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DEHYDRATION INDUCED INTERACTIONS OF EGG YOLK LIPOPROTEINS AND LOW MOLECULAR WEIGHT CARBOHYDRATES

by

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TABLE OF CONTENTS

INTRODUCTION

Termohlen et al. (1938) reported that the first egg drying plant began operations in the United States in 1878. Since those early days in the egg drying industry, many substances have been added to the liquid egg prior to drying in attempts to improve the shelf-life, quality, and palatability of the dried product. Some of the early additives included salt, sugar, sodium phosphate, corn starch, wheat flour, rice, farina, malt sugar, lemon juice, and glycerine. During the tremendous expansion of the egg drying industry in World War II, it was firmly established that the addition of sugars to liquid whole egg or yolk prior to drying would yield a dried product of superior functional performance and of improved flavor stability.

The rapid growth of the dehydrated convenience food market in the United States has increased the demands for functional ingredients which are capable of supplying the classical culinary functions of foaming, emulsifying, leavening, lubricating, and coagulating. These dried products must be capable of remaining on retail store shelves for long periods of time with no loss in functional performance and with little or no deterioration in sensory properties. The improved sanitary properties of dehydrated

egg products, as compared with fresh or frozen products, have led to their increased use in institutions. Here the dried egg products must be able to perform as well as their fresh or frozen equivalents. Baking technology has been advanced by the use of many formulated products, such as the newer type emulsifiers, which have superior performance characteristics over the formerly available natural ingredients. Highly specialized formulations, such as the modern cake doughnut mixes, require properties not usually associated with egg products (i.e., the ability to control viscosity in the reconstituted batter).

Dehydrated whole egg and yolk products to which sugars have been added prior to drying help meet the demands mentioned above and are found to be increasingly utilized by the baking and confectionery industries. The use of sugar-dried products has increased faster than the use of other products, either dried or frozen. Every commercial company in the egg industry today markets empirical formulations containing egg products and varying proportions and types of carbohydrates. These formulations are based on only fragmentary scientific evidence and improvement of the products is difficult without a better understanding of the processes involved. Several investigations have been conducted to elucidate the interactions between the dried egg yolk components

and the added carbohydrates, but the interactions are still incompletely understood. This study was undertaken to further investigate these biochemical interactions between sugars or corn carbohydrate derivatives and egg yolk lipids or lipoproteins in dried egg yolk.

REVIEW OF LITERATURE

Egg Yolk Composition and Structure

The yolk of an egg accounts for approximately 36% of the fresh weight of the total egg contents. However, because the yolk is about 51% solids, while the albumen is only about 12% solids, the yolk contains over 70% of the total solids in whole egg (Romanoff and Romanoff, 1949). On a dry weight basis yolk contains approximately 64% lipid and 35% protein. It also contains a small amount of carbohydrate and inorganic material. Table number 1 shows the approximate protein and lipid content of various fractions of egg yolk. It was compiled from data reported by Cook, 1961; Burley and Cook, 1961; Martin et al., 1964; Saari, 1963; Romanoff and Romanoff, 1949; and Privett et al, 1962.

Proteins and lipoproteins

At least eight different proteins and lipoproteins have been isolated from egg yolk. These proteins include phosvitin, α-, β-, and γ-livetin, α- and β-lipovitellin, and two low-density lipoproteins. Figure number 1 shows the distribution of these proteins in fresh egg yolk. This diagram was compiled from the same references as were used to compute the data in table 1.

Table 1. Approximate protein and lipid composition of egg yolk fractions

	Fresh Egg Yolk	Egg Yolk Solids	Egg Yolk Proteins	Egg Yolk Granules (Fresh Wt.)	Egg Yolk Plasma (Fresh Wt.)	Molecular Weight
Solids Content	51%	100%	100%	56%	50%	--
Moisture Content	49%	0%	0%	44%	50%	--
Protein Content	18%	36%	100%	40%	13%	--
Lipid Content	33%	64%	0%	15%	37%	--
α-Livetin	1.1%	2.2%	6%	0%	1.4%	80,000
β-Livetin	2.7%	5.3%	15%	0%	3.4%	42,000
γ-Livetin	1.6%	3.1%	9%	0%	2.0%	150,000
Phosvitin	1.9%	3.7%	10%	9%	0%	40,000
Lipovitellin	8.3%	16.1%	35%	39%	0%	450,000
α-Lipovitellin	4.1%	8.1%	17.5%	19.5%	0%	450,000
β-Lipovitellin	4.1%	8.1%	17.5%	19.5%	0%	450,000
Low-density Lipoprotein Fraction	35.5%	69%	24%	7%	43%	500,000 to 34,000,000 Avg.=4,800,000
LDF_1 [a]	8.9%	17%	5%	--	11%	500,000 to 34,000,000 Avg.=10,300,000
LDF_2 [b]	26.6%	52%	19%	--	32%	500,000 to 34,000,000 Avg.=3,300,000
Granules	21%	23%	47%	100%	0%	--
Plasma	79%	77%	53%	0%	100%	--

[a] Low-density lipoprotein fraction 1

[b] Low-density lipoprotein fraction 2

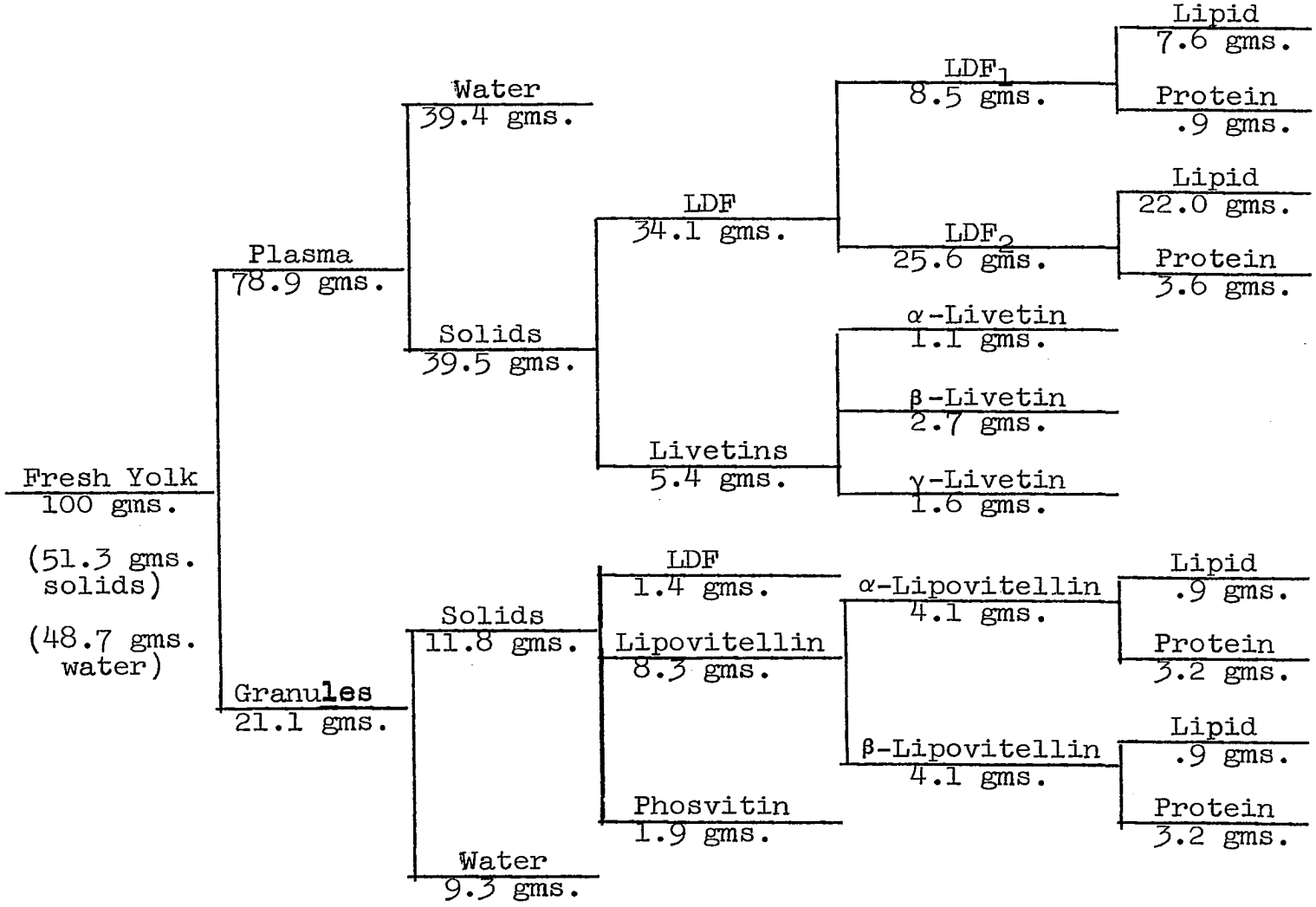


Figure 1. Distribution and amount of proteins in fresh egg yolk

In one of the earliest references to egg yolk proteins, Fourcroy in 1782 stated that "yolk is chiefly albumin with fat being the substance second in importance" (Jukes and Kay, 1932). According to Fevold (1951), Bence-Jones first demonstrated the presence of heat coagulable proteins in egg yolk in 1841 and Dumas and Calhours applied the term vitellin to these proteins in 1842. Fevold (1951) also reported that one of the first recorded attempts to fractionate egg yolk proteins was made by Lehman and Messerschmidt in 1842. By diluting egg yolk with water they precipitated a protein which could be dissolved in sodium or ammonium chloride and again precipitated by dilution with water. In 1856 Denis showed that these proteins were soluble in acid or alkali but that prolonged contact with water caused them to gradually become insoluble.

Lipovitellin In 1865 Hoppe-Seyler reported that the protein vitellin was actually a conjugate of protein and lipid (Fevold, 1951). Osborne and Campbell (1900) devised a method for isolating this lipid-protein complex and reported that the complex contained from 15 to 30% lipid which was believed to be lecithin. They prepared this lipoprotein by diluting fresh egg yolk with an equal volume of saturated sodium chloride, shaking the mixture with one-third of its volume of ether (to which a small

amount of alcohol had been added), separating the aqueous layer, and dialyzing out the salt to precipitate the lipoprotein. This precipitate was redissolved in 10% sodium chloride and reprecipitated by dialysis. It was noted that the lipoprotein was stable to ether, but that it was readily broken down and that all of its complexed lipid was extracted by contact with 80% aqueous alcohol. The isolated lipoprotein was reported to have the solubility of a globulin and the name lecithin-vitellin was suggested for it.

Using a similar procedure, Chargaff (1942) prepared a similar lipoprotein and purified it by repeated solution and precipitation from a 10% solution of sodium chloride. The resulting lipoprotein was found to contain about 18% phospholipid which was stable to ether but which could be extracted with alcohol. Chargaff renamed this lipoprotein, lipovitellin.

Alderton and Fevold (1945) described a simplified method for preparing lipovitellin which consisted of first diluting the yolk with two volumes of water and then centrifuging the mixture in a Sharples centrifuge to sediment crude lipovitellin. The sedimented lipovitellin was dissolved in 10% sodium chloride and was precipitated three times by dialysis against water. This reprecipitation reduced its phosphorus content from 2.4 to 1.3%. Cold

ether extraction of the original sediment, however, yielded a substance similar to the one obtained by Chargaff (1942). Fevold (1951) reported that vitellin is the protein portion which results from the extensive extraction of lipovitellin with 80% alcohol to remove all of its lipid. Warner (1954) stated that vitellin probably should be considered as denatured since it is soluble only in alkali solvents.

A modification of Chargaff's (1942) method yielded a product which Lea and Hawke (1951) assumed was lipovitellin, but since it contained from 25 to 35% lipid, it was probably a mixture of lipovitellin and lipovitellenin. [Lipovitellenin is a lipoprotein containing from 36 to 41% lipid and is derived from the low-density lipoproteins by ether extraction (Cook, 1961).]

This "lipovitellin", when dispersed in a 15% sodium chloride solution at pH 6.8 and extracted with diethyl ether, was found to lose about 3% of its lipid to the ether during each hour it was extracted (Lea and Hawke, 1952). Approximately 1% of the total lipid was extracted by ether in 20 minutes from a 10% suspension of the lipovitellin in water. The addition of sodium chloride to bring the molarity of the solvent to approximately 0.20 increased the amount of lipid extracted to about 22% of the total lipids. Addition of more sodium chloride again reduced the amount extracted by ether to 2 or 3%. About 30% of

the total lipid was readily extractable from dry, unstored, lyophilized lipovitellin. Upon rehydration the amount of lipid extracted with ether was again reduced to 1 to 2%.

Vandegaer et al. (1956) reported that the earlier methods of preparing lipovitellin, which had employed the use of ether, changed the solubility of the lipoprotein. As evidence of this, it was pointed out that lipovitellin is not precipitated by dilution until it is treated with ether and that ether extracted lipovitellin is more soluble in aqueous salt solutions which are saturated with ethyl ether. Consequently, to avoid this ether induced change, Vandegaer et al. (1956) diluted fresh egg yolk with 25 volumes of a 5% sodium chloride solution and examined this solution in an analytical ultracentrifuge. They found both a rising (R) and a sedimenting (S) fraction. By centrifuging a solution consisting of egg yolk diluted with water in a preparative ultracentrifuge at 105,000XG for 5 hours at room temperature, they were able to separate the R-fraction from the S-fraction but were not able to separate the R-fraction from excess "free" lipid. The S-fraction, which was obtained after removing the supernatant R-fraction, was diluted with 10% sodium chloride and was recentrifuged. The precipitate from this centrifugation was redissolved in 5% sodium chloride and was examined in an analytical ultracentrifuge. A major, faster sedimenting fraction, S_1, and a minor, slower sedimenting fraction, S_2,

were found. Upon dialysis, the S_1 fraction was found to precipitate at a sodium chloride concentration of about 1.6%, while the S_2 fraction remained in solution. The S_1 fraction had a lipid content of approximately 18%, 70% of which was lecithin, and was assumed to be lipovitellin. Analytical ultracentrifugation indicated its molecular weight to be 400,000 while light scattering techniques indicated its molecular weight to be 330,000.

In a later paper Joubert and Cook (1958a) reported that this material, which Vandegaer et al. (1956) thought to be purified lipovitellin, was actually a mixture containing lipovitellin and phosvitin. Sugano (1957) separated the proteins of egg yolk by first extracting them with ether and then subjecting them to moving boundary electrophoresis. He also reported that lipovitellin contains considerable quantities of phosvitin. He separated two lipoproteins electrophoretically and called them α- and β-lipovitellin (Sugano, 1958a). However his α-lipovitellin is probably a degradation product of lipovitellin since the α-lipovitellin contained only 6.5% lipid. What he called β-lipovitellin closely resembles lipovitellenin since it contained 38% lipid. Sugano (1958b) demonstrated that lipid, which was not extracted from lipovitellin or lipovitellenin with alcohol and ether in a 10% sodium chloride solution, could be extracted from the lipoproteins if they were dissolved

in water. In a later paper (Sugano, 1959) he reported that both his α- and β-lipovitellin appeared to be heterogeneous by analytical ultracentrifugation.

McCully et al. (1962) showed that phosvitin moves with lipovitellin during paper electrophoresis, unless the yolk is first diluted with 1.0 M sodium chloride, whereupon the phosvitin migrates as a single, fast-moving band.

Bernardi and Cook (1960a) were able to separate six different proteins, including two lipovitellins, from egg yolk by combining preparative ultracentrifugation with free boundary electrophoresis. For centrifugation the yolk was suspended in a series of sodium chloride and magnesium sulfate solutions. The two lipovitellins were named α- and β- on the basis of their electrophoretic mobility, α-lipovitellin having the higher mobility. Bernardi and Cook (1960b) fractionated α- and β-lipovitellin on hydroxyapatite columns with a pH 6.8 phosphate buffer. β-lipovitellin was eluted with 0.6 M buffer and α-lipovitellin with 2 M buffer. Both lipoproteins were reported to contain approximately 20% lipid, and to have the same nitrogen content, amino acid composition, and molecular weight (approximately 400,000). The two lipoproteins were found to differ in protein phosphorus content (α-lipovitellin contained 0.50% and β-lipovitellin

0.27% protein phosphorus), electrophoretic mobility, absorption on hydroxyapatite, ultracentrifugal behavior in alkaline solvents, and solubility. Both α- and β-lipovitellin tended to dissociate into two ultracentrifugally indistinguishable sub-units in alkaline solutions, but β-lipovitellin began to dissociate at a lower pH than α-lipovitellin (Burley and Cook, 1962). That these are the only lipoproteins known to reversibly dissociate was noted by Kratohvil et al. (1962). This association-dissociation phenomena suggested that the lipovitellins closely resemble globular proteins and have a more rigid and regular structure than would be expected from a protein-lipid micelle.

That α- and β-lipovitellin are indistinguishable on the basis of their N-terminal amino acids was reported by Neelin and Cook (1961). The major N-terminal amino acids from both, recovered as their dinitrophenyl derivatives, were arginine and lysine, thus indicating that more than one polypeptide chain must comprise the protein portion of each egg yolk lipoprotein. The amino acid composition of α- and β-vitellin was determined by Cook et al. (1962) and the two proteins were found to differ only in their histidine contents. The molecular weight was revised upward to 450,000 by Cook and Wallace (1965). With methods currently employed, Wallace (1965)

reported that the lipid content of the lipovitellins can only be established as being between 16 and 22%.

α- and β-lipovitellin were found to absorb chloroform to the extent of 13 and 22%, respectively, of their dry weights from a chloroform saturated aqueous buffer (Burley, 1963). This absorption caused aggregation of the molecules which could not be completely reversed by the removal of the chloroform. This chloroform absorption suggested that the lipovitellin structure was flexible enough to accommodate large amounts of certain substances. Phospholipase D from Clostridium perfringens was found to have little effect on untreated lipovitellin, but readily hydrolyzed a large proportion of the phospholipid in lipovitellin which had absorbed chloroform (Burley and Kushner, 1963). This was interpreted as indicating that chloroform caused a change in the location and/or orientation of the phospholipids in the lipovitellin molecules so that the enzyme could effectively attack them.

Glick (1963) demonstrated that papain would hydrolyze mixed lipovitellins into two components; one containing the lipid portion plus a small amount of the protein present in the original lipovitellin, the other containing only free amino acids and small molecular weight peptides. Therefore it appears that lipovitellin digested with papain still has some of its proteins associated with the lipid portion of the molecule.

Low-density lipoproteins The supernatant liquid remaining after precipitation of lipovitellin by the method of Alderton and Fevold (1945) was extracted with ether by Fevold and Lausten (1946). A solid phase layered between the water and the ether. It contained another lipoprotein which was quite different from lipovitellin. This lipoprotein contained from 36 to 41% lipid which, like lipovitellin, could be extracted with alcohol but not with ether. However this lipoprotein, unlike lipovitellin, was unstable to acetone. The authors named this new lipoprotein, lipovitellenin. Shephard and Hottle (1949) prepared a lipoprotein by a slightly modified precipitation method which appeared to be quite similar to lipovitellenin. It was homogeneous by moving boundary electrophoresis at pH 7.95.

Lipovitellenin was prepared by Turner and Cook (1958) by diluting fresh egg yolk with 10% sodium chloride and centrifuging at 105,000XG for 5 hours to float and compact the low-density fraction. Eighty percent of the dry weight of this transparent, yellow, compacted material was removed by prolonged ethyl ether extraction at -1° C. When the remaining material was placed in a 10% saturated solution of ammonium sulfate a portion of it sedimented, but the rest remained in solution. The insoluble portion contained 43% lipid, while the soluble portion contained

about 53% lipid. It was presumed that the difference in solubility was simply caused by more lipid being extracted from the lipoproteins of the insoluble fraction rather than having two different kinds of lipoproteins present. As evidence for the existence of only one kind of lipoprotein molecule, Smith and Turner (1958) demonstrated that the major N-terminal amino acid from both the soluble and insoluble fractions was lysine. Neelin and Cook (1961) reported that the major C-terminal amino acid of vitellenin was glutamic acid. The total amino acid content of the three lipoproteins present in yolk was determined by Cook et al. (1962). Vitellenin was found to differ statistically from the vitellins in its content of leucine, aspartic acid, lysine, arginine, proline, threonine, phenylalanine, tyrosine, methionine, and histidine. Therefore the lipovitellins cannot be converted to the low-density lipoproteins (from which vitellenin is derived), or vice versa, by a mere gain or loss of lipid.

While earlier workers reported that diethyl ether extracts "free" lipid from lipovitellenin, Turner and Cook (1958) reported that the decreased solubility and behavior of the extracted lipoprotein indicated that, in fact, stabilizing lipid was removed by the ether.

McIndoe (1959) isolated the lipoprotein without ether extraction and reported that it contained 12% protein and

88% lipid. Prolonged ether extraction removed 90% of the lipid, leaving a substance containing about 40% lipid; in all probability the lipovitellenin of Fevold and Lausten (1946).

Martin et al. (1959) prepared vitellenin, the protein portion of lipovitellenin, by extracting all of the lipid from lipovitellenin in the cold with methanol. By analytical ultracentrifugation the molecular weight of vitellenin was estimated to be 5.5×10^4, about half the estimated size of the protein portion of the initial lipid containing substances. It was thus hypothesized that the protein portion of this lipoprotein contained two or more polypeptide chains, either combined directly or through forces weaker than the peptide linkage. The micellular weight of the parent lipoprotein from which lipovitellenin was derived was estimated to be 4.8×10^6, but a great deal of polydispersity was indicated. The molecular weight of vitellenin in formic acid was determined to be 9.3×10^4 by Martin (1961).

Ultracentrifugation of yolk diluted with varying concentrations of sodium chloride was used by Sugano and Watanabe (1961) to sediment the high-density lipoproteins and to float and compact the low-density lipoproteins. The isolated low-density lipoproteins were further resolved into two fractions, L_1 and L_2 which were estimated to have

hydrated densities of 0.969 and 0.980, respectively. From viscosity measurements and ultracentrifugal floatation rates, L_1 and L_2 were calculated to have hydrated diameters averaging 310 and 250 A^{o} and molecular weights of 9.0×10^{6} and 4.8×10^{6}, respectively. The lipid content of both fractions was 84%. Both lipoproteins migrated as one zone during agar gel electrophoresis.

Phospholipase D was found to rapidly hydrolyze much phospholipid from native low-density proteins as well as from low-density lipoproteins which had absorbed chloroform (Burley and Kushner, 1963). Thus, in contrast to the lipovitellins, the low-density lipoproteins must have considerable phospholipid exposed which the enzyme can readily attack.

Martin et al. (1964) succeeded in separating the low-density lipoproteins, the parent material from which lipovitellenin was originally derived, into two polydisperse fractions. These two fractions had small but significant and consistent differences in lipid content. LDF_1 (low-density lipoprotein fraction one) contained 86.8% lipid and LDF_2 contained 83.2% lipid. The separation of the lipoproteins was accomplished through a series of flotations in water and was based on a difference in flotation rates of the lipoprotein particles. Flotation rate is determined by both the size and the partial specific volume of the particles. LDF_1 had -S values in 1 M sodium chloride

ranging from 4 to 665, micellular weights ranging from 0.5×10^6 to 34×10^6 and micellular diameters ranging from 117 to 480 A^o. Under the same conditions LDF_2 had -S values ranging from 4 to 385, micellular weights from 0.5×10^6 to 14×10^6 and micellular diameters ranging from 117 to 350 A^o. The weight average micellular weight of LDF_1 was estimated to be 10.3×10^6, while for LDF_2 it was estimated to be 3.3×10^6. The micellular weight of the protein moiety in LDF_1 and LDF_2 was computed to be about 1.4×10^6 and 0.6×10^6, respectively. Assuming a spherical shape and a film of protein 8 A^o thick, this is about enough protein to cover two-thirds of the surface of each type of lipoprotein micelle, which suggested to the authors that a certain proportion of the particle surface must be covered with protein in order to confer stability to the lipoprotein micelle.

LDF_1 and LDF_2 were found to yield derived products with similar sedimentation patterns upon extraction with ethyl ether (Augustyniak et al., 1964). These derived products are formed sequentially from one another as their lipid content decreases. The sedimentation coefficients during ether extraction are in order 7S, 4S, and 13S. These fractions contain 67%, 50%, and 44% lipid, respectively. This is explained by hypothesizing that the stable sizes of lipoprotein particles are represented

by LDF_1, LDF_2, 7S, and 4S. The 13S component is thought to be an aggregation of 4S components, which indicates that a lipid content of about 50% is the lowest percentage of lipid which can be obtained in the particle and still have it maintain a stable structure. The amount of protein in each successive particle in the series LDF_1, LDF_2, 7S, and 4S is about half that of the preceding one, suggesting that the particles successively halved as the lipid content is reduced. The molecular size of the protein portion of the 4S component was estimated to be about 1.7×10^5, approximately three times the size of the vitellenin component isolated by Martin et al. (1959). However, no evidence for the existence of these smaller sub-units in the presence of lipid was found.

Saari et al. (1964) isolated two low-density lipoproteins from egg yolk plasma, which were apparently quite similar to those isolated by Martin et al. (1964) and named them LPL_1 and LPL_2 (low-protein lipoprotein one and two). These two fractions contained 89% and 86% lipid, respectively. The electrophoretic migration rate on paper of LPL_1 was shown to be much less than that of LPL_2. The concentration of TCA-soluble nitrogen in solutions of each lipoprotein which had been treated with papain indicated that proteolysis had occurred in the solution of LPL_2 but not in the LPL_1 solution. Three percent

added sodium chloride was found to enhance the aggregation of solutions of LPL_1 and LPL_2. This was interpreted as indicating that the sodium chloride decreased the positive charges on the surfaces of the micelles and allowed aggregation to take place.

Since vitellenin demonstrates an abnormally high affinity for lipid and is insoluble in aqueous solvents, Augustyniak and Martin (1965) suspected a large number of N-acetyl groups were present in the protein. If present, these would increase the nonpolar character of vitellenin by reducing the effective number of charged amino groups. While they found some acetyl groups, theoretical calculations demonstrated that less than 10% of the potential sites were acetylated. It was reported to be unlikely that these acetyl groups exerted much influence on vitellenin's solubility.

Phosvitin A protein or peptide of high phosphorus content was extracted from vitellin with 12% ammonium hydroxide by Levene and Alsberg in 1900 (Lipman and Levene, 1932). After neutralization with acetic acid, addition of picric acid, and filtration, the phosphoprotein was precipitated by the addition of alcohol and was called vitellenic acid. When precipitated as its copper salt, it contained from 9.7 to 10.0% phosphorus which was alkali labile.

Digestion of vitellin yielded three fragments of high phosphorus content for Swigel and Posternak in 1927 (Warner, 1954). They named these three components α-, β-, and γ-ovotyrine. β-ovotyrine was found to be similar to the vitellenic acid reported by Levene and Alsberg in 1900. It was found to contain a large amount of serine, which led to the suggestion that the phosphorus containing unit of this protein was phosphoserine.

Lecithovitellin was digested with trypsin-kinase and pepsin by Blackwood and Wishart (1934). These enzymes left materials of high molecular weights which had high phosphorus contents. They stated that Bunge in 1885 had reported the presence of a similar material in a peptic digest of egg yolk which he had named "haematogen" because of the resemblance of its empirical chemical composition to hemoglobin.

Mecham and Olcott (1948) extracted a phosphoprotein, which they termed "phosvitin," from precipitated lipovitellin. The phosphorus was found to be alkali-labile, 96% being removed by 0.25 N NaOH at 35° C. in 24 hours. The phosvitin prepared by these workers was found to be homogeneous by analytical ultracentrifugation but inhomogeneous by electrophoresis. Aggregation occurred in the presence of magnesium ions. It was found to contain no cystine, cysteine, methionine, or threonine. Mecham and Olcott (1949) found

phosvitin to be resistant to peptic or tryptic digestions. It was found to be soluble in salt solutions of moderate concentration such as 0.4 M ammonium sulfate, but was precipitated by dialysis or by saturation of the solution with ammonium sulfate.

Ultracentrifugal analyses and determination of osmotic pressure, as reported by Mecham and Olcott (1949), indicated that solutions of phosvitin undergo dimerization upon the addition of 0.25 M magnesium chloride; presumably because of the formation of magnesium bridges between two phosvitin monomers. They reported phosvitin had a high degree of affinity for other proteins, probably because of its ability to form a large number of electrostatic bonds, and that it would precipitate bovine serum albumin.

Using P^{32} labeled phosvitin, Francis (1952) demonstrated that phosvitin which was added to a crude lipovitellin solution would undergo exchange reactions with phosvitin which was initially complexed with the lipovitellin.

Joubert and Cook (1958b) purified phosvitin by precipitating it and redissolving it in magnesium sulfate solutions. They found it contained 9.6% phosphorus, 12.6% nitrogen, and no lipid. It showed only a single component during electrophoresis and its titration curve showed it to be negatively charged from pH 4.0 to 9.0.

Phosvitin's low specific volume of 0.545 was attributed to its large number of acidic phosphate groups and the compensating gegenions. It had a frictional ratio indicating it was an elongated molecule with dimensions estimated to be 14 A^{o} by 280 A^{o}. Because of its high negative charge, it should interact strongly with proteins of opposite charge.

Phosvitin, the lipovitellins, and a small amount of low-density lipoprotein make up the granules of egg yolk. Burley and Cook (1961) suggested that the granules may simply be amorphous agglomerates which are insoluble at the pH and ionic strength of yolk.

Phosvitin was separated from diluted whole egg yolk by means of paper electrophoresis by McCully et al. (1959). They demonstrated that, depending upon the solvent employed, phosvitin might run as one, two, or three zones during electrophoresis. Mok et al. (1961) prepared phosvitin from both hen's serum and hen's egg yolk and found the two to be identical in all their physical measurements. Each had alanine as its major N-terminal amino acid, a sedimentation constant of $S^{o}_{20,w} = 3.7$, a weight-average molecular weight of 4.2×10^{4}, a number-average molecular weight of 4.0×10^{4}, and they both had the same paper electrophoretic pattern. Heald and McLachlan (1963) also isolated phosvitin from the plasma of laying hens and found it to be identical with yolk

phosvitin. Because of the difficulty in isolating phosvitin from serum, however, they reported that it probably does not exist in the same complex in serum as it does in yolk (i.e., complexed with lipovitellin). McCully et al. (1962) found that high ionic strength solvents allowed the phosvitin to separate from the lipoproteins of egg yolk during electrophoresis and migrate as a faster zone.

Greengard et al. (1964) isolated phosvitin and demonstrated that it was the major iron-binding protein in yolk. High ionic strength solvents dissolve the granules, and then phosvitin can be separated from the lipovitellins. Wallace (1963) found that there were two phosvitin molecules for every lipovitellin molecule in the granules.

Livetins Fevold (1951) states in his review that Valenciennes and Fremy in 1854 recognized the presence of another protein in egg yolk in addition to the one precipitated by dilution by Lehman and Messerschmidt in 1842. They termed this protein an "albumin." Plimmer (1908) demonstrated that the aqueous supernatant solution remaining after the precipitation of lipovitellin by the method of Osborne and Campbell (1900) contained material which gave a positive biuret reaction. He found that protein coagulated and precipitated when the aqueous

solution was boiled. It was believed that this protein was derived from vitellin and so was named livetin. Their livetin had a much lower phosphorus content than vitellin.

Livetin was characterized as a pseudoglobulin by salting it out with half-saturated ammonium sulfate by Kay and Marshall (1928). On the basis of its solubility behavior they suggested that livetin contained more than one protein.

Electrophoretic examination of the livetins, reported by Shepard and Hottle (1949), showed three components. Sugano (1955), also using electrophoresis, found three components in the livetin fraction of egg yolk. He confirmed that all three components were pseudoglobulins. Martin et al. (1957) reported that three components were demonstrable electrophoretically from a gross livetin fraction, and these three were named α-, β-, and γ-livetin in order of decreasing electrophoretic mobility. Isolated α- and β-livetins were found to have the solubility characteristics of a pseudoglobulin. α-livetin was estimated to have a molecular weight 4.2×10^4 and β-livetin a molecular weight of 8.0×10^4.

γ-livetin, isolated by Martin and Cook (1958), had the solubility of an euglobin. It irreversibly lost its solubility upon prolonged dialysis against water or upon lyophilization. It had an axial ratio of about 8 and a molecular weight of 1.5×10^5.

Williams (1962a) identified α-livetin as serum albumin, β-livetin as an α_2-glycoprotein found in serum, and γ-livetin as serum γ-globulin by immunoelectrophoresis. Another protein isolated from the livetin fraction of egg yolk was demonstrated to be transferrin, the iron-binding β-globulin of serum, by Williams (1962b).

Enzymes and minor constituents Lineweaver et al. (1948) reported that yolk contained tributyrinase, peptidase, catalase, amylase, and phosphatase. Neither lipases active on lipids with fatty acids containing six or more carbon atoms nor oxidative enzymes were found in egg yolk. Immunological investigations by Marshall and Deutsch (1951) indicated conalbumin and ovalbumin were present in extremely small amounts in egg yolk.

Lipids

Lipids account for nearly two-thirds of the weight of egg yolk solids. Many investigations determining the typical classes of lipids and the fatty acids occurring within each lipid class have been conducted on egg yolk (Cruickshank, 1934; Hawke, 1959; Rhodes and Lea, 1956, 1957; Reiser, 1951a; Shorland, 1951; Riemenschneider et al., 1938; Privett et al., 1962). The report by Privett et al. (1962) is one of the most reliable since modern techniques of separation were employed. They showed that in eggs produced by hens on a "typical" diet

65% of the lipids are triglycerides, 28% are phospholipids, and 5% are sterols. There are also small amounts of other lipid constituents. Approximately two-thirds of the total fatty acids of egg yolk is saturated and one-third unsaturated. However, this ratio of saturated to unsaturated can be altered, within certain limits, by changes in the dietary fats of the hen (Reiser, 1951b; Fisher and Leveille, 1957; Feigenbaum and Fisher, 1959; Evans et al., 1960) and differs between breeds and strains of hens (Edwards, 1964).

Privett et al. (1962) found that egg yolk contains about 5 times as much phosphatidylcholine (lecithin) as phosphatidylethanolamine (cephalin). Egg yolk contains very little phosphatidylserine, the other component of cephalin. The lecithin found in the yolk which was investigated by Privett et al. (1962) was predominantly (88%) α-saturated β-unsaturated with the rest of the lecithin being about equally distributed among α-unsaturated β-saturated, disaturated, and diunsaturated. There was a very small percentage (2%) of the triglycerides which was trisaturated, the rest were about equally distributed among monounsaturated-disaturated, monosaturated-diunsaturated, and triunsaturated.

Brooks and Hawthorne (1944) demonstrated that petroleum ether will extract less than 0.5% of the lipid of fresh egg yolk while chloroform-methanol will extract

nearly all of the lipid. Despite this demonstration that the lipid of fresh egg is not readily extracted by non-polar lipid solvents, many authors have written of "free" lipid being present in fresh egg yolk (Chargaff, 1942; Alderton and Fevold, 1945; Romanoff and Romanoff, 1949; Vandegaer et al., 1956). Weinman (1956) found that lipids did not rise to the surface upon ultracentrifugation, and suggested that nearly all of the egg yolk lipids are bound to the proteins in complexes having densities greater than that of chylomicrons. In a sample of reconstituted spray dried egg, about 5% of the lipids floated on centrifugation. McIndoe (1958) reported that egg yolk and hen plasma contains a small amount of lipoprotein which is alcohol-stable; that is, the lipid will not be extracted even after repeated extractions with chloroform and methanol.

The amounts of the various classes of lipids and their fatty acid compositions in lipovitellin and the low-density lipoproteins were studied by Evans and Bandemer (1960, 1961). However, because their methods of preparing the lipoproteins vary from those of most other workers, their results are difficult to interpret. Martin et al. (1963) did a similar study on α- and β-lipovitellin and the low-density lipoproteins which were prepared by more conventional means. The lipovitellins contained approximately 60% phospholipid and 40% neutral lipid. About 75%

of the phospholipid was lecithin, 18% phosphatidylethanolamine, and 7% sphingomyelin, lysolecithin, and lysocephalin. The low-density lipoproteins contained approximately 75% neutral lipid and 25% phospholipid. The distribution of phospholipids was about the same in the low-density lipoproteins as in the lipovitellins.

Carbohydrates Romanoff and Romanoff (1949) reported that the total amount of carbohydrate in egg yolk is nearly 1%; 70% of which was free and 30% of which was bound to the phospholipids or proteins. The free carbohydrate was believed to be mainly glucose and the combined carbohydrates were thought to be mannose and galactose. Abraham et al. (1960) isolated the low-density lipoproteins from both egg yolk and hen serum and found they contained galactose, mannose, glucosamine, and sialic acid as the only carbohydrate constituents. They were present in the lipoproteins from both sources in the identical ratio of 1 hexose to 0.5 glucosamine to 0.3 sialic acid. They were present at levels of about 0.25% of the weight of the total lipoproteins.

Structure Egg yolk consists of concentric layers of yellow and white yolk (Romanoff and Romanoff, 1949). Little is known about these layers or the composition of the two types of yolk, but the white yolk is believed to be the yolk which is laid down in the early morning hours

when the blood of the hen is depleted of the usual materials necessary for yolk formation. The amount of white yolk does not exceed 5% of whole yolk. White yolk contains 88% water, 4.6% protein, and 3.5% lipid. Yellow yolk contains 46% water, 15% protein, and 36% lipid. It is interesting to note the ratios of lipid to protein in the two types of yolk.

Romanoff and Romanoff (1949) reported that yolk contains numerous globules which can be observed under a microscope. The globules in the yellow yolk range from 0.025 to 0.150 mm. in diameter and some of them contained numerous highly refractive granules. The globules in the white yolk ranged from 0.004 to 0.075 mm. in diameter and contained one or more refractive bodies of varying sizes suspended in a clear fluid. These structures, which Romanoff and Romanoff (1949) referred to as globules, Bellairs (1961) called yolk spheres. She called the particles in the spheres, subdroplets, and reported that the ones in the white yolk spheres were highly refractile while those in the yellow yolk spheres were not. The subdroplets in the white yolk spheres were larger and less numerous than those in the yellow yolk spheres. She also reported the presence of free-floating lipid drops in the dispersed (continuous) phase which were much smaller than yolk spheres, more numerous, and highly refractile. The

subdroplets in the yolk spheres and the free-floating lipid drops were reported to be stained by Sudan III. Using electron microscopy Bellairs (1961) found the lipid drops and the yolk sphere subdroplets to be about 2μ in diameter and to contain, in turn, small dense particles from 30 to 60 A^{o} in diameter. She found the continuous phase of yolk to contain small spherical particles about 250 A^{o} in diameter. These particles were considered by her to be the granules in contrast to the nomenclature of the Romanoffs. The yolk spheres were believed to have a naked surface with no unit membranes surrounding them.

Burley and Cook (1961), as previously mentioned, isolated yolk granules (as described by Romanoff and Romanoff, 1949) and found that they contained the lipovitellins, phosvitin, and a smaller amount of low-density lipoprotein. Wallace (1963) reports that Colvin and Cook believe these granules to be the lipid drops and yolk sphere subdroplets of Bellairs (1961). However, since Bellairs (1961) apparently found the yolk sphere subdroplets to be similar to the free-floating lipid drops, and since Burley and Cook (1961) reported their sedimented granules were free of contaminating yolk spheres (which they called globules), there appears to be some confusion as to which proteins or lipoproteins are found in each of the various microscopic structures.

Characteristic Properties of Dried Eggs

The relative merits of the various methods which had been used for determining the quality of dried eggs were discussed by Thistle et al. (1943). These methods included foam volume of reconstituted egg beaten under standardized conditions, pH of reconstituted egg, percentage of total nitrogen soluble in water, percentage of total nitrogen soluble in a solution of 10% potassium chloride, fluorescence values, the palatability as determined by a taste panel, and the amount of creaming in reconstituted dried eggs.

Foaming ability

One of the functions of eggs in cakes is that of exerting an aerating and binding effect during the mixing and baking of the batter. Eggs aid in retaining the air beaten into the mix and in maintaining the cell structure during the baking, thus imparting lightness and texture to the cake. Bennion et al. (1942) described a method which they had developed for estimating this aerating ability of spray dried egg. It consists of beating together, under specified conditions, a mixture of egg and sugar and measuring the volume of foam obtained. This foam volume was found to be highly correlated with the baking quality of the egg. In a later paper, Hawthorne

and Bennion (1942) detailed a modification of this original beating test which involved doing the mixing at 120° F. instead of at room temperature. This higher temperature was found to give substantially greater foam volumes. They suggested that higher mixing temperatures be used in commercial bakeries to give sponge goods of greater volume and lighter texture.

Sponge cakes were used to demonstrate that dehydrated egg containing 2% moisture did not deteriorate as rapidly as egg containing 5% moisture by Miller et al. (1947). Sponge cakes were also used by Boggs et al. (1946) to show the advantages of acidifying eggs prior to drying them. Bollman (1953) described a method for evaluating dried eggs in sponge cakes and stated that sponge cakes worked excellently for evaluating the aerating ability of dehydrated eggs.

pH

Stuart et al. (1942a) measured the pH of reconstituted spray dried egg immediately after drying and after six months of storage at 75 to 80° F. and found that the pH decreased approximately 1 pH unit during storage. Bate-Smith et al. (1943) also observed this decrease in pH during storage and attributed it to the liberation of free fatty acids during storage, although bacterial spoilage of the egg pulp prior to drying can also cause low pH values. Brooks

and Hawthorne (1943b) reported that the pH of freshly dried whole egg, reconstituted 3 to 1 with distilled water, will range from 8.5 to 8.9, while egg yolk that has been stored for quite some time under rather adverse conditions will have pH's ranging from 5.9 to 7.0.

Solubility

The solubility of dried egg is considered to be a measure of the deterioration suffered by the egg proteins, either during the spray drying or during storage. The loss in solubility during storage is generally considered to be due mainly to Maillard reactions (Kline and Sonoda, 1951). While there is sometimes a correlation between solubility and palatability, this relationship does not cover all types of deterioration.

Hawthorne (1944) states that a dried egg sample of poor solubility will disperse readily in water to form a viscous fluid, or in extreme cases will remain in suspension as a paste, whereas a sample of good solubility is more difficult to reconstitute and will give a comparatively thin fluid. Stuart et al. (1942b) developed two tests relating the degree of solubility to the quality of dried egg as measured by its performance in pound cakes. The first test gives a "centrifuged heat-coagulable solubility index" and consists of mixing a given amount of powder with an acetate buffer, dissolving under

specified conditions, heating in a boiling water bath, centrifuging and measuring the volume of precipitate. The second test gives what is called "Esbach's sedimentation solubility index" and is treated in a manner similar to the first sample except that the dissolved sample is mixed with Esbach's reagent and the tube allowed to stand. The amount of material which has precipitated after a specified time is then recorded.

Two types of insolubility were noted by Stuart et al. (1942b). One type is due to improper drying methods and the other kind is due to prolonged storage or storage under improper conditions. It was found that if two samples had equally low indices of solubility as measured above, that the one that had suffered damage during drying would give worse performance in baked goods than the one with a low index due to adverse storage conditions.

Hawthorne (1944) considered the standard method of determining solubility to be the one that measures the solubility of the powder, under specified conditions, in a 10% potassium chloride solution. It consists of dissolving the powder in a 10% potassium chloride solution and measuring the total soluble nitrogen by the Kjeldahl method. The soluble nitrogen is then expressed as a percentage of the total nitrogen in an equal weight of the same sample. A sample of good dried egg will have a

solubility index of 95 to 98%. The main disadvantage of this method is that it is too laborious for routine analyses.

Thistle et al. (1943) published two methods for determining the solubility of dried whole egg. One involved shaking a weighed amount of powder with distilled water, while the other method used a 10% solution of potassium chloride. Then, in both cases, the solutions were filtered and an aliquot of the filtrate coagulated, dried, and weighed. The weight of the dried material was expressed as a percentage of the original dry weight of powder and the result was expressed as the "water value" or "potassium chloride value."

Hawthorne (1944) also reported on a method proposed, but never published, by Dr. E. O. Haenni of the U.S. Food and Drug Administration. It involved measuring the refractive index of a specially prepared solution of dried egg. The refractive index showed an excellent correlation with the potassium chloride solubility index.

Palatability

Even today, taste panels appear to be the only method used for evaluating and classifying off-flavors and odors in dried eggs. In a typical situation samples of the dried egg are evaluated by several experienced tasters in

reconstituted scrambled eggs or in custards. Standardized methods of reconstituting and cooking dried eggs for evaluation by a taste panel were described by Wilson and Slosberg (1942). Bate-Smith et al. (1943) detailed a numerical system of scoring flavor in scrambled eggs based on 10 as equivalent to the flavor of fresh shell eggs and 0 as being inedible or repulsive. Marcuse (1947) described a statistical method for evaluating dried eggs and selecting a panel of tasters.

Bate-Smith et al. (1943) list fishy, burnt, storage, and acid or cheesy flavors as being the most commonly occurring off-flavors found in dried eggs. Fishy flavors are usually found in powders of relatively low moisture content (2% or below) or in powders which have been contaminated with copper. This fishy flavor is generally considered to arise from the oxidation of fats. Burnt flavors are caused by excessively high drying temperatures or by storage at elevated temperatures which leads to an excessive amount of Maillard browning. Storage flavors, also sometimes described as cardboard, arise in egg yolk stored for excessively long times at moderate temperatures. These flavors are probably caused by a combination of browning reactions and a general loss of flavor components. Acid or cheesy and sour flavors are caused by extensive bacterial growth in the liquid egg prior to spray drying.

Other types of off-flavors are usually picked up from foreign materials by eggs stored in non-airtight containers. For this reason it is important to select an airtight packaging material which will not impart a foreign flavor to the egg.

Fyrd and Hanson (1944) reported a formula, based on the Haenni value, the fluorescence value, the water-solubility, and the amount of free fatty acids, which was used to estimate the flavor score that a taste panel would give the sample. Later (Fyrd and Hanson, 1945) they found that the glucose level was highly negatively correlated with the flavor score (presumably because the glucose was involved in reactions leading to the loss of palatability in dried eggs) and incorporated this factor into their flavor estimating formula.

Eggs were fractionated into egg white, egg yolk, lipovitellin, livetin, acetone-soluble lipids, and crude acetone-insoluble phospholipids by Fevold et al. (1946). These fractions were then dried separately, subjected to storage tests, recombined with other fractions, and evaluated in palatability tests. The yolk, and more specifically the yolk phospholipids, were found to be the source of the typical off-flavors which develop during the storage of dried eggs. The neutral lipids, when stored alone developed definite off-flavors and

odors, but not typical "stored-egg" flavors. The phospholipids tended to prevent the development of off-odors in the neutral lipids, but were pinpointed as the source of typical "stored-egg" flavors and odors themselves. It was suggested that the cause of the off-flavors was aldehyde groups, possibly arising from fat oxidation, reacting with the phospholipid-amino groups in Maillard reactions. Kline et al. (1951a) also reported that aldehydes react with phosphatidylethanolamine, but found the source of the aldehydes to be the glucose in egg and not aldehydes which arose from oxidized lipids.

Fluorescence

Pearce and Thistle (1942) described a test for measuring the amount of fluorescence of a potassium chloride extract of defatted egg powder; a parameter which they reported to be highly correlated with flavor. Pearce (1943) reported that fluorescence increased during storage in dried whole egg, defatted dried whole egg, dried egg yolk, and dried egg white. He also found some of the fluorescent materials in dried whole egg to be soluble in fat solvents.

A method for directly recording the fluorescence of dried egg yolk which was pressed between two microscope slides was reported by Fyrd and Hanson (1944). There was a high correlation between this fluorescence value and the flavor score of the dried egg.

Olcott and Dutton (1945) reported that the source of the salt water fluorescence in dried eggs comes from Maillard reactions between free aldehyde groups and the free amino groups in the proteins. Similar reactions between the cephalin amino groups and free aldehyde groups were reported by Edwards and Dutton (1945) to be the source of the fat soluble fluorescence. Alkaline pH values were found to increase the rate of the development of fluorescence by Dutton and Edwards (1945). The color change in dried egg yolk from yellow to brown was followed with reflectance and absorption spectrophotometry by Dutton and Edwards (1946). They reported that the reaction between the lipid amines and free aldehydes was accelerated by increasing temperatures and increasing moisture levels. Lipid soluble fluorescence was found by Boggs et al. (1946) to be more highly correlated with loss of palatability in dried egg powder than was salt water soluble fluorescence.

Creaming

Bate-Smith et al. (1943) reported that a carefully dried, freshly-prepared sample of dried egg will remain homogenous when reconstituted and allowed to stand. Egg which has been overheated during drying or stored at relatively high storage temperatures will "cream" on reconstitution and standing. This cream layer is composed

of fat globules which have separated from the yolk lipoproteins and fused together. A rough measure of the structural damage can be obtained by measuring the height of cream in a column of reconstituted egg which has been allowed to stand overnight and expressing the result as a percentage of the total height of the column.

Factors Influencing Dried Egg Quality

Some of the factors which influence the ultimate quality of the dried egg include moisture content, predrying treatments, and storage conditions.

Moisture content

Stuart et al. (1942c) recommended a moisture content of below 5% in order to minimize solubility loss during storage. A moisture content as low as can be attained with the particular dryer being used, without prejudice to the initial quality of the product, was recommended by Hawthorne (1943). He showed that deteriorations in solubility, flavor, and beating power proceeded more rapidly in powder with a moisture content of 5% or more. By initially taking a single sample and adjusting it to different moisture contents, he demonstrated that it was the moisture content during storage that affected the deterioration rate and not the differences in drying conditions.

The concentration of orthophosphate and acid-soluble phosphate, and the acidity of the ether extract were reported to increase more rapidly in samples of dried egg containing greater than 4 or 5% moisture by Brooks (1943). He attributed these increases to lipases and lecithinases present in the egg yolk. However, Lineweaver et al. (1948) reported that they could find no lipases in egg yolk. If these enzymes were present in the dried yolk which Brooks (1943) studied, their probable source was bacterial spoilage prior to spray drying.

White and Thistle (1943b) recommend that dried egg should contain no more than 5% moisture and preferably 2% or less. Boggs and Fevold (1946) found a progressive increase in shelf-life as the moisture content decreased from 6.0 to 0.5%. Other workers have found a tendancy toward oxidative deterioration and the development of fishy flavors in egg powders with very low moisture contents (Bate-Smith et al., 1943). Gane (1943) determined the moisture content of dried whole egg, dried yolk, and dried albumen which was stored at temperatures of 10°, 37°, 60°, and 80° C. and at relative humidities ranging from 5 to 80%. The method of drying (ie., spray drying under varying conditions or lyophilization) was found to have very little effect on the final moisture content of dried samples which were stored under similar conditions. The

protein content had the most effect on the moisture content of the sample, and on a lipid-free basis dried albumen and dried yolk had similar moisture contents when stored under similar conditions. The water content of dried egg stored at a known temperature and humidity could be calculated from the relative amount of yolk and albumen solids in the sample.

Stewart et al. (1953) studying samples of dried whole egg stored at 50° C. and ranging from 0.29 to 4.6% in moisture content found that fluorescence values increased during storage in direct relation to increasing moisture contents. In the same samples solubilities decreased as moisture contents increased. Results from palatability tests confirmed the fluorescence and solubility tests.

Thistle et al. (1944) found that dried egg deteriorated even at moisture levels as low as 1.4%. They recommended that as low a moisture content as was practicable, be attained in commercially dried eggs but cautioned that even very low moisture contents did not render the products imperishable.

Pre-drying treatments

Acidification The pH of a series of liquid whole egg samples was adjusted to 7.5, 6.5, and 5.5 by Stewart et al. (1953) using lactic acid. These samples were then spray dried and stored. The lowered pH was found to

decrease greatly the solubility loss ordinarily encountered during the storage, but was found to have no effect on the fluorescence value. Pearce et al. (1946), however, found no beneficial effect from adjusting the pH of whole egg prior to drying to 6.8 and then storing the dried product in an inert atmosphere of nitrogen or carbon dioxide.

The pH of whole egg was lowered to 4.5, 5.5, and 6.0, lyophilized, and enough dry sodium bicarbonate added to the dried samples to bring their pH up to 8.5 when reconstituted by Boggs and Fevold (1946). The samples were stored at 97° F. for periods ranging from 8 to 16 weeks in air, nitrogen, and carbon dioxide. Palatability scores indicated that the egg dried from the liquid egg of pH 4.5 or 5.5 and stored under either nitrogen or carbon dioxide had a shelf-life of 5 to 6 times longer than unacidified dried egg stored in air.

Salwin and Mitchell (1953) found that the conditions of reconstitution significantly effected the pH of the freshly reconstituted egg. They found that cooking the reconstituted samples led to reproducible pH values, no matter what method or condition of reconstitution was used. Furthermore, cooking increased the sensitivity of the measurements to variations in acidity or alkalinity. They recommended that in acidified whole egg 1.5% sodium bicarbonate be added to the dried pulp prior to

reconstitution. This was equivalent to a pH in the reconstituted cooked egg of from 7.0 to 9.0.

Kline et al. (1953b) reported that acidification owed its effectiveness to decreasing Maillard-type reactions, but that it did not completely eliminate the Maillard reactions.

Glucose removal A lowering of pH produced by the bacterial fermentation of whole egg prior to drying was considered to be the reason for an improvement in the solubility of the dried product which was observed by Stewart et al. (1953). Brooks and Hawthorne (1943b) also observed that certain commercial "acid" samples which had undergone unintentional bacterial fermentation before drying retained their solubility during storage better than unfermented samples. Since they were aware that the reason egg white is stabilized by fermentation is that the glucose is removed, they reasoned that the same phenomenon might be occurring in whole egg. Consequently the glucose of fresh whole egg (about 0.5%) was removed by yeast fermentation. Although the resulting product had a foreign "yeasty" off-flavor, it was much more resistant to deterioration as measured by creaming, fluorescence, foaming ability, and solubility than was untreated egg. The pH of this egg remained unchanged from the control product, thus demonstrating that the glucose removal of

itself improved the solubility of the dried egg upon storage and that it was not solely an effect of lowered pH as had been reported by Stewart et al. (1953).

By using from 0.07 to 0.15 gms. of dry yeast (Saccharomyces cerevisiae or Saccharomyces carlsbergensis) per 100 mls. of egg melange and incubating at 30° C. for 2 to 3 hours Kline and Sonoda (1951) were able to reduce residual "yeastiness" to the point where it was undetectable by a trained panel.

Flavor stability of whole egg powder stored at 100° F. was shown to be primarily dependent on glucose induced reactions by Kline et al. (1951b). While non-glucose deteriorations occurred at this storage temperature, they were neither as rapid nor as marked as the glucose-type of deteriorations.

Kline et al. (1953a) found that removal of the glucose present in whole egg pulp by yeast fermentation prior to spray drying retarded storage deterioration to a much greater extent than did acidification of the liquid egg to pH 5.5 prior to drying. In eggs stored at 100° F. the superiority of glucose removal was obvious in powders with moisture contents of both 2 and 5%. Glucose oxidase had no apparent advantages or disadvantages in removing glucose as compared with yeast-fermentation (Kline et al., 1953a).

From sensory evaluations, Hanson (1953) found that yeast fermented dried whole egg was more than 4 times as stable as acidified dried egg when stored at moisture contents of 2% and temperatures of 100° F. Mitchell (1953) also found that glucose-free dried eggs were clearly superior to acidified dried eggs. He also found that glucose removal by glucose oxidase and yeast fermentation yielded products of about equal stability and quality, but reported that in samples of greater than 3.5% moisture content, the yeast fermented eggs had a greater tendancy to develop off-odors during storage.

Egg yolk solids which had been enzyme treated to remove glucose were found to produce higher quality cake doughnuts than unstabilized control samples after prolonged storage times at elevated temperatures by Paul et al. (1957).

Pasteurization Pasteurization of liquid yolk for 4 minutes at temperatures ranging from 140 to 146° F. was found to have no adverse affects on egg yolk used in mayonnaise production by Miller and Winter (1951).

Pasteurization prior to drying was reported to cause no changes in the functional performance of egg yolk solids as used in cake doughnuts by Paul et al. (1957). Four different treatments, ranging in time from 0.5 to 4 minutes and in temperature from 140 to 144° F., were

found to produce no change in fat absorption or specific volume of the doughnuts.

Additives Over the years many products have been added to eggs prior to drying in attempts to improve the storage life or functional performance of the dried product. Sodium bicarbonate, which is added to acidified eggs, has already been discussed and sugar as an additive will be discussed under the section "Properties of Sugar-Dried Yolk." Termohlen et al. (1938) reports that in the early days of the egg drying industry in the United States such things as salt, sodium phosphate, corn starch, wheat flour, rice flour, farina, lemon juice, and glycerine were added to egg which was to be dried. At times formaldehyde was reported to be added in an attempt to cover the smell of rotten or stale eggs.

The effects of sodium chloride, citric and lactic acid, sodium bicarbonate, sodium acetate, benzoate, salicylate, and tartrate on the keeping quality of dried egg were studied by Pearce et al. (1944). Sodium chloride in combination with citric or lactic acid was found to accelerate the rate of fluorescence development. Sodium bicarbonate decreases deterioration as measured by fluorescence development and palatability scores. The other substances had no apparent effect on the rate of deterioration. Hay et al. (1947) added soya lecithin,

egg lecithin, heated whole egg powder, heated dried egg white, heated dried yolk, and heated and unheated cystine and methionine to liquid egg prior to drying and found that these substances had little effect on the deterioration rate of the spray dried egg prepared from them. Whey solids were found to yield baked goods of inferior quality and to accelerate the rate of deterioration of the dried egg.

Forsythe (1964) reported that while numerous substances are added to dried albumen as whipping aids, the number of compounds added to whole egg or egg yolk is more limited. He noted that a survey of bakers indicated that they would like to see the Food and Drug Administration approve the use of standardized yolk color additives.

Forsythe et al. (1964) added colloidal silica, sodium silicoaluminate, and diatomaceous earth in attempts to improve the flowability of spray dried egg yolk powder. The substances were added to the dried yolk. It was found that the commercial colloidal silica preparation added at levels ranging from 2.0 to 2.5% was the most effective agent in increasing the flowability of the dried yolk. Electron micrographs showed that the additives exercised their effect by coating the surface of the spray dried particles and thus reducing caking in the dried yolk.

Storage conditions

Temperature The temperature of storage was found to be the main factor influencing the rate of deterioration in dried egg by Bate-Smith et al. (1943). The rate of pH drop in dried egg was reported to be more rapid in dried egg stored at 37° than that stored at 15° by Brooks and Hawthorne (1943b). White et al. (1943) recommended a storage temperature of 60° F. or less to maintain reasonable quality during the storage and transport of dried egg. White and Thistle (1943a) found that if dried egg yolk is not cooled fast enough after drying that deterioration can proceed rapidly. They recommended that dried egg powder be cooled to 80° F. or less within 3 hours after drying. White and Grant (1944) found that dried egg stored at over 95° F. quickly lost its bright yellow color and turned brown. Thistle et al. (1944) reported that dried egg deteriorated slightly even at temperatures as low as -40° F.

Atmosphere Storing of dried egg in inert atmospheres of N_2 or CO_2 was reported to be beneficial only when the temperature of storage is less than 15° C. by Bate-Smith et al. (1943). Dried egg was stored under N_2, CO_2, and under a vacuum by White et al. (1943). Only CO_2 was found to exhibit a beneficial effect on the storage life. CO_2 was found to afford some measure of protection against high temperature deterioration of

dried egg stored at 37° and 48° C. by Thistle et al. (1944). Its action was especially apparent in preserving the solubility of the dried egg.

Boggs and Fevold (1946) found no significant difference in the rate of deterioration of acidified egg powder stored under CO_2 and N_2. Kline et al. (1953a) confirmed the report of Boggs and Fevold (1946) and suggested that since CO_2 is so rapidly absorbed by the dried egg that the reason CO_2 preserves non-acidified dried egg better than N_2 is that it lowers the pH slightly. Kline (1953) also stated that anti-oxidants used in place of inert atmospheres were found to yield no improvement in the keeping quality of the dried egg.

Properties of Sugar-Dried Yolk

Knowing that carbohydrates can protect proteins against heat denaturation and that sugars protect yolk against gelation during freezing, Brooks and Hawthorne (1943a) experimented with adding different sugars to liquid egg before it was spray dried. They found that the addition of lactose, sucrose, dextrin, and gum arabic retarded high temperature deterioration of dried eggs. They also found that sorbitol, glucose, fructose, and arabinose accelerated deterioration and that starch and

mannitol had little effect on the rate of deterioration. Quite unexpectedly they found that all of the added sugars greatly increased the aerating ability of the dried yolk. Plain dried yolk has very little foaming power and consequently produces inferior quality sponge goods. The egg yolk dried with sugar was found to yield excellent sponge goods. Interestingly, glycerol was found to yield dried products with good foaming ability, while products dried with glyceraldehyde added had extremely poor foaming ability.

Brooks and Hawthorne (1944) suggested that sugar stabilizes some kind of complex between lipids and proteins which tends to make the lipid unextractable from the sugar-dried yolk. Bate-Smith and Hawthorne (1945) determined that the loss of solubility in dried eggs occurs in two steps. The first step involves the condensation of a free aldehyde group with the amino group of a protein and was found to have no effect on the solubility of the dried egg. The second step involves further reactions of the condensation products and leads to insolubility in the dried egg. The sugars which prevent the development of insolubility during storage are thought to first interact with the free-amino groups and then not lend themselves to further reactions leading to the development of the insoluble compounds. Inasmuch as glycine will also decrease the

development of insolubility in dried eggs, it is thought that the amino groups of glycine interact with the free aldehyde groups of glucose and thus render them unavailable for reactions with the protein amines which would lead to further reactions causing insolubility. Bate-Smith and Hawthorne (1945) were unable to explain why the added sugar improved the aerating ability of the dried egg, however.

The addition of 15% sucrose to liquid egg prior to spray drying was found to inhibit the development of fluorescence in powder stored at 24° C., to have less effect in reducing fluorescence in powder stored at 37° C., and to have no effect in powder stored at 48° C. by Pearce et al. (1944). Hay et al. (1947) found that sucrose and lactose exhibited a positive effect on the baking quality of dried egg powder stored at 27° and 38° C.

Dawson et al. (1947) tested the effects of 10% sucrose, 20% sucrose, 10% lactose, 10% glucose, and 10% dextrin on the keeping quality of dried egg. They evaluated the egg on the basis of taste panels, specific gravity of foam produced upon beating, volume of sponge cakes, solubility, volume of popovers, and height of baked custard. The sample containing 10% lactose was generally superior to the other samples in most of the tests. The samples containing 10 and 20% sucrose were superior to

the samples containing dextrin in most tests, and the samples containing glucose and dextrin were only superior to a control dried without any additives in a few of the tests and in many tests were even inferior to the control sample.

By removing the free lipid present in dried egg yolk with acetone, Joslin and Proctor (1954) demonstrated that this lipid prevented the dried egg from foaming upon rehydration and beating. They attributed this free lipid to the breakdown upon drying of the natural fat emulsion present in fresh egg yolk. The addition of a small amount of dried egg to a foam produced with fresh eggs, causes the collapse of the foam.

An extensive study on the effects of adding carbohydrates to whole egg and egg yolk prior to spray drying was conducted by Kline et al. (1964). Sucrose and corn syrup solids with dextrose equivalents of 24 and 42 were added to the liquid egg prior to drying. The authors commented that low-dextrose-equivalent corn syrup solids have been substituted for sucrose at the same level in commercial practice, presumably to avoid the fishy flavors which sometimes developed in dried egg with 10% added sucrose. Kline et al. (1964) found all three carbohydrates would yield products of similar flavor stabilities, but

at different additive levels. Despite containing substantial quantities of reducing sugars, the corn syrup solids protected against browning as well as the sucrose did. As the amount of each carbohydrate was increased, the flavor was gradually improved until a maximal level of flavor stability was reached. Additional amounts of carbohydrates caused an abrupt transition to a marked flavor instability. The optimum amount of each carbohydrate to be added to the liquid whole egg was 5% sucrose, 7.5% 42-DE corn syrup solids, and 10% 24-DE corn syrup solids. The flavor transistion corresponded to a change in the physical state of the lipids, from one in which the lipids were coalesced and easily extracted with non-polar solvents to a state in which they were finely dispersed or in an emulsified condition and virtually unextractable.

Kline et al. (1964) also reported that the foaming ability of dried egg gradually, but continuously, increased from 0 to 15% added sucrose. Thus, the dilemma arises wherein the level of added sugar which will yield maximum functional performance cannot be used because of the ensuing flavor instability.

As an interesting sidelight, Kline et al. (1964) reported that spray dried whole egg powder retains most of its foaming ability if it is rehydrated and beaten within one hour after being spray dried. The foaming

ability was nearly completely lost after three hours or more, however.

Privett et al. (1964) reported that under many conditions the usual chemical indicators of rancidity, such as peroxide number, carbonyl number, and thiobarbituric acid number, do not correlate well with organoleptic tests. It was determined that the ultraviolet absorbancy of volatiles collected by vacuum distillation from dried egg yolk with and without added carbohydrates was more highly correlated with organoleptic scores than were the earlier mentioned chemical tests. The gas-liquid chromatography patterns of the volatiles from high quality dried yolk, sugar-dried yolk, and various types of deteriorated dried yolk showed great differences, but no particular chromatographic peaks could be correlated with any particular type of deterioration or with the onset of rancidity.

METHODS AND MATERIALS

Materials

Laboratory reagents

Analytical reagent-grade chemicals were used in all of the laboratory experiments. The sugars added to laboratory broken eggs were reagent-grade, but the corn syrup used in the commercially dried products was of commercial quality. Water used in connection with these experiments was double deionized and glass distilled. Its electrical conductivity indicated an ion contamination, expressed as sodium chloride, of less than 0.2 PPM.

Eggs

Fresh eggs were procured from the University Poultry Farm. They were stored in a laboratory refrigerator and used within one week after being laid, usually within 48 hours. The eggs were broken and the albumen was separated from the yolk. Adhering albumen was removed from the vitelline membrane by rolling the yolk on a paper towel. The chalazae were removed with tweezers and the yolk membrane was punctured to allow the yolk contents to drain into a glass beaker. The yolk from several eggs was usually pooled in a tared beaker, the beaker was then weighed and covered with parafilm, and if the yolk was not used immediately it was stored in a refrigerator.

Samples of spray dried yolk were obtained from liquid yolk which had been commercially prepared from machine

broken eggs. The liquid yolk was dried in a commercial, modified Rogers spray dryer. The fresh liquid yolk contained 44% solids. The sugar-dried yolk was prepared from the same batch of liquid yolk as the plain dried yolk. The final sugar-dried product contained 74.5% egg yolk solids, 20.5% 30DE corn syrup solids, and 4.2% moisture.

Samples of plain and sugar-dried yolk which had different viscosities were prepared by varying the feed rate of the dryer during the drying run. Three different viscosities of plain yolk and two different viscosities of sugared yolk were prepared. Viscosities were measured on samples which were prepared by slowly stirring 80 mls. of water into 60 gms. of plain dried egg yolk and an equivalent amount of sugar-dried yolk (based on total egg yolk solids). This mixture was then stirred thoroughly but slowly with a glass stirring rod and allowed to stand for 6 to 7 hours at about 2° C. in a cold room. The samples were brought to 25° C. and again stirred before viscosity measurements were taken. All samples were prepared in 200 ml. tall beakers and viscosity measurements made with the sample remaining in the beaker. The viscosities were measured with the model LVH Brookfield viscometer using the number 2 and number 3 spindles at 30 rpm and are reported as dial readings. Conversion

of the dial readings to centipoises would be meaningless when the type of viscosity exhibited by the rehydrated samples is considered.

Lyophilized samples were prepared in a small laboratory freeze dryer. Yolk, from laboratory broken eggs with the proper additives, if any, was frozen in a thin film on the inside of a 2 or 3 liter round bottomed vacuum flask by swirling the flask containing the proper amount of yolk in a dry-ice-acetone bath. When dry the yolk was scraped from the flasks, put in glass stoppered bottles, and stored at -30° C. until used.

Other samples of yolk from laboratory broken eggs were dried by rotary evaporation. Forty gms. of yolk were weighed into a 2 liter round bottomed vacuum flask and the flask was rotated in a water bath at 40° C. Initially a vacuum of 50 mm of mercury absolute pressure was maintained on the yolk during drying. It took about 30 hours under these conditions to dry the yolk to a moisture content of 5%. Two mls. of a mixture containing penicillin G and oxytetracycline each at a level of 0.1% were added to the yolk to prevent bacterial degradation. Later experiments indicated that the time or vacuum under which yolk was dried had little or no effect on the amount of easily extractable lipid in the dried yolk, so the yolk was rapidly dried by gradually increasing the vacuum during drying.

the yolk was rapidly dried by gradually increasing the vacuum during drying.

Preparations

Methods

Preparative ultracentrifugations High speed preparative ultracentrifugation was carried out in a Spinco model L-2 50 preparative ultracentrifuge. The number 30 and titanium 50 rotors were used at top speeds. Maxium centrifugal force developed in the 30 rotor was 105,650XG and in the titanium 50 rotor it was 226,400XG. Unless otherwise specified, the temperature was maintained at 2-3° C.

The low-density lipoproteins were isolated from the various samples by the procedures illustrated in the flow diagram presented in figure 2. The procedures used were a modification of those described by Martin et al. (1964). Fresh or dried yolk equivalent to 30 gms. of yolk solids was diluted with enough water to give 90 gms. of water in the final mixture. The mixture was prepared in a 250 ml. beaker, the beaker was covered with parafilm, and the mixture was stirred in a cold room at 2° C. with a magnetic stirrer for 3 to 4 hours. This solution was poured into the centrifuge tubes and spun at top speed in the 30 rotor for 2 hours in order to sediment and pack the granules. The supernatant plasma, which contained most of the low-density lipoproteins, was poured into a

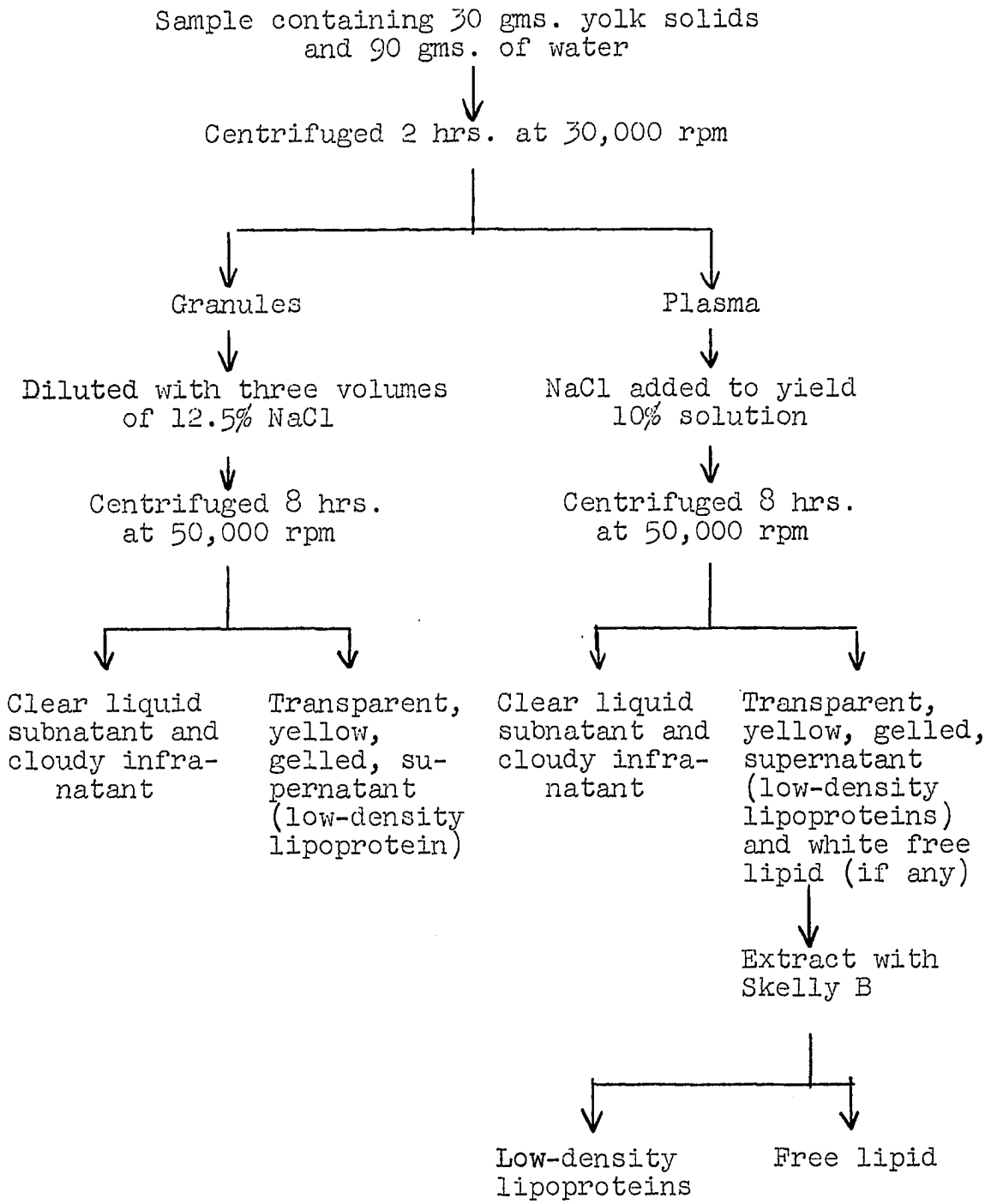


Figure 2. Flow diagram for isolating low-density lipoproteins and free lipid from fresh and dried yolk

beaker and diluted with enough solid sodium chloride to yield a final concentration of 10%. This was again stirred in a cold room for 1 or 2 hours and then poured into centrifuge tubes and centrifuged at top speed in the titanium 50 rotor. After 8 hours the low-density lipoproteins had floated and sufficiently compacted to allow the clear subnatant solution to be drained from the tube by puncturing the bottom of the tube. In addition to the gelled supernatant and the clear subnatant, there was also a very small amount of a cloudy infranatant solution just below the semi-solid supernatant. This probably also contained some low-density lipoproteins along with other proteins (c.f. Saari, 1963), but under our centrifugation conditions there was such a small amount of it that we did not consider it to be worth the effort required to separate and purify it. The transparent, yellow, gelled supernatant was scraped from the centrifuge tube and weighed. The moisture content of this low-density fraction was determined, and the amount of moisture-free low-density lipoproteins in the plasma, as a percentage of the total egg yolk solids, was calculated.

The granules, which were sedimented in the first step, were dissolved in three volumes of 12.5% sodium chloride by stirring on a magnetic stirrer in a cold room for 5 to

6 hours. This solution was poured into centrifuge tubes and centrifuged at top speed in the titanium 50 rotor. After 8 hours the low-density fraction had compacted at the top of the tube, and this fraction was treated the same as the low-density fraction from the plasma. The total amount of moisture-free lipoproteins as a percentage of egg yolk solids was then determined.

The low-density lipoproteins which were analyzed by analytical ultracentrifugation were further purified by repeatedly suspending them in a 10% sodium chloride solution and floating them by ultracentrifugation until no bands could be detected in the separating gel by disc electrophoresis.

Moisture determinations were made in a vacuum oven at 98-100° C. At least duplicate determinations were always made and often triplicate or quadruplicate samples were averaged.

Electrophoresis Disc electrophoresis was done on the Canalco model 12 disc electrophoresis apparatus, which is manufactured by Canal Industrial Corporation, Rockville, Maryland. Gels were made up from the basic ingredients as described by the Chemical Formulation for Disc Electrophoresis sheet published by Canal Industrial Corporation in April 1965. The standard 7% gel with a pH of 9.5 was used. The "upside-down technique" described in the above mentioned instructions was used in

these experiments. From 200 to 500 micrograms of proteins or lipoproteins were used. The gels were stained with analine blue black to detect proteins and with Sudan Black B to detect lipoproteins.

Analytical ultracentrifugations Analytical ultracentrifugations were made with a Beckman model E analytical ultracentrifuge. Epon-aluminum, $2\frac{1}{2}^{\circ}$, 12 mm, double sector centerpieces were used in an AN-D rotor at a speed of 29,500 rpm. Schlieren optics were used and the rotor was maintained at a temperature of 26° C. A lipoprotein concentration of 1.5% (W/W) was dissolved in a 1.745 molal sodium chloride solvent (γ = 1.063 gms./ml. at 26° C.).

Photomicrography Photomicrographs were made with a Zeiss photomicroscope equipped with an automatic camera exposure device. The drops of yolk photographed were placed on a microscope slide, which was on the microscope stage, and dried by shining a desk lamp on the yolk drops. The drops were then photographed at intervals during the time they were drying.

Heat treatments Egg yolk and isolated low-density lipoproteins were heat treated to determine the amount of lipid released due to heat denaturation of the proteins. The samples to be heat treated were weighed into tared 250 ml. beakers, a teflon covered magnetic stirring bar

was added to the beaker, and the beaker was covered with parafilm. The beaker was then submerged to about two-thirds of its depth in a bath of glycerol maintained at the proper temperature by means of a heated magnetic stirring plate. The yolk or lipoprotein was stirred and heated for 10 minutes in this manner, and then the beaker was removed and cooled. The yolk or lipoprotein was then extracted with petroleum ether.

Free lipid extraction The free lipid was extracted from the heat treated samples, as well as from certain dried samples, by a rapid extraction with re-distilled petroleum ether (Skelly B, B.P. = 66 to 68° C.). The Skelly B was mixed with the sample as thoroughly as possible with a glass stirring rod and then this mixture was carefully transferred to a teflon lined 450 ml. Lourdes homogenizer container. This was connected to a Lourdes model VM volu-mix homogenizer and was mixed at a speed of approximately 10,000 rpm for one minute. The mixed solution was then carefully transferred to a funnel and filtered through Whatman number 1 filter paper into a tared 250 ml. beaker. The residue on the filter paper was rinsed three successive times with aliquots of Skelly B. After filtration was completed, the solvent was evaporated on a steam bath and the lipids dried in a forced air oven for 30 minutes at 105° C. The beaker

containing the fat was then weighed and the percentage of theoretical fat extracted was calculated. (The term "free lipid" will be applied to lipid which is extracted by this technique throughout the remainder of this thesis.)

Phosphorus analyses The phosphorus in the samples was estimated by a modification of the procedure described by Taussky and Shorr (1953). From 10 to 90 mg. of sample (depending on the estimated phosphorus content) was weighed into a 16 x 150 mm culture tube. Three-fourths of a ml. of an acid mixture (consisting of 70% concentrated perchloric acid, 20% concentrated nitric acid, and 10% concentrated sulfuric acid) was added to the tube along with one or two clean glass boiling beads. The tubes were placed on an electrically heated micro-kjeldahl digestion rack. The mouths of the digestion tubes were placed in a fume tube which was connected to a polyethylene water aspirator. The heat was controlled by means of a rheostat. The solutions were digested for 10 minutes at a rheostat setting of 80 volts, and then for 45 minutes on a rheostat setting of 115 volts. After the tubes were cooled, 12 mls. of water was added to each tube and the contents thoroughly mixed. Two mls. of this solution was then transferred to a clean test tube and one ml. of color reagent was added. The mixture was shaken thoroughly and after five minutes the absorbancy

was measured at 820 mμ in a Spectronic 20 colorimeter equipped with the red phototube and red filter. Standards made from KH_2PO_4 were run with each set of determinations. A blank, prepared by mixing 0.75 ml. of digestion mixture with 12 mls. of water and combining 2 mls. of this with 1 ml. of color reagent, was used to zero the instrument. The amount of phosphorus, as a percentage of the total lipid extracted, was then calculated. The percentage of phosphorus was then multiplied by 26 to obtain the percentage of phospholipid extracted.

RESULTS AND DISCUSSION

Laboratory Dried Samples

Photomicrography

The English group working on dried eggs during World War II demonstrated that one of the major effects of sugars, which were added to whole egg, was to minimize the loss of foaming power which otherwise occurs shortly after the eggs are spray dried. Joslin and Proctor (1954) demonstrated that free lipid is present in plain dried yolk, and that this free lipid is the material which prevents dried egg yolk from foaming upon rehydration and beating. They presented microscopic evidence that lipid is released and coalesces during the drying of egg yolk, but conducted no studies on the effects of the addition of carbohydrates to egg yolks prior to their being dried.

The photomicrographs on page 71 are similar to those published by Joslin and Proctor (1954). Figure 3 is of fresh egg yolk immediately after it has been placed on the microscope slide; essentially before any drying has taken place. The structures described by Romanoff and Romanoff (1949) as being yolk globules can be seen, but no free lipid is visible. What Romanoff and Romanoff (1949) described as granules can be seen within some of the yolk globules. Figure 4 is of the same drop of yolk after it has been dried for a short time. Small fat

Figure 3. Fresh egg yolk before drying begins (300X)

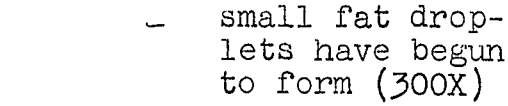


Figure 4. Fresh yolk after drying has begun and small fat droplets have begun to form (300X)

Figure 5. Drying yolk showing fat droplets beginning to coalesce (300X)

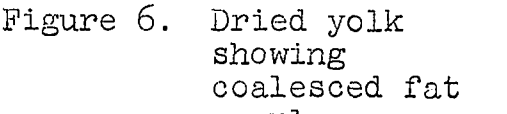
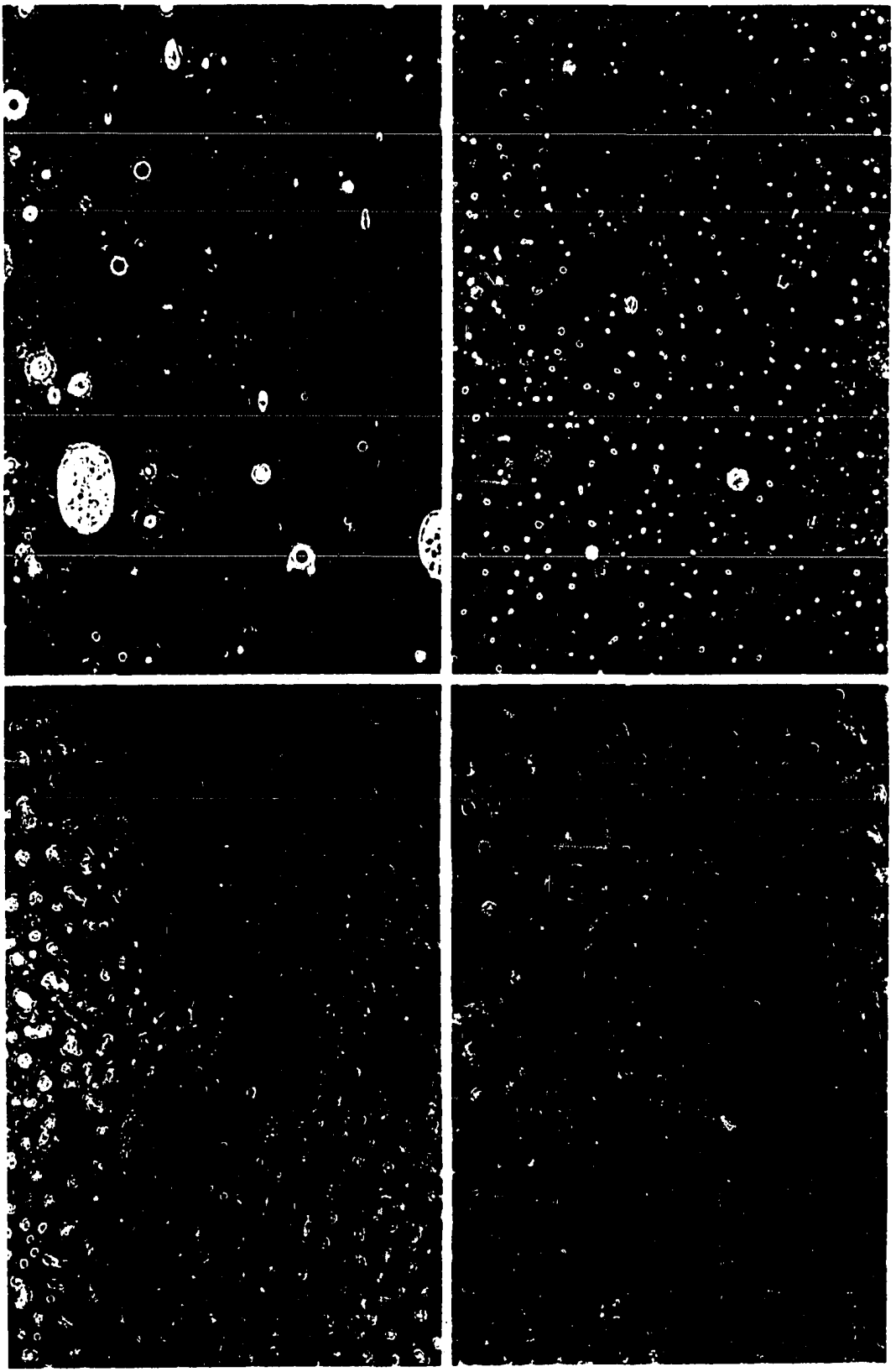


Figure 6. Dried yolk showing coalesced fat nearly covering surface of drop of yolk (300X)



droplets which were beginning to form on the surface of the drying yolk can be observed. In figure 5 the fat droplets are beginning to coalesce and in figure 6 the fat has coalesced until it has nearly covered the surface of the yolk. It was established that the coalesced material was lipid by demonstrating that osmium tetroxide vapors would stain the material black.

The pictures on page 74 are of drying yolk and drying yolk lipoproteins. Figure 7 is of yolk dried to about the same extent as the yolk in figure 6, but the yolk in figure 7 had 10% maltose added to it prior to being dried. Even though the yolk is so dry that a surface film has hardened over the drop of yolk, it is readily apparent that no lipid was released to coalesce as it was in the pictures on page 71. Figure 8 shows yolk again dried to about the same extent as the yolk in figure 6, but in this case 4% maltose was mixed with the yolk prior to drying. Some lipid can be seen to have coalesced, but it is apparent that there is not nearly as much visible lipid present as in figure 6. The yolk in figure 9 had 2% maltose added to it, and the amount of coalesced lipid on the surface of the drying yolk appears to be somewhere between the amount in figure 6 and that in figure 8.

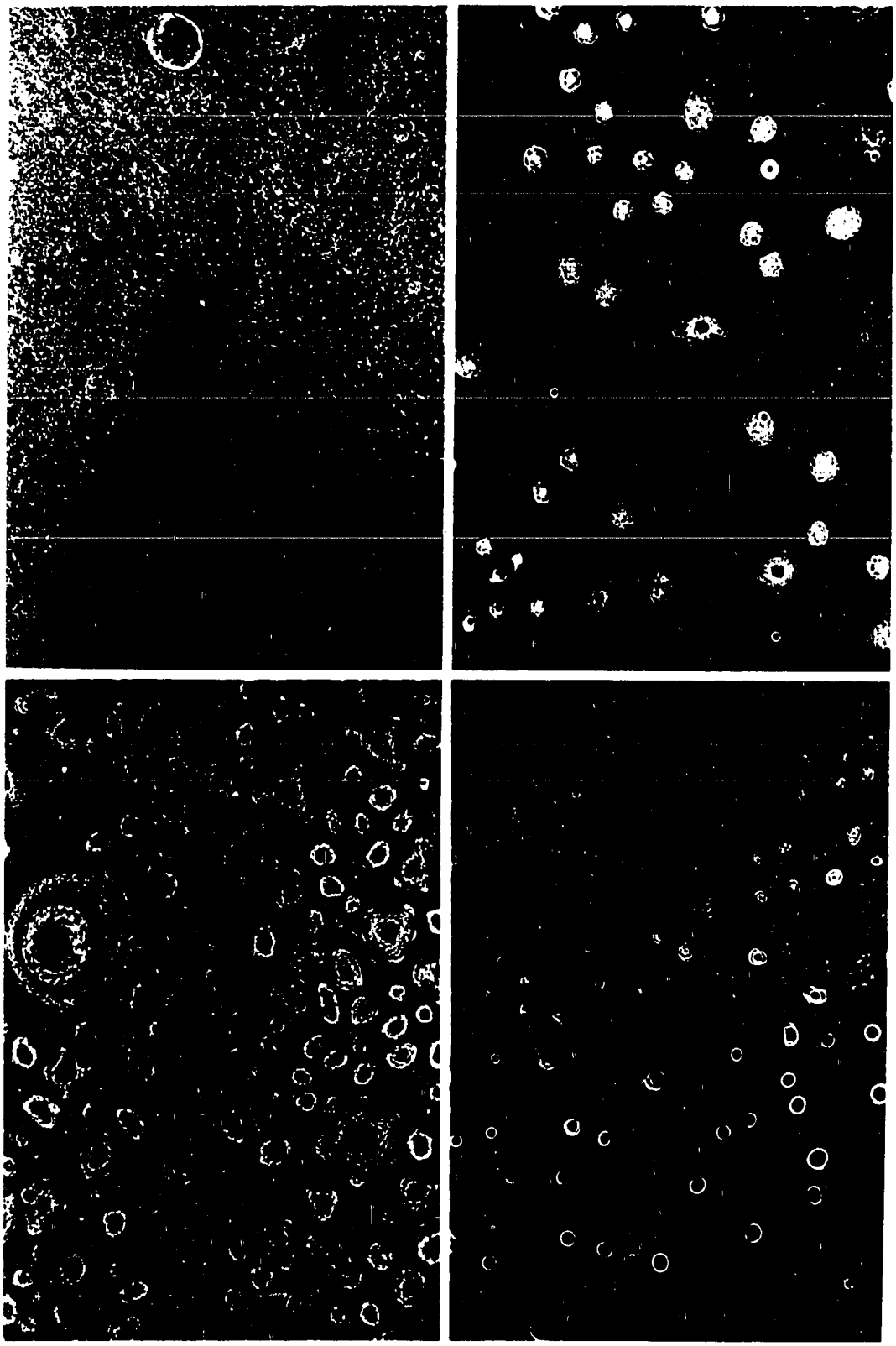
Figure 10 is a photomicrograph of low-density lipoproteins isolated from fresh egg yolk. Its stage of

Figure 7. Dried yolk with 10% maltose added prior to drying illustrating that no fat is released to coalesce (300X)

Figure 8. Dried yolk with 4% maltose added prior to drying illustrating that only a small amount of fat coalesces (300X)

Figure 9. Dried yolk with 2% maltose added prior to drying showing an intermediate amount of fat coalesced on the yolk surface (300X)

Figure 10. Low-density lipoproteins isolated from fresh egg yolk which are in an intermediate stage of drying (300X)



drying is between the stage of drying seen in the yolk in figure 4 and the stage seen in the yolk in figure 6. As it dries lipid droplets form on the surface and coalesce as they do in fresh egg yolk. The low-density lipoproteins isolated from fresh yolk, from rehydrated plain spray dried yolk, and from rehydrated spray dried yolk with added carbohydrates were all found to look and behave similarly when examined and dried under a microscope. Since the lipoproteins isolated from the sugar-dried yolk and the plain spray dried yolk both tend to release lipid upon drying, the sugar apparently does not undergo an irreversible association with these lipoproteins. Instead, when the yolk is rehydrated and the low-density lipoproteins isolated, the sugars must dissociate themselves from the lipoproteins, for if the sugars remained with the lipoproteins upon drying the fat would not be expected to be released and coalesce. The major noticeable difference between the low-density lipoproteins and the fresh yolk, when examined under a microscope, is the lack of globules in the low-density lipoprotein fraction.

As the water of hydration is removed from the surface of a low-density lipoprotein micelle the micelle loses its ability to maintain its micellular structure. As it breaks down, most of the neutral lipids and some of the phospholipids, which formerly were in the center of the

micelle, are released and begin to coalesce with lipids which are released from other micelles. This is the lipid which can be seen forming lipid droplets under the microscope and is the free lipid which inhibits the foaming ability of dried yolk or dried whole egg. Since no lipid could be seen to have coalesced on the dried yolk which had 10% maltose added to it prior to drying and since less free lipid coalesced on the yolk containing 4% and 2% added maltose (than coalesces on the surface of yolk dried without any added maltose), it was hypothesized that the added sugars preserve the foaming ability of dried yolk by preventing the release of free lipid.

Heat treatments

Since the low-density lipoproteins have a hydrated protein coat on their micellular surfaces, an experiment was designed to determine if heat denaturation of the protein portion of the lipoprotein micelle would release free lipid. Preliminary experiments indicated that no lipid is extracted from fresh egg yolk with Skelly B using the extraction procedure described in the section on methods and materials. Samples of fresh yolk and fresh yolk plus 10% maltose, fresh yolk plus 10% sucrose, fresh yolk plus 10% lactose, and fresh yolk plus 10% glucose were heated at 60°, 70°, 80°, 90°, 100°, and 110° C. for 10 minutes. Figure 11, which was taken from table 3 in the appendix, shows the amount of lipid, expressed as a percentage of total theoretical lipid present,

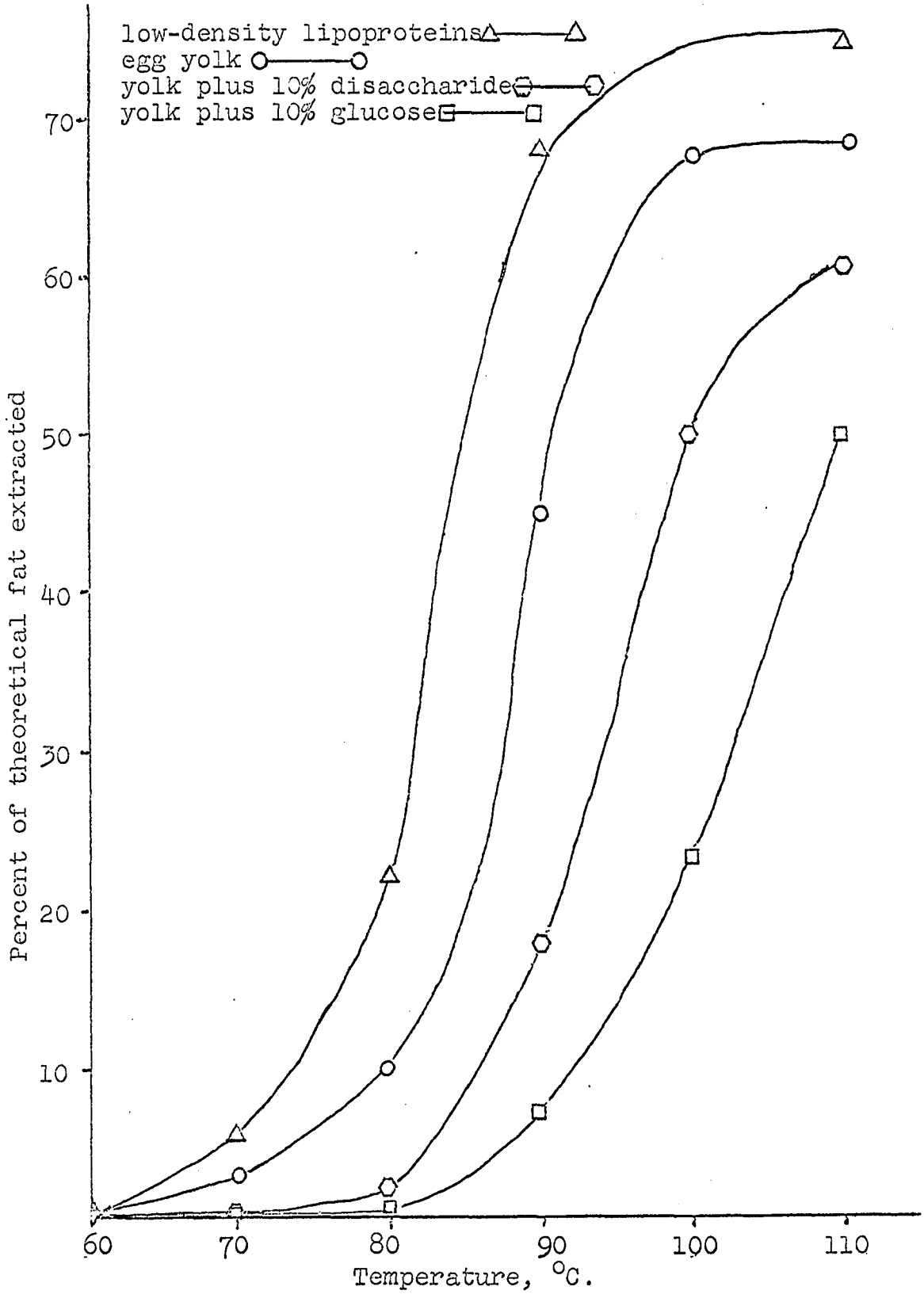


Figure 11. Heat treatment vs. lipid extractability

which was readily extracted from the heated samples with Skelly B. Only one line was drawn to represent the lipid extracted from the yolk samples with the added maltose, sucrose, or lactose since the results with these three sugars were all within experimental error of each other.

The yolk which has had sugar added to it must be heated to approximately a 10° C. higher temperature than yolk without the added sugar before it begins to release free lipid. At each temperature, the sugared yolk releases less lipid than the non-sugared yolk. The samples containing the three disaccharides all released equivalent amounts of lipid at equal temperatures, but the sample containing the monosaccharide released considerably less lipid than the others. Therefore, one way in which sugars probably protect egg yolk against the damaging effects of spray drying is by protecting the protein portion of the lipoprotein micelle against heat denaturation. Kline et al. (1964) reported that free lipid extraction in sugar-dried egg exhibited a dependancy which was based on the molecular size of the sugar added to the liquid egg as well as on the amount of the sugar added. The results reported here indicate a similar phenomenon occurs in heated yolk since a smaller molecular weight sugar prevents free

lipid release to a greater extent than the same amount of a larger molecular weight sugar.

It should be kept in mind that the temperatures to which the yolk was heated in these experiments are not the temperatures to which the yolk is heated during spray drying. Since the yolk is dispersed in a fine spray during spray drying the outside of the droplets of egg probably receive more heat, and consequently the proteins on the outside would undergo more heat denaturation than the inside of the droplets. However, whether or not heat denaturation is a major factor in releasing free lipid in spray dried yolk, these experiments show that if the lipoproteins undergo enough heat denaturation free lipid will be released. They also indicate that added sugar will reduce the amount of free lipid released at a given temperature.

Since over 90% of the total lipid in egg yolk is present in the low-density lipoproteins and the amount of lipid easily extracted from dried egg yolk (in the dried state) is about 70% of the lipid theoretically present, it is obvious that most of the free lipid in dried egg has to come from the low-density lipoproteins. Since Lea and Hawke (1951) demonstrated that only 30% of the theoretical lipid present in dry lipovitellin

(which contains about 10% of the total lipid present in yolk) is extractable with ether, it is apparent that this amount of lipid could not be responsible for the large amount of lipid easily extracted from dried yolk.

Isolated low-density lipoprotein was also heated and extracted with Skelly B. Its pattern of free lipid release was similar to that of fresh yolk, but at equivalent temperatures even more lipid was released than in the fresh yolk. The ultimate amount of lipid released is also somewhat greater for the low-density lipoproteins than for the fresh yolk. The greater percentage of lipid released from the low-density lipoproteins is not surprising when the amount of lipid associated with the high-density lipoproteins (lipovitellins) is considered.

Moisture levels

The next series of experiments indicate that the moisture content of egg yolk or isolated low-density lipoprotein can affect the lipid extractability of these materials to about the same extent as can heat denaturation of the proteins. Rotary evaporation was used to dry samples of yolk and samples of isolated low-density lipoproteins to various moisture contents. Since the temperature was maintained at 40° C. during the drying, it was assumed that the proteins were not damaged by heat. Figure 12, which was taken from table 4 in the appendix,

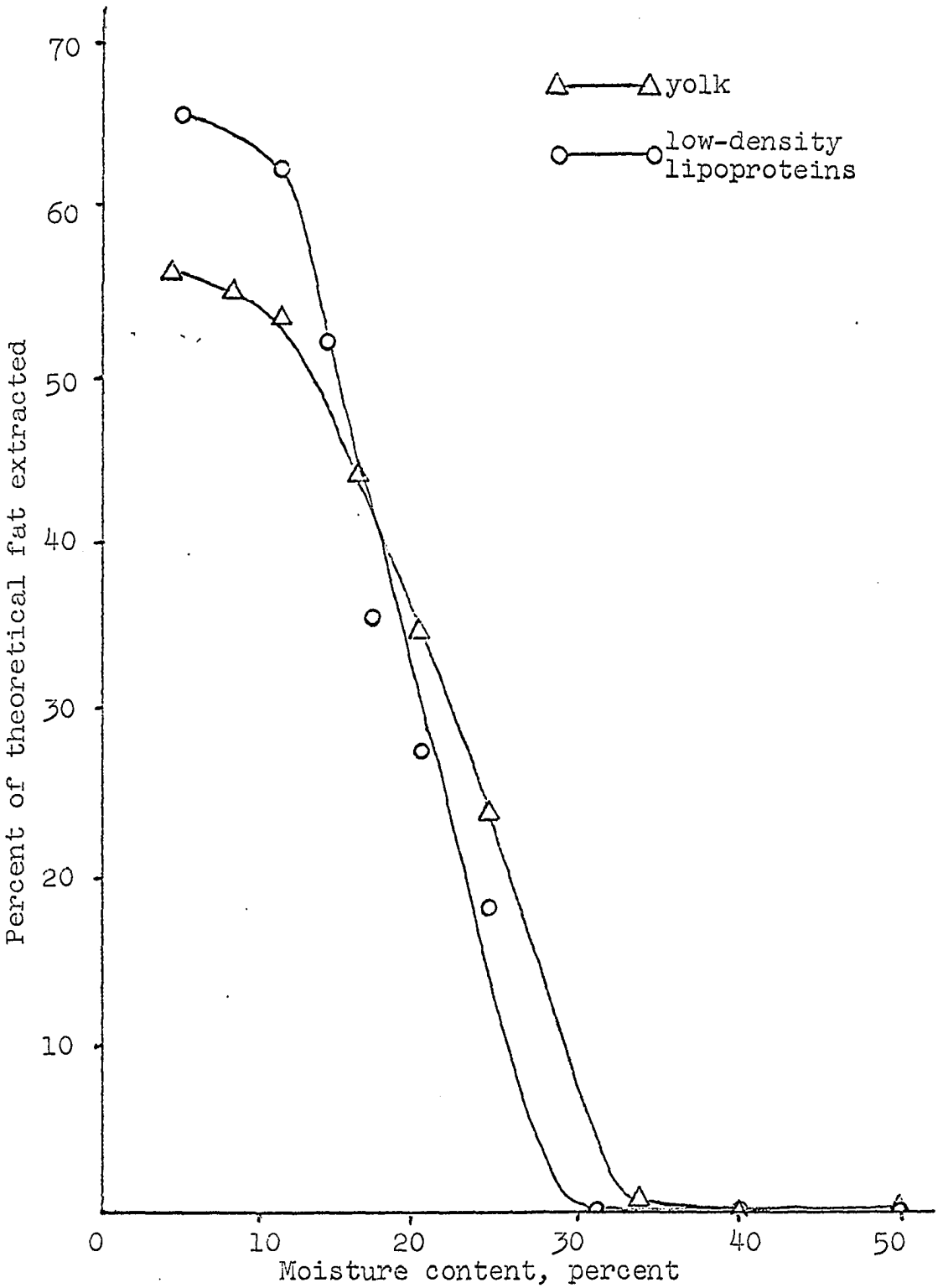


Figure 12. Moisture content vs. lipid extractability in yolk and low-density lipoproteins

shows a graph of the amount of lipid easily extracted from egg yolk and isolated low-density lipoprotein plotted against various moisture contents.

The curves comparing the percentage of theoretical lipid extracted by Skelly B against the moisture content of the sample are very similar for the low-density lipoproteins and for egg yolk. The low-density lipoproteins do not begin releasing lipid until they reach a slightly lower moisture level than the moisture level where fresh yolk begins to release free lipid. The ultimate amount of lipid released by the low-density lipoproteins is also somewhat greater than the amount released by the yolk, but this is not surprising considering the differences in the two. Preliminary trials demonstrated that the drying time could be decreased by increasing the vacuum during rotary evaporation without changing the moisture content vs. free lipid pattern. Thus this was done in these experiments to conserve time as well as to decrease the danger of bacterial degradation.

Martin et al. (1964) found that with extremely high speed centrifugation for prolonged times they were able to reduce the moisture content of the low-density fraction lipoproteins to 34% and speculated that somewhat less than this amount of water must be present as water of hydration on the surface of the micelles. Saari (1963)

found that approximately 40% of the hydrated weight of the low-density lipoproteins he had isolated was water. He speculated that a moisture content somewhat lower than this was required to alter the properties of the lipoproteins.

In this study the isolated lipoproteins also contained approximately 40% water. If the release of free lipid is considered to be a criterion of lipoprotein alteration, from the results reported here, it would appear that a moisture content of about 30% is required for the lipoprotein micelle to maintain the type structure which prevents the lipid from being easily extracted with non-polar lipid solvents. It can be concluded that the moisture content of egg yolk plays a significant role in determining the amount of easily extractable lipid in egg yolk and in low-density lipoprotein isolated from egg yolk.

When egg yolk is dried, it is believed the added sugar replaces the water of hydration at its binding site in the lipoprotein micelles thus preserving the micellular structure. The graph in figure 13 illustrates that if enough sugar is added to yolk prior to drying, the amount of lipid extracted by Skelly B after drying will be significantly reduced. The yolk used in this experiment was lyophilized and extracted in the dried

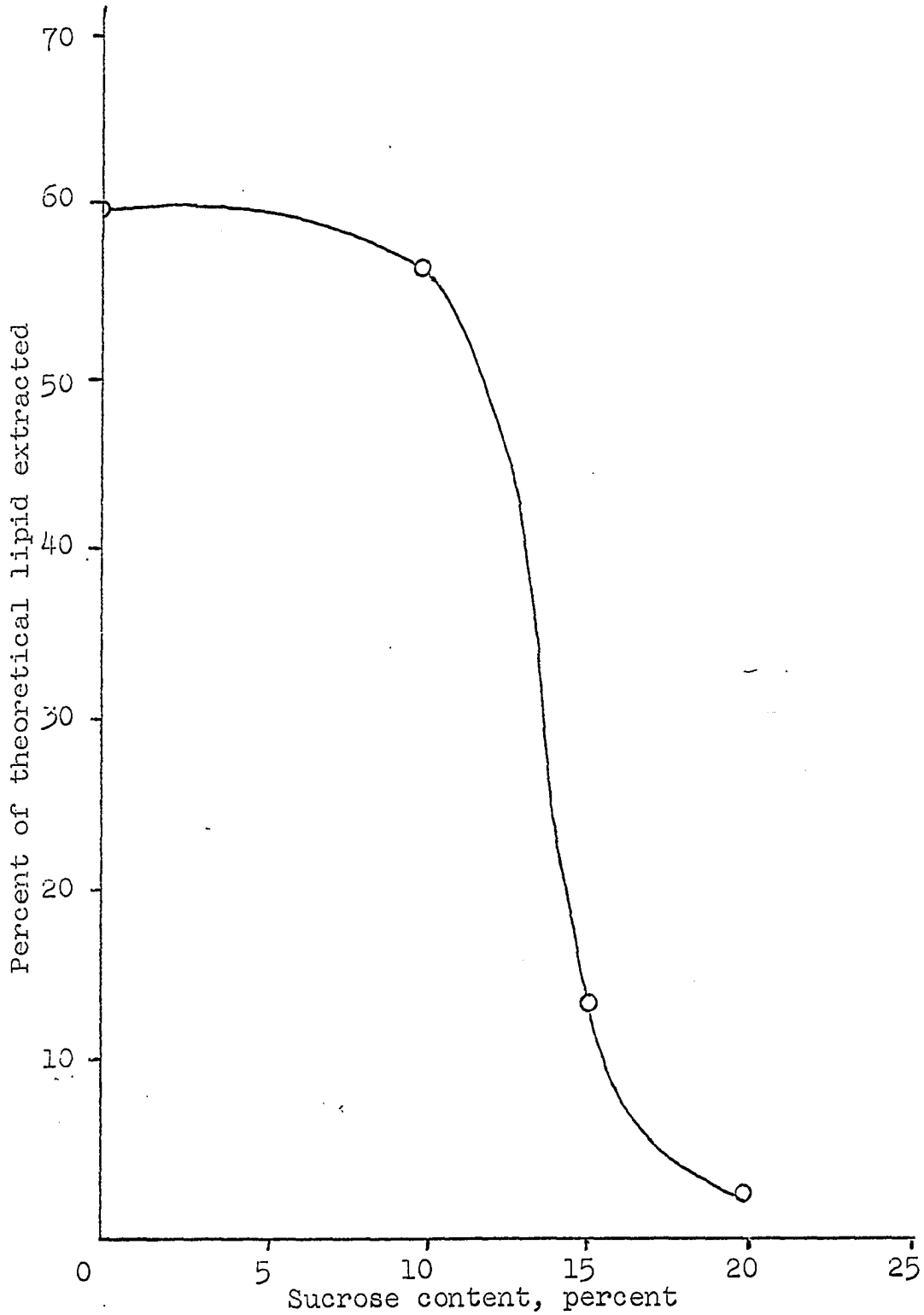


Figure 13. Sucrose content vs. lipid extractability

state. In figure 13 the "breakpoint", that is the level of added sucrose where the lipid extractability is sharply reduced, occurs when between 10 and 15% sucrose, on a liquid yolk weight basis, is added to the egg yolk. These results are in general agreement with those of Kline et al. (1964). It is not necessary to add sugar past this breakpoint to preserve most of the functional performances of the dried egg. This is fortunate since egg which contains enough added sugar to exceed this breakpoint has been reported to be highly susceptible to oxidative rancidity (Kline et al., 1964).

Commercially Spray Dried Samples

Lipid extractability

In the commercially dried yolk it was believed that the severity of the drying conditions might alter the lipid extractability in the dried egg yolk. Kline et al. (1964) had reported that plain dried yolk retains most of its original aerating ability if it is rehydrated and beaten within one hour after being spray dried, but that after three hours it loses its aerating ability. However, they had found the lipid extractability of the samples to be virtually identical immediately after spray drying and after several days of storage. Since we were using a lipid extractability procedure which varied considerably from that used by Kline we also

checked the extractability of lipid against storage time after drying. Samples of high, medium, and low viscosity plain spray dried yolk and samples of high and low viscosity sugar-dried yolk were extracted. After drying the dried yolk was collected in 5 pound bags, the first sample taken immediately, and the rest of the bag stored in a cold room at about 1° C. The samples were thereafter stored in a cold room at about 1° C. during the test period.

Table 2 shows the amount of lipid, expressed as a percentage of the total lipid theoretically present, extracted from the samples after various storage times.

Table 2. Percent of lipid extracted from spray dried yolk[a]

Time After Drying	Plain			Sugared	
	High Viscosity	Medium Viscosity	Low Viscosity	High Viscosity	Low Viscosity
10 min.	73%	71%	70%	67%	68%
1 hr.	74%	72%	70%	68%	69%
3 hrs.	73%	71%	70%	69%	68%
4 days	72%	71%	70%	69%	68%
12 days	71%	69%	69%	69%	68%
31 days	71%	71%	70%	69%	68%

[a]Average of two determinations

In this experiment the extractions were made on the dried samples. There are no large differences in the amount of extractable lipid between any of the samples. There does seem to be a slight tendancy for the sugared samples to have

slightly less free lipid than the plain samples. The sugared samples had from 68 to 69% free lipid, while the plain samples generally had from 70 to 73% free lipid. The low viscosity plain dried yolk had a tendancy to have slightly less lipid extracted from it (70%) than did the high viscosity plain dried yolk (71-73%).

While the differences in the amount of total lipid extracted from the plain and sugar-dried yolk were small, there were considerable differences in the amounts of phospholipids extracted from the two types of dried yolk. The amount of phospholipid extracted with Skelly B averaged about 35% of theoretical from the plain dried yolk and about 2% of theoretical from the sugar-dried eggs. (See table 5 in the appendix.)

The amount of lipid readily extracted from the spray dried yolk without added carbohydrates is somewhat greater than the amount of lipid extracted from yolk dried by rotary evaporation or lyophilization (when both are extracted in the dried state; 70 to 74% vs. 55 to 60%). The greater amount of lipid extracted from the spray dried product is probably because more protein damage occurs in the spray drying process than occurs during drying by the other methods. The protein damage during spray drying then releases more lipid.

The viscosity of the plain spray dried yolk was measured with the number 3 spindle in the Brookfield viscometer. The viscometer dial reading was 18.2 for the low viscosity sample, 34.5 for the medium viscosity sample, and 62.0 for the high viscosity sample. The viscometer dial readings, using the number 2 spindle, were 12.7 for the low viscosity sugar-dried yolk and 44.8 for the high viscosity sugar-dried yolk. Since the number 2 spindle is much larger than the number 3 spindle, the viscosity of the sugar-dried yolk was much less than the viscosity of the plain dried yolk. These viscosities vary considerably more than commercial samples ordinarily would; even from different sources.

In addition to the differences in viscosities between different samples, there were also differences in the amount of precipitate sedimented in the first step of the isolation of the low-density lipoproteins. (See table 6 in the appendix.) The amount of precipitate on a dry weight basis, sedimented from 30 gms. of egg yolk solids was 11.3 gms. of precipitate from the low viscosity yolk, 12.6 gms. from the medium viscosity yolk, and 14.3 gms. from the high viscosity yolk. The amount of precipitate is assumed to be an indication of the amount of protein denatured. Since the amount of precipitate in the samples increases in the same order as

the viscosity of the samples, it was concluded that both precipitate and viscosity are related to protein denaturation in spray dried yolk.

While the total time of extraction for the procedure used here did not exceed 20 minutes, we found that Skelly B extracted as much free lipid from freshly spray dried yolk as it did from yolk stored for longer periods of time. Qualitative observations indicated that the freshly dried egg yolk foamed much more voluminously than did egg yolk which had been dried for some time. Since the sugar-dried yolk also foamed well even after long storage times, these findings would appear to be in conflict with the conclusion that free lipid is the substance which inhibits the foaming ability of plain spray dried yolk. The most logical explanation for this apparent contradiction is that in the very freshly dried egg yolk the lipoproteins have not yet undergone irreversible changes, and when water is added to the system they rehydrate and reassociate with lipid which would be readily extracted from the spray dried yolk with Skelly B. If the dried yolk is stored for a short time, irreversible changes occur and not all of the lipid is reabsorbed by the lipoprotein micelles when rehydrated. Apparently a similar phenomenon occurs in the sugar-dried yolk, but in sugar-dried yolk the sugar prevents the irreversible changes from occurring and the

lipoproteins are able to reassociate with all, or nearly all, of the lipid, thus the yolk will foam when rehydrated even after prolonged storage times.

Isolation of low-density lipoproteins

Low-density lipoproteins were isolated from fresh yolk and from plain and sugared samples of dried yolk. Figure 14 illustrates the amount of low-density lipoprotein extracted from the yolk and the amount of lipid easily extracted from the rehydrated and purified lipoproteins. Both the lipoprotein and the lipid are calculated on the basis of the percentage of lipoprotein or lipid theoretically present. The amount of low-density lipoproteins as a percentage of total dry weight is slightly greater in these experiments than in those reported by Martin et al. (1964); (i.e., 72% in this case as compared to 69% as reported by them). This small difference is probably not of any significance, however.

Fresh yolk has a considerably greater amount of low-density lipoprotein than does the plain spray dried yolk and has a tendancy to have a slightly greater amount of lipoprotein than the sugar dried yolk. It is also of interest that the rehydrated lipoproteins from the plain dried yolk have a considerable amount of easily extractable lipid, ranging from about 3 to 5%. The rehydrated and isolated low-density lipoproteins from the low viscosity

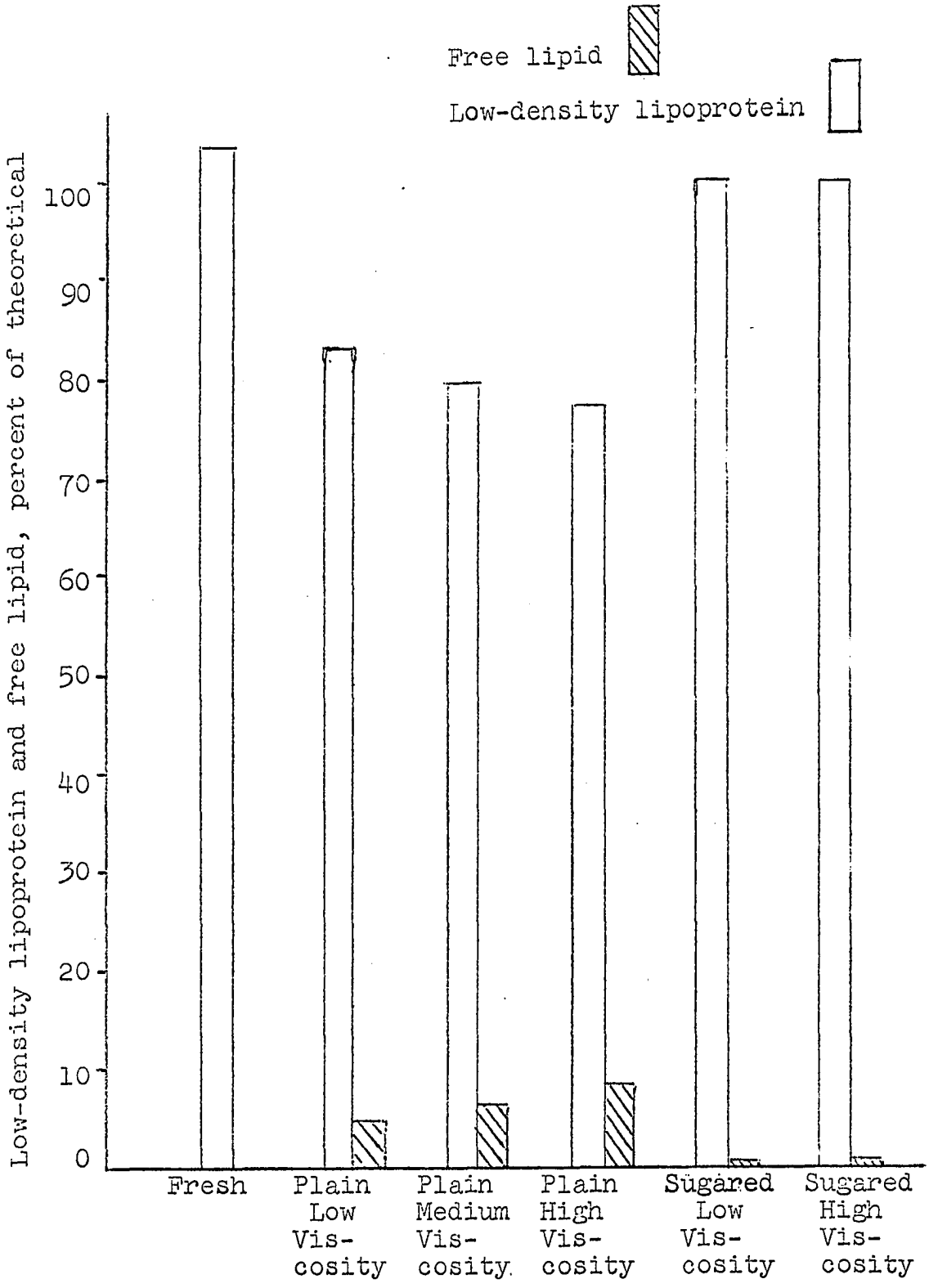


Figure 14. Lipoprotein and free lipid extracted from fresh and rehydrated yolk

yolk had the most free lipid. Thus it appears that the conditions of drying do affect the amount of lipid which remains free after rehydration.

This dried yolk (when extracted in the dry state) had about 70 to 72% of its lipid extracted by petroleum ether, however after rehydration, it only had from 5 to 8% of its lipid extracted by Skelly B. Thus it is obvious that most of the lipids which were extractable from the dried yolk are unextractable with Skelly B from the rehydrated yolk. Some of the lipid does remain free, however, and this lipid, which is seen to be much greater in the plain dried yolk than in the sugar-dried yolk, is what inhibits the foaming ability of the plain dried yolk.

Since spray dried yolk which is rehydrated immediately after being dried has been shown to foam (Kline et al., 1964), it is hypothesized that if the low-density lipoproteins were isolated from this rehydrated yolk that there would be nearly as much lipoprotein in it as in the fresh yolk. Since it does foam well, it is also hypothesized that there would be very little free lipid found in the rehydrated yolk or in the lipoproteins isolated from this yolk.

Electrophoresis

The low-density lipoproteins isolated from egg yolk were checked for purity by disc-gel electrophoresis. When

subjected to electrophoresis in polyacrylamide gel, fresh egg yolk is resolved into 15 or 16 bands of varying intensity. Under the same electrophoretic conditions, purified low-density lipoprotein shows no bands in the separating gel. Instead the sample gel at the top of the disc-gel column will be stained by a protein or a lipid stain. Thus the purified lipoproteins do not migrate into the polyacrylamide. This is probably because the pore-size of the 7½% polyacrylamide is too small to allow penetration by the large low-density lipoprotein micelles. When the lipoproteins from the first floatation step were checked by disc-gel electrophoresis, there were several light bands in the separating gel as well as heavily staining material in the sample gel. After redissolving the low-density lipoprotein in a 10% sodium chloride solution and refloating them 3 or 4 different times, the separating gel showed no bands. The low-density lipoprotein was then considered to be freed from contaminating proteins. When purified the low-density lipoproteins were then analyzed with an analytical ultracentrifuge.

Analytical ultracentrifugations

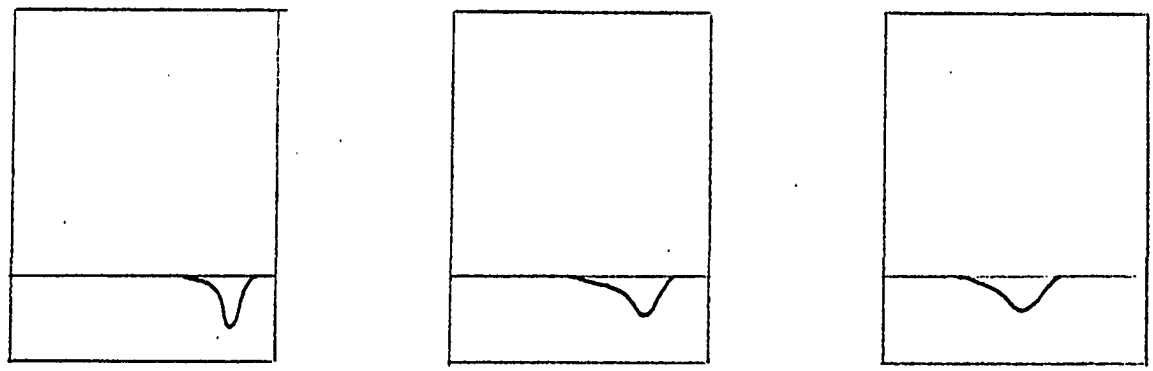
Figures 15, 16, and 17 show the analytical ultracentrifugal patterns of isolated low-density lipoproteins from fresh egg yolk, from plain spray dried egg yolk, and from spray dried egg yolk with added carbohydrates.

Figure 15. Ultracentrifugal flotation pattern of low-density lipoprotein isolated from fresh yolk

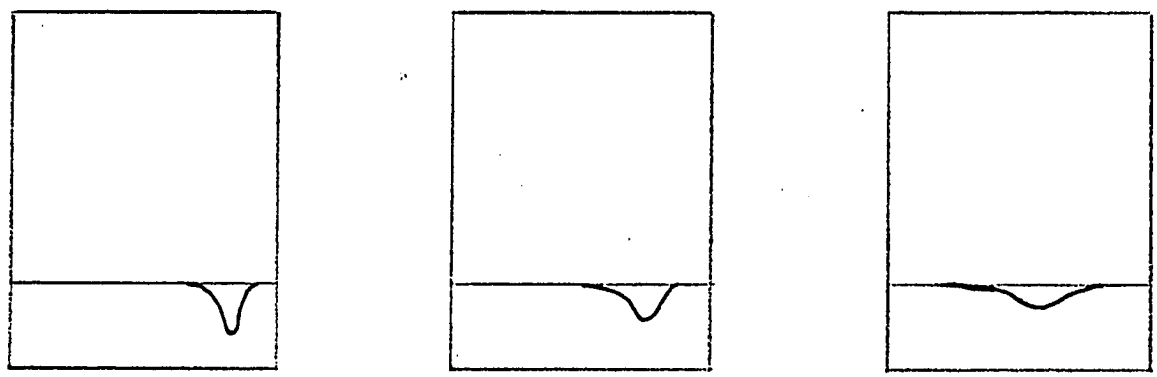
Figure 16. Ultracentrifugal flotation pattern of low-density lipoprotein isolated from rehydrated spray dried yolk with added carbohydrates

Figure 17. Ultracentrifugal flotation pattern of low-density lipoprotein isolated from rehydrated spray dried yolk without added carbohydrates

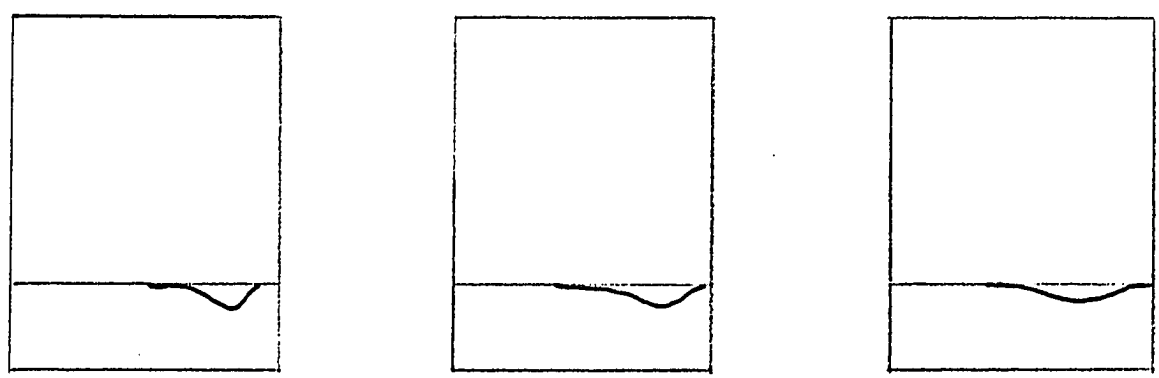
Direction of flotation



2 Min. 6 Min. 10 Min.



2 Min. 6 Min. 10 Min.



2 Min. 6 Min. 10 Min.

All pictures were taken at the same time after centrifugation was begun. Because of excessive spreading in the peaks of the lipoproteins isolated from the dried egg yolk, no flotation coefficients were calculated. Visual inspection of the peaks, however, indicates that the lipoproteins isolated from the dried egg yolk do spread more extensively than do the lipoproteins isolated from the fresh yolk. The greater spreading in the lipoproteins isolated from the dried yolk indicates a greater heterogeneity in lipoprotein density or micellular size. This heterogeneity probably occurs because of damage done to the lipoproteins during drying or storage.

If small amounts of lipid actually leak out of the lipoproteins during or after drying, and then become reassociated with the lipoproteins upon rehydration, this might lead to a more heterogeneous distribution of micellular sizes in the lipoproteins from dried yolk than is normally found in native low-density lipoproteins. Since spray dried yolk with greater amounts of carbohydrates have very small amounts of lipid extracted by fat solvents it would be interesting to study the lipoproteins isolated from yolk with these high amounts of sugar added prior to spray drying. If the flotation patterns of the lipoproteins isolated from yolk with these higher amounts of added sugars more closely

resembled the flotation patterns of native lipoproteins, it would indicate that little intermicellular lipid exchange had taken place.

In any event the flotation peaks of lipoprotein isolated from yolk with added carbohydrates spread less than the peaks from the plain dried yolk. This indicates that less damage has been done and/or that less lipid exchange has taken place between the lipoproteins isolated from the sugared yolk than has occurred in the lipoproteins from the plain dried yolk.

SUMMARY AND CONCLUSIONS

When observed through a microscope, fresh egg yolk can be seen to release free lipid while it dries. This free lipid can be seen as a coalescing substance on the surface of the drying yolk. Microscopically, fresh yolk and isolated low-density lipoproteins appear to release lipid in similar manners during drying. The addition of sufficient amounts of sugar to egg yolk before drying has been shown to decrease the amount of, or eliminate, the lipid which can be seen coalescing on the surface of the drying yolk.

Slightly over 90% of lipid present in fresh egg yolk is associated with the low-density lipoproteins. These lipoproteins have the neutral lipid in a core in the center of the micelle, a layer of phospholipid surrounding this neutral lipid, and have a hydrated protein overwrap covering the phospholipid. Upon drying the water of hydration on the outside of the lipoprotein micelle is removed and the micelle is no longer able to completely maintain its original structural integrity. It is hypothesized that the carbohydrates, through their hydroxyl groups, are able to partially substitute for the water of hydration and thus lend an added degree of stability to the lipoproteins during drying and during storage in the dried state.

Added sugars have been shown to protect the lipoproteins against heat denaturation. This effect of protecting the lipoproteins against heat denaturation has been shown to be associated with the molecular size of the added sugar as well as with the amount of sugar added. Since some heat is encountered during spray drying, the sugars may partially protect the proteins against heat denaturation in commercially spray dried yolk.

The removal of water from egg yolk without the addition of heat (by lyophilization or by rotary evaporation) has also been shown to release free lipid. The moisture level where free lipid begins to be released by the low-density lipoproteins has been considered to be an indication of a change in the micellular structure of the lipoprotein. This change occurs when the lipoproteins contain about 30% moisture on a wet weight basis. Sufficient amounts of sugar, when added to the liquid yolk, prevent the release of free lipid upon drying.

However, commercially it is not practical to add enough sugar to completely prevent the lipid from being extracted with non-polar fat solvents since these high levels of added sugar lead to rapid oxidative deteriorations in the dried egg. Since upon rehydration most of the lipid which was easily extracted from the yolk in

the dried state, again becomes much less extractable with non-polar lipid solvents, most of this lipid must become reassociated with the lipoproteins. Some lipid remains free upon rehydration, however, and this free lipid inhibits the aerating ability of the plain spray dried yolk. Even though not enough sugar is added to prevent the release of free lipid from the dried egg, the added sugar promotes the reassociation of the lipid with the lipoprotein micelles upon rehydration, and only a very minimal amount of lipid is easily extractable upon rehydration.

This sugar was also shown to protect the lipoproteins by analytical ultracentrifugal flotation. The flotation patterns of low-density lipoproteins isolated from spray dried yolk with added sugar were shown to indicate less heterogeneity of micellular structure than the flotation patterns of low-density lipoproteins isolated from spray dried yolk without added sugar. In the ultracentrifuge the lipoproteins from sugar dried yolk resemble the lipoproteins from fresh yolk, but differences indicated that some changes in micellular size or structure had taken place.

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APPENDIX

Table 3. Weight of lipid extracted from heated samples of yolk and lipoprotein[a]

Temp. °C.	Sample[b]					
	Plain Yolk	Yolk Plus 10% Maltose	Yolk Plus 10% Sucrose	Yolk Plus 10% Lactose	Yolk Plus 10% Glucose	Low-density Lipoprotein
60	0.01	0.02	0.02	0,02	0.02	0.03
70	0.11	0.01	0.02	0.02	0.01	0.45
80	0.26	0.09	0.04	0.05	0.02	0.73
90	1.46	0.53	0.55	0.59	0.35	5.81
100	2.18	1.68	1.66	1.77	0.74	6.49
110	2.20	1.97	1.89	2.04	1.65	6.69

[a]Average of 4 determinations

[b]Sample equivalent to 10 gms. of fresh yolk or low-density lipoprotein used in each determination

Table 4. Weight of lipid extracted from partially dried yolk and low-density lipoprotein[a]

Moisture Content Percent	Yolk[b]	Low-density Lipoprotein[c]
49	.00	.01
40	.00	.00
34	.04	.00
32	.18	.02
30	--	.05
26	1.52	.90
20	2.24	1.43
16	2.91	1.85
12	3.50	3.29
10	3.53	--
5	3.70	3.55

[a]Average of 2 determinations

[b]Sample was originally 20 gms. of fresh yolk

[c]Sample was originally 10 gms. of 40% moisture low-density lipoprotein plus 5 gms. of water added to dissolve sample

Table 5. Percent of theoretical phospholipid extracted from spray dried yolk[a,b]

Plain			Sugared	
High Viscosity	Medium Viscosity	Low Viscosity	High Viscosity	Low Viscosity
36.2%	38.1%	35.8%	2.46%	2.35%

[a]There was no difference between time of storage and percentage of phospholipid extracted; values are from yolk stored 4 days at 1° C.

[b]Average of 2 determinations on each of 2 different samples

Table 6. Percentage of various fractions in the isolation of low-density lipoproteins

	Fresh Yolk	Plain			Sugared	
		High Viscosity	Medium Viscosity	Low Viscosity	High Viscosity	Low Viscosity
Equivalent wt. of yolk solids	30 gms.	30 gms.	30 gms.	30 gms.	30 gms.	30 gms.
Wt. of ppt solids	7.2 gms.	14.3 gms.	12.6 gms.	11.3 gms.	--[a]	--[a]
Wt. of LDF[b] solids in supernatant	19.7 gms.	15.0 gms.	15.5 gms.	16.1 gms.	20.0 gms.	20.1 gms.
Wt. of LDF[b] solids in ppt.	1.9 gms.	1.1 gms.	1.0 gms.	1.1 gms.	1.0 gms.	1.0 gms.
% of lipid extracted from LDF	0.0%	7.7%	6.3%	4.8%	0.4%	0.5%

[a]Wt. not listed because of added sugar

[b]Low-density fraction lipoproteins