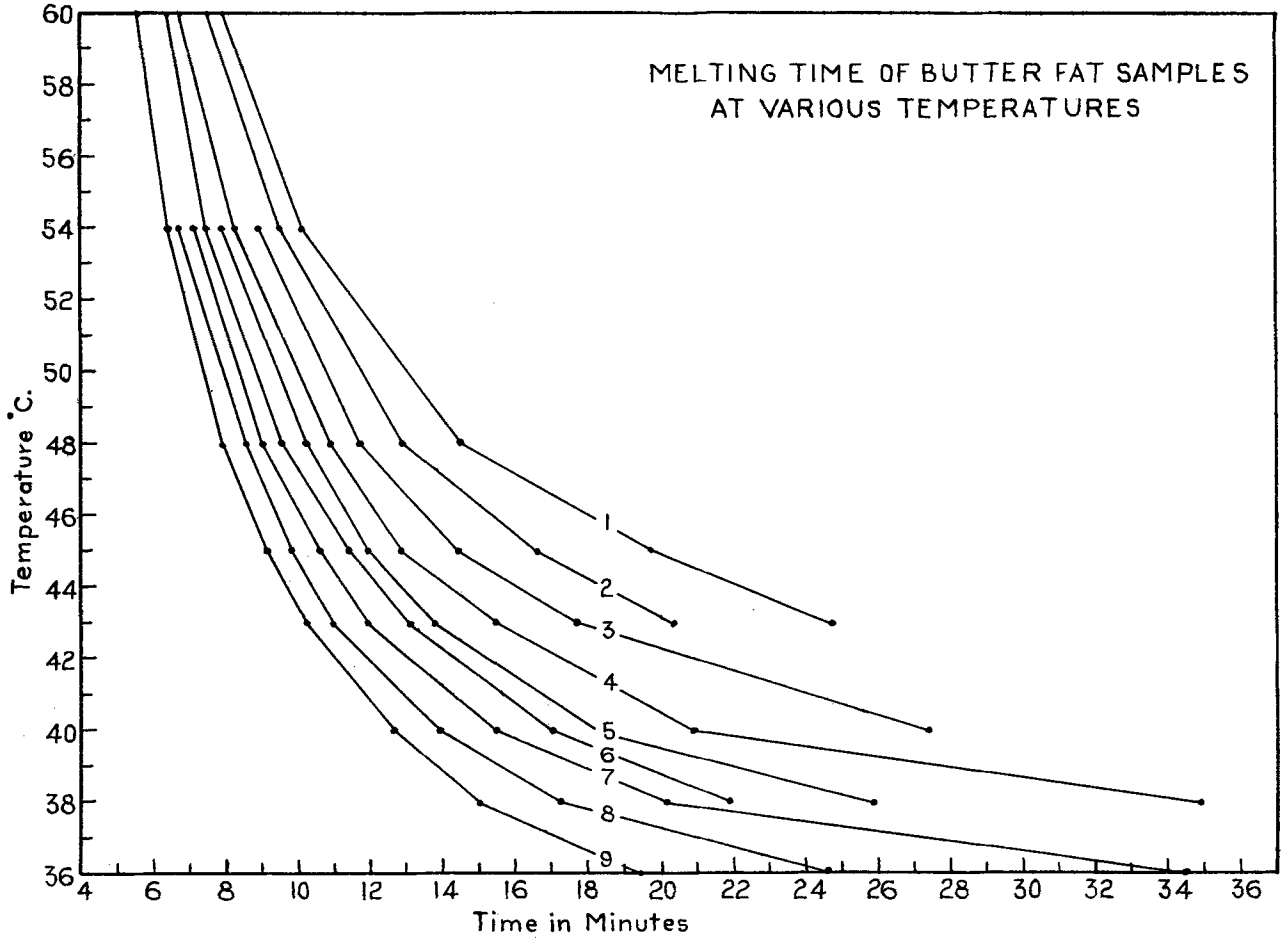

FIGURE 2

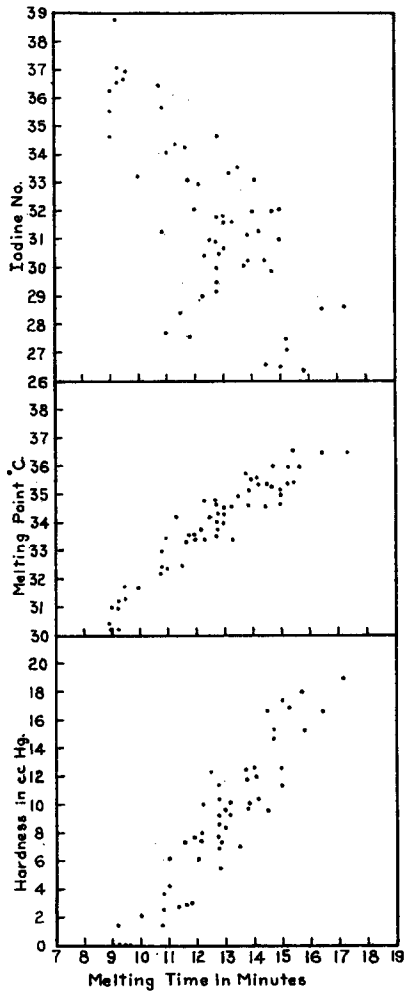

FIGURE 3

one end, are inserted through holes in the rubber stoppers.

The selection of 45° C. as the temperature at which the melting point determinations are carried out was made after comparing the results of tests in which the values of nine different fat samples were determined for each of several temperatures ranging from 36° C. to 60° C. A graphical representation of the results is shown in Figure 2. Each curve represents an individual sample; each point on the curve was located by plotting the temperature at which the bath was held against the melting time of the sample at that temperature. At temperatures above 45° C. the differences between the melting rates of the various samples were not great. Below 45° C. some samples did not form a clear transparent liquid on melting.

The index of hardness, determined by the method of Gallup (3), is expressed as the number of cc. of mercury required to force a plunger 5 mm. in diameter through a butter fat disk 6 mm. in thickness held at a temperature of 20° C. Values for duplicate samples varied as much as 10% from the mean; for this reason the average of either two or three samples was taken as the index.

Iodine number was determined by the Hanus method and melting point by the alcohol-water method; both are official A.O.A.C. methods (1).

Origin of Butter Fat Samples

The butter fat samples used were extracted from butter churned from the cream produced by individual cows in the college herd. The cows were selected from four different breeds and were fed various rations; the butter fat samples, therefore, varied widely in physical properties, due probably to differences in composition. Determinations made on 61 samples were used in comparing the four constants studied by the authors.

Discussion of Results

The graphs in Figure 3 show the results of plotting index of hardness, melting point and iodine number as ordinates against melting time as abscissa for each individual sample.

In Figure 3 hardness and melting point are each shown to be directly proportional to melting time; an inverse relationship, not as definite as the two previously mentioned, is shown to exist between iodine number and melting time. These correlations are not surprising, for the constants compared are measures of the same or similar properties. The scattered points obtained upon plotting iodine number against melting time indicate that a chemical property other than degree of unsaturation is a factor in determining the consistency of butter fat.

By use of these data, indexes of hardness and melting points of butter fat samples may be estimated from the melting time values.

Summary

1. A method for the determination of the melting time of butter fat samples has been devised.

2. Definite correlations between hardness and melting time and between melting point and melting time have been shown.

REFERENCES

1. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Fourth Ed. 1935.

2. Coulter, S. T., and Hill, O. J., J. of Dairy Sci. 17, No. 8 (1934).

3. Gallup, Willis D., Ind. Eng. Chem., Anal. Ed., 8, 123 (1936).

4. Haglund, E., Wode, G., and Olsson, T., Meddel, 387, Centralanst. Försoksv. Jordbruksomradet (Sweden). (1930).

5. Hunziker, O. F., Mills, H. C., and Spitzer, G. Purdue Agr. Expt. Sta. Bul. 159, 285-360 (1912).

SOYBEAN PHOSPHATIDES*

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Introduction

THE present investigation is concerned with the phosphatides extracted from soybeans by hot ethyl alcohol. Evidence will be given on the existence of two types of phosphatides not related to cephalin and lecithin, as well as on the nature of the extracted lecithin complex.

In a previous investigation (1) it was found that the phosphatides which separate from the expressed oil upon standing, after purification by repeated solution in ether and precipitation by acetone, still contained much less phosphorus than that calculated from the soybean lecithin formula (2).

Formerly it was believed that lecithin was the only phosphatide in plants. Later, the presence of cephalin was shown. The soybean investigations of Levene and Rolf (2) and Suzuki and Yokoyama (3) indicated that the cephalin and lecithin are quite similar to the cephalin and lecithin isolated from egg yolk. Levene and Rolf also separated another compound insoluble in alcohol and acetone from soybean phosphatides, similar to cuorin. They believed that this substance resulted from the partial hydrolysis of ceph-

*The experimental data present in this paper were taken from the thesis submitted by Robert S. McKinney to the Graduate School of American University in 1936 in the partial fulfillment of the requirements for the degree of Doctor of Philosophy.