acid has been added to the ninhydrin indicator solution for this purpose. Formate, however, acts as its own reducing agent and can be detected with a ninhydrin solution free from added reducing compounds, thus providing a means of differentiating it from acetic acid.

This property of formic acid was confirmed with known mixtures containing either formate or acetate. However, when a chromatogram of the unknown volatile acids from menhaden oil was dipped in the ninhydrin solution free from ascorbic acid, all seven spots appeared, indicating that some reducing impurities were carried along all through the chromatogram.

Spot 1, with an R_f value of 0.13, is unknown. It is somewhat more polar than formic or acetic acid, but less so than keto (pyruvic) or hydroxy (lactic) acids which do not migrate from their point of application with the solvents used here. The other spots correspond, respectively, to formic (or acetic), acrylic, propionic, crotonic, butyric, and valeric acids.

The presence of these acids in menhaden oil is easily explained through autoxidation of aldehydes which, in turn, result from degradation of hydroperoxides. Loury (*11*) has suggested that aldehydes with six to nine carbon atoms readily undergo a stepwise degradation through autoxidation to the corresponding moloxide, followed by scission to yield formic acid and the next lower aldehyde. The ease with which this scission occurs decreases with decreasing chain length and is almost nil with acetaldehyde and propionaldehyde. Based on these findings, therefore, spot 2 is more likely to be formic than acetic acid. Toyama and Matsumoto (*17*) and Toyama *et al.* (*18*) isolated saturated acids from formic to caproic from oxidized methyl esters of highly unsaturated sardine oil fatty acids. The conditions of oxidation and of peroxide decomposition were considerably more severe than those used to collect the volatiles studied here, perhaps explaining why no unsaturated acids were found by these workers.

Branched-chain acids such as isobutyric and isovaleric have the same R_f values as their straight-chain isomers. Therefore, spots 6 and 7 could represent branched isomers also, but this possibility appears rather remote. Presumably, the precursors for these compounds would have to be the branched-chain fatty acids. Although these compounds are known to occur in marine lipids, they comprise only about 1.5% of the total fatty acids of menhaden oil. Furthermore, they are not highly unsaturated and they would not be expected to undergo rapid oxidative degradation. Thus, the volatile acidic compounds expected to result from autoxidation of highly unsaturated fatty acids of fish oils are the straight-chain saturated and unsaturated acids of low molecular weight, tentatively identified in this study. These acids have strong, sharp, unpleasant odors, and their presence should contribute significantly to the over-all odor of menhaden oil, although none has a characteristic fishy odor.

Undoubtedly, acids of longer chain length occur also in oxidized menhaden oil but because of their low volatility and solubility in water they are not found in the fraction studied here. Valeric acid was detected only when large amounts of the acidic material were chromatographed. The higher homologs are either completely absent or present in quantities too small to be detected.

Acknowledgment

The authors are grateful to Maurice Stansby and Eric Gauglitz of the Seattle Technological Laboratory of the Bureau of Commercial Fisheries for supplying the starting material.

Literature Cited

(1) Burness, A. T. H., King, H. K., *Biochem. J.* **68,** 32P (1958).
(2) Chang, S. S., Masuda, Y., Mookherjee, B. D., *Chem. Ind.* **1962,** p. 1023.
(3) Davies, W. L., Gill, E., *J. Soc. Chem. Ind.* **55,** 141-T (1936).
(4) Day, E. A., Lillard, D. A., *J. Dairy Sci.* **43,** 585 (1960).
(5) Ellman, G. L., *Arch. Biochem. Biophys.* **74,** 443 (1958).
(6) El-Negoumy, A. M., Miles, D. M., Hammon, E. G., *J. Dairy Sci.* **44,** 1047 (1961).
(7) Forss, D. A., Dunstone, E. A., Stark, W., *J. Dairy Res.* **27,** 211, 373 (1960).
(8) Kalbe, H., *Hoppe Seyler's Z. Physiol. Chem.* **297,** 19 (1954).
(9) Kennedy, E. P., Barker, H. A., *Anal. Chem.* **23,** 1033 (1951).
(10) Lillard, D. A., Day, E. A., *J. Dairy Sci.* **44,** 623 (1961).
(11) Loury, M., *Compt. Rend.* **256,** 2870–1 (1963).
(12) Mukerjee, H., *Anal. Chem.* **31,** 1284 (1959).
(13) Pont, E. G., Forss, D. A., Dunstone, E. A., Gunnis, L. F., *J. Dairy Res.* **27,** 205 (1960).
(14) Privett, O. S., Chipault, J. R., Schlenk, H., Lundberg, W. O., *Com. Fisheries Rev.* **20** (11a), 18 (1958).
(15) Stansby, M. E., *Food Technol.* **16,** No. 4, 28–32 (1962).
(16) Stark, W., Forss, D. A., *J. Dairy Res.* **29,** 173 (1962).
(17) Toyama, Y., Matsumoto, I., *Mem. Fac. Eng., Nagoya Univ.* **5** (2), 335 (1953).
(18) Toyama, Y., Suzuki, K., Nakagami, T., Yoshida, K., *Ibid.*, **9** (1), 125 (1957).
(19) Wyatt, C. J., Day, E. A., *J. Food Sci.* **28,** 305–12 (1963).
(20) Yu, T. C., Day, E. A., Sinnhuber, R. O., *Ibid.*, **26,** 192–7 (1961).

Received for review March 9, 1964. Accepted April 16, 1964. Study supported by the U. S. Department of the Interior, Fish and Wildlife Service, and by the Hormel Foundation.

MILK STALING

Phospholipids of Fresh Milk and of Sterile Whole Milk Concentrate

H. W. SPRECHER, F. M. STRONG, and A. M. SWANSON

Departments of Biochemistry and Dairy and Food Industries, College of Agriculture, Madison, Wis.

SEVERAL investigators have suggested that phospholipids may be involved in detrimental reactions in dairy products (*7, 16, 29, 30*). The susceptibility of these components to oxidative attack in model systems has been established for some time (*13, 28*). Lack of a better understanding of their behavior in various dairy products can be attributed to the difficulty encountered in separating them from the large amount of neutral lipids.

Investigators to date have taken advantage of the fact that upon separating milk, about 60% of the phospholipids go into the cream. Upon churning, these can readily be recovered from either the buttermilk (*23, 25*) or butter serum (*2, 3, 14, 19*) and it is these milk phospholipids which have been mainly studied to date. However, approximately 40% of the total milk phospholipids are not recovered by this procedure. While Rhodes and Lea (*23*) have presented evidence that no selective partitioning occurs when the cream is separated, it has never been established whether there might be selectivity in regard to the fatty acid composition of the phospholipids which do or do not go

The phospholipids as obtained from whole milk were fractionated into cephalin, lecithin, sphingomyelin, and a minor inositol-containing component. The fatty acid composition of the various constituents was determined by gas chromatography. Phospholipids isolated from sterilized milk concentrate which had developed a pronounced stale flavor showed about a threefold increase in the amount of phosphorus present in the inositol-containing lipid. Close agreement of the fatty acid composition with that found in the phospholipids from fresh whole milk indicates that the staling reaction does not involve oxidative attack on phospholipid unsaturated fatty acids.

into the cream. A more serious drawback to the above procedure is the inability to isolate and study these components from any processed dairy product which can no longer be satisfactorily separated.

This work was therefore undertaken to develop a procedure for isolating and studying the chemical nature of the phospholipids from whole milk. The same procedure was then employed to ascertain whether appreciable phospholipid oxidation was involved in the so-called "staling" of sterilized milk concentrate. Upon prolonged storage of such concentrates the stale flavor becomes so pronounced that the product can be highly undesirable as a marketable commodity. Since the chemistry of this off-flavor is not known, it cannot be defined or described in terms of a definite standard. However, experienced taste panel members are able to recognize and reproducibly evaluate it in various dairy products (*20*).

Experimental

Analytical Methods. Phosphorus was determined according to King's procedure (*11*). The micro-Kjeldahl method with copper sulfate as catalyst was used to measure total nitrogen content. Ester equivalents were measured as the ferric hydroxamate complexes as described by Lands (*15*). Methyl stearate of 99% purity or better (Hormel Foundation, Austin, Minn.) was used as the standard. Choline was measured as the reineckate as described by Glick (*6*). Choline chloride, twice recrystallized from 2-methyl-1-propanol and dried over phosphorus pentoxide in a vacuum desiccator, was used as the standard. Inositol was measured microbiologically as described by Atkin *et al.* (*1*), after hydrolysis of the lipid by heating the sample at 110° C., in a sealed tube, for 48 hours in 6*N* HCl.

Alpha-amino nitrogen was determined by a modification of the procedure of Lea and Rhodes (*17*). A sample containing 0.05 to 0.30 μmole of alpha-amino nitrogen was dissolved in ethanol and 0.5 ml. of 0.2*M* citrate buffer pH 5.0 was added. After mixing, 0.2 ml. of 5% (w./v.) ninhydrin in methyl Cellosolve was added, followed by 1.0 ml. of KCN dissolved in methyl Cellosolve. The stock KCN solution was 0.01*M* and was diluted 1 to 50 for the working reagent. The samples were mixed and heated for 15 minutes in a boiling water bath. After cooling, 7.3 ml. of 60% ethanol was added and the absorbancy wasmeasured at 570 mμ. Serine was used as the standard for this procedure.

Chromatography. Mallinckrodt silicic acid, 100- to 200-mesh, lot 2847, was activated at 110° C. for 7 hours. Forty grams of the acid was slurried with chloroform, poured into the column, and allowed to settle by gravity. The column was equipped with a fritted disk and Teflon stopcock at one end, while the other was fitted with a standard-taper joint for attachment to the gradient device (*24*). This consisted of a 2000-ml. bulb filled with methanol, connected in series to a 500-ml. and a 1000-ml. bulb, each filled with chloroform at the start of the chromatogram and continuously stirred throughout the run. Development of the chromatogram was terminated when the first bulb was empty. The gradient produced varied from zero to 75% methanol in chloroform.

The column was jacketed and cool tap water (about 15° to 20° C.) circulated through it during development. A circle of Whatman No. 1 filter paper was placed on the fritted disk and another on top of the packed column. The packed column was 27.5 cm. high and 2 cm. wide. Chloroform was allowed to run through it until the silicic acid was transparent. The lipid load applied was between 0.4 and 0.6 mg. of phospholipid phosphorus per gram of adsorbent. After the sample was placed on the column, about 300 ml. of chloroform was allowed to run through to elute any neutral lipid before the gradient device was attached (*24*). The progress of elution was followed by determining the phosphorus content of 1-ml. aliquots from alternate tubes. The volume delivered to each tube was 10.5 ml.

Thin Layer Chromatography (TLC). Silica Gel G as well as the thin layer equipment were purchased from Brinkman Instruments, Great Neck, Long Island, N. Y. The plates were heated for 2 hours at 120° C. prior to use. The developing solvent was chloroform-methanol-water, 65:25:4 (v./v.). For general staining either exposure to iodine vapors or spraying with 2′,7′-dichlorofluorescein and viewing under ultraviolet light proved to be satisfactory (*18*). Lipids containing alpha-amino groups were detected by spraying with a 0.25% solution of ninhydrin in acetone-lutidine 9 to 1 (v./v.). To detect phosphorus-containing components, the plates were sprayed with a modified Hanes and Isherwood reagent (*10*) consisting of 5 ml. of 60% perchloric acid, 10 ml. of 1.0*N* HCl, and 25 ml. of 4.0% (w./v.) ammonium molybdate.

Gas Chromatography. The methyl esters of fatty acids were prepared by use of anhydrous methanolic HCl as described by Stoffel, Chu, and Ahrens (*26*). Gas chromatography was carried out on an Aerograph Model 600 B unit equipped with a hydrogen flame detector. A 5-foot, 1/8-inch-diameter column packed with 15% diethylene glycol succinate on Chromosorb W 60- to 80-mesh was used. The column temperature was maintained at 180° C. and the nitrogen flow rate at 30 ml. per minute. The methyl esters were identified by comparing retention times with standards obtained from the Hormel Foundation, plotting log of retention time against carbon number, and hydrogenating the esters and comparing the chromatograms with those obtained before reduction. Hydrogenation was carried out in methanol with PtO_2 catalyst.

Results and Discussion

The procedure used to extract and purify the phospholipids is shown in Figure 1. The first aqueous layer, which contained 40% of the phosphorus of the original chloroform-methanol extract (Figure 1), gave positive ninhydrin and anthrone tests, and all of the material remained at the origin when this fraction was examined by TLC. Therefore these phosphorus-containing components were more polar than phospholipids. Billimoria, Curtis, and Maclagan (*3*) have reported that butter serum contains a number of phospholipid-like components with high carbohydrate and nitrogen content.

Figure 2 shows the results of chromatographic separation of the chloroform-soluble phospholipids (Figure 1) on silicic acid columns. For further analysis the tubes comprising each peak were pooled. TLC was used to determine where peaks 3 and 4 overlapped and the contents of these tubes (151 to 158, inclusive) were discarded.

Since phospholipids fractionate on silicic acid columns according to unsaturation (*22*), it is possible that the fatty acid composition of the discarded material may have been somewhat different from that in the main portion of peaks 3 and 4. However, little error

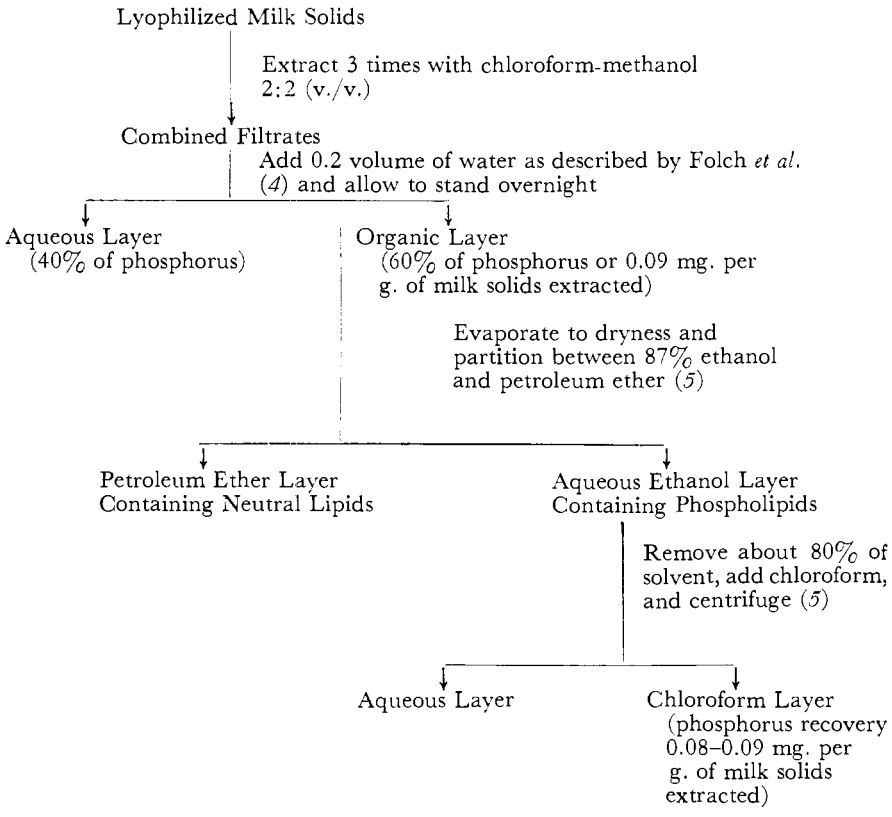

Figure 1. Flow diagram for extraction and purification of milk phospholipids

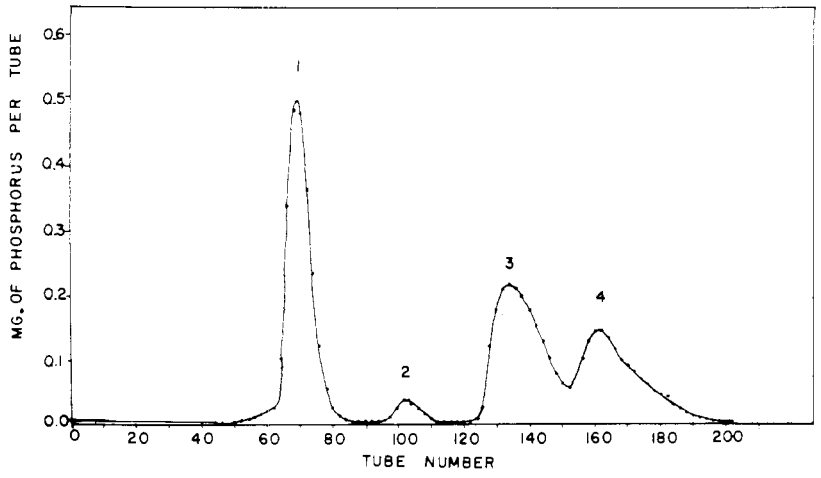

Figure 2. Gradient elution separation of phospholipids of whole milk on silicic acid column

would be expected, as only 12% of the phosphorus of these two peaks was discarded.

TLC showed only one component each in the pooled fractions corresponding to peaks 1, 3, and 4. Of these, only the peak 1 material stained positively with ninhydrin. Peak 2, when stained to detect phosphate esters, displayed considerable streaking. Upon staining the same plate with ninhydrin, a component was visible in the center of the phosphorus-positive area. Further analytical results (Table I) agree closely with theoretical values for cephalin (peak 1), lecithin (peak 3), and sphingomyelin (peak 4).

The high content of inositol in peak 2 indicates that one of the principal components of this fraction is probably a phosphoinositide. The large amount of contaminating nitrogenous material, as shown by the ratio of phosphorus to alpha-amino nitrogen, makes it impossible to determine the nature of the postulated phosphoinositide. Attempts to purify this fraction by rechromatographing it on silicic acid were unsuccessful. The presence of a large amount of nitrogenous material in this fraction is not without precedent. As Hanahan points out, the phosphoinositide fraction, as obtained from silicic acid chromatography, is frequently contaminated by tenaciously bound nitrogenous material (*8*). The nature of these components is not known with certainty, but it has been suggested that they may include lysocephalin or phosphatidyl peptides (*8, 9*).

The fatty acid compositions of the three major phospholipid classes are presented in Table II. The values shown were obtained in a single run. Duplicate runs gave closely similar values, except that there was some difficulty with the longer chain components of the sphingomyelin fraction. In this case variations up to about 10% of the values shown in Table II were encountered. Cephalin is characterized by the finding that about 70% of the fatty acids exhibit some degree of unsaturation, with oleate comprising almost 50% of the total. While stearate is the principal saturated fatty acid in cephalin, palmitate is the chief saturated component in lecithin.

Sphingomyelin is readily characterized by the finding that about 95% of the acids are saturated, with a preponderance of the long-chain acids. Of particular interest was the finding of 23 to 25% tricosanoic acid (Tables II and III). No authentic sample of this acid was available for use as a standard, so its identity was assigned exclusively by its position in the log plot and by the fact that this position was not altered by hydrogenation. Other workers have also reported appreciable percentages of tricosanoic acid in the fatty acids of sphingomyelin from human serum (*21*) and from cow's milk (*24*), and of rat brain cerebrosides (*12*).

The procedure used in this investigation provides, for the first time, a direct method for quantitatively isolating the phospholipids from a high-fat material such as milk. It was found that the molar percentages, based on phosphorus content, of lecithin, cephalin, sphingomyelin, and inositol-containing fractions were, respectively, 39, 32, 26, and 3. In their investigation of the phospholipids of buttermilk, Smith and Lowry (*25*) found that the molar percentages of cephalin, lecithin, and sphingomyelin were 37, 31, and 23, respectively. Their fatty acid analyses agree well with the results obtained in this investigation. It can therefore be concluded that no selective partitioning in regard to either class or fatty acid content occurs when a selective starting material, such as buttermilk, is used for the study of milk phospholipids.

Phospholipids of Sterilized Milk Concentrate

The 3 to 1 sterilized milk concentrate used in the present study was prepared according to the general procedure described by Swanson and Seehafer (*27*). The processing conditions were forewarming, 185° F. for 30 minutes; poly-

Table I. Composition of Milk Phospholipids

	Percentages of Dry Weight			
	Peak 1	Peak 2	Peak 3	Peak 4
Phosphorus				
Calculated[a]	4.15	3.58	3.93	3.93
Found	3.78	2.23	3.80	3.70
Nitrogen				
Calculated	1.88	...	1.78	3.55
Found	1.75	Not run	1.64	3.39
Choline				
Calculated (as chloride)	...	...	17.7	17.7
Found	...	...	16.1	14.8
Inositol				
Calculated	...	20.8	...	...
Found	...	6.47	...	...
	Molar Ratios			
Nitrogen/phosphorus	1.02	Not run	0.96	2.03
Fatty acid ester/phosphorus	1.97	1.75	2.04	...
Phosphorus/choline	...	...	1.06	1.12
Phosphorus/α-amino nitrogen	1.01	1.45	...	...
Phosphorus/inositol	...	2.01	...	...

[a] To calculate theoretical molecular weights it was assumed that peak 1 was oleyl-steraryl-phosphatidyl ethanolamine, $C_{41}H_{80}O_8NP$, mol. wt. 746. For peak 2 the values are for a monophosphoinositide with stearic and oleic acids, $C_{45}H_{85}O_{13}P$, mol. wt. 865. Peak 3 was assumed to be oleyl-stearyl-phosphatidyl choline, $C_{44}H_{87}O_8NP$, mol. wt. 789. Assuming peak 4 to be sphingomyelin with behenic acid, the formula would be $C_{45}H_{92}O_6N_2P$, mol. wt. 788.

Table II. Fatty Acid Composition of Milk Phospholipids as Determined by Gas Chromatography

(Mole per cent)

Component	Cephalin Fraction (Peak 1)	Lecithin Fraction (Peak 3)	Sphingomyelin Fraction (Peak 4)
12:0[a]	0.5	0.5	0.8
13:0	0.2	0.2	...
14:0	1.9	8.1	4.0
14:1	0.3	0.5	...
15:0	0.5	1.9	1.1
16:0	9.4	33.2	24.7
16:1	2.2	3.5	1.2
17:0	0.6	1.1	1.6
18:0	12.2	8.4	4.6
18:1	48.3	28.2	2.8
19:0	0.7	0.7	0.8
18:2	14.3	8.2	0.7
20:0	0.5	0.3	1.3
18:3	3.3	1.6	...
21:0	1.6	...	1.4
22:0	0.7	0.5	16.7
20:4	1.2	0.5	...
23:0	...	...	23.0
24:0	...	...	14.3
Unidentified	2.4	2.4	1.2

[a] Carbon atoms: double bonds.

phosphates, 0.05% of the whole milk; pH of the concentrate, adjusted to 6.55 to 6.65. A 9-month-old sample of the concentrate which had developed a pronounced stale flavor was extracted and the phospholipids were fractionated as described above. The amount of phospholipid phosphorus obtained was 0.082 mg. per gram of solids extracted, compared to 0.087 mg. from fresh milk. The only visible difference between the two preparations was a pronounced turbidity of the chloroform solution of the phospholipids from the stale concentrate.

The chromatographic separation is shown in Figure 3. The phosphorus recovery from the column was only about 80%, in comparison to a 90 to 100% recovery for the fresh milk phospholipids. The amount of phosphorus increased from 3% to about 11%, of the total eluted, in the second peak. The phosphorus distribution among peaks 1 to 4 was 33, 11, 31, and 25%, respectively.

TLC of the four fractions did not reveal any difference from previous results. The only significant difference was that the percentage of inositol in the second peak on a dry weight basis had decreased from 6.47 to 4.28%, while the phosphorus-inositol molar ratio increased from 2.01 to 3.08. This change is attributed solely to the presence of more nonphosphoinositide material rather than the additional extraction of any other phosphoinositide with a higher phosphorus-inositol ratio. For every 100 grams of fresh milk solids extracted, 0.24 mg. of phosphorus and 0.73 mg. of inositol were found in the second peak. In the case of sterilized milk concentrate, 0.73 mg. of phosphorus and 0.79 mg. of inositol were so obtained.

Table III. Fatty Acid Composition of Phospholipids of Sterilized Milk Concentrate after Staling and Comparison with Respective Components from Fresh Milk as Determined by Gas Chromatography

Component	Cephalin Fraction	Delta Cephalin[a]	Lecithin Fraction	Delta Lecithin[a]	Sphingomyelin Fraction	Delta Sphingomyelin[a]
12:0[b]	0.4	+0.1	0.5	0.0	0.7	+0.1
13:0	0.2	0.0	0.1	+0.1	...	...
14:0	1.6	+0.3	8.7	−0.6	3.1	+0.9
14:1	0.3	0.0	0.7	−0.2	...	...
15:0	0.3	+0.2	2.0	−0.1	0.9	+0.2
16:0	9.2	+0.2	32.3	+0.9	21.3	+3.4
16:1	1.9	+0.3	4.2	−0.7	1.1	+0.1
17:0	0.7	−0.1	1.3	−0.2	1.4	+0.2
18:0	14.8	−2.6	8.5	−0.1	3.7	+0.9
18:1	47.2	−1.1	27.3	+0.9	2.5	+0.9
19:0	0.5	+0.2	0.7	0.0	0.6	+0.2
18:2	12.0	+2.3	8.2	0.0	0.7	0.0
20:0	0.8	−0.3	0.4	−0.1	1.0	+0.3
18:3	3.9	−0.6	1.8	−0.2	...	...
21:0	0.7	+0.3	...	...	0.7	+0.7
22:0	1.8	−1.1	0.3	+0.2	19.1	−2.4
20:4	1.4	−0.2	0.5	0.0	...	...
23:0	...	...	...	...	24.9	−1.9
24:0	...	...	...	...	16.2	−1.9
Unidentified	1.9		2.4		1.6	

[a] Mole per cent of component in whole milk minus mole per cent of respective component in stale sterilized milk concentrate.
[b] Carbon atoms: double bonds.

In an attempt to ascertain whether the change in the peak 2 material occurred during the prolonged storage period or during the processing of the concentrate, the phospholipids were isolated from a freshly prepared concentrate. This preparation behaved in every way like that from fresh milk. The phosphorus distribution among peaks 1 to 4—39, 3.5, 32, and 26%, respectively—was in good agreement with that found for the phospholipids of fresh milk.

Examination of the fatty acid content of the phospholipids of the stale concentrate, as depicted in Table III, shows very little difference from fresh milk. In particular, the unsaturated content was very similar. If appreciable oxidation had occurred, the content of unsaturates would have been expected to show a marked decrease. This appears to be true only in the case of the 18:2 fatty acid in the cephalin fraction. However, examination of the 18:3 and 20:4 components, which are more susceptible than linoleate to oxidation, shows very little difference. In view of these findings it appears that the staling reaction, in sterilized milk concentrate, does not involve oxidation of the phospholipids. If oxidation does take place, it either occurs to such a small

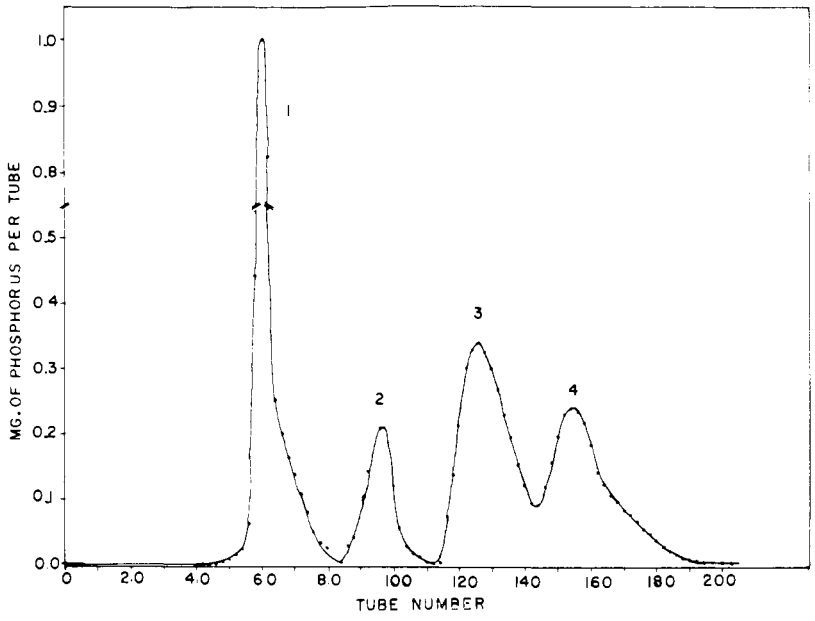

Figure 3. Gradient elution separation of phospholipids of 3 to 1 sterilized milk concentrate on silicic acid column

extent that it cannot be detected in a study of this type or else some other lipid or other substance must serve as the principal point of oxidative attack.

Acknowledgment

The authors thank the Wisconsin Alumni Research Foundation Laboratories for microbiological determinations of inositol.

Literature Cited

(1) Atkin, L., Schultz, A. S., Williams, W. L., Frey, C. N., *Ind. Eng. Chem., Anal. Ed.* **15,** 141 (1943).
(2) Badings, H. J., *Neth. Milk Dairy J.* **16,** 217 (1962).
(3) Billimoria, J. D., Curtis, R. G., Maclagan, N. F., *Biochem. J.* **78,** 185 (1961).
(4) Folch, J., Lees, M., Sloane Stanley, G. H., *J. Biol. Chem.* **226,** 497 (1957).
(5) Galanos, D. S., Kapoulas, V. M., *J. Lipid Res.* **3,** 134 (1962).
(6) Glick, D., *J. Biol. Chem.* **156,** 643 (1944).
(7) Greenbank, G. R., Pallansch, M. J., *J. Dairy Sci.* **44,** 1597 (1961).
(8) Hanahan, D. J., "Lipide Chemistry," pp. 28–32, Wiley, New York, 1960.
(9) Hanahan, D. J., Watts, R. M., Pappajohn, D., *J. Lipid Res.* **1,** 421 (1960).
(10) Hanes, C. S., Isherwood, F. A., *Nature* **164,** 1107 (1949).
(11) King, E. J., *Biochem. J.* **26,** 292 (1932).
(12) Kishimoto, Y., Radin, N. S., *J. Lipid Res.* **1,** 72 (1959).
(13) Koops, J., *Neth. Milk Dairy J.* **11,** 53 (1957).
(14) *Ibid.*, **12,** 226 (1958).
(15) Lands, W. E. M., *J. Biol. Chem.* **231,** 883 (1958).
(16) Lea, C. H., *J. Sci. Food Agr.* **8,** 1 (1957).
(17) Lea, C. H., Rhodes, D. N., *Biochem. J.* **56,** 613 (1954).
(18) Mangold, H. K., *J. Am. Oil Chemists' Soc.* **38,** 716 (1961).
(19) Mattsson, Sonja, *XVI Intern. Dairy Congr.* **2,** 537 (1962).
(20) Nawar, W. W., Lombard, S. H., Dall, H. E. T., Ganguly, A. S., Whitney, R. McL., *J. Dairy Sci.* **46,** 674 (1963).
(21) Nelson, G. J., *J. Lipid Res.* **3,** 71 (1962).
(22) Rhodes, D. N., Lea, C. H., "Biochemical Problems of Lipids," G. Popjak, and E. Leberton, eds., p. 73, Butterworth, London, 1956.
(23) Rhodes, D. N., Lea, C. H., *J. Dairy Res.* **25,** 60 (1958).
(24) Silhacek, D. L., Enzyme Institute, University of Wisconsin, personal communication.
(25) Smith, L. M., Lowry, R. R., *J. Dairy Sci.* **45,** 581 (1962).
(26) Stoffel, W., Chu, Florence, Ahrens, E. H., Jr., *Anal. Chem.* **31,** 307 (1959).
(27) Swanson, A. M., Seehafer, M. E., *Dept. Agr. Economics*, University of Illinois, Bull. **6,** 14–26 (September 1963).
(28) Swanson, A. M., Sommer, H. H., *J. Dairy Sci.* **23,** 201 (1940).
(29) Tarassuk, N. P., Koops, J., *Ibid.*, **43,** 93 (1960).
(30) Thurston, L. M., Brown, W. C., Dustman, R. B., *Ibid.*, **18,** 301 (1935).

Received for review May 28, 1964. Accepted September 11, 1964. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Research supported in part by grants from the American Dairy Association and the National Science Foundation (G 22249). Based on the Ph.D. dissertation of Howard W. Sprecher, University of Wisconsin, 1964.

RADIONUCLIDES IN MILK

Determination of Picocurie Concentrations of Iodine-131 in Milk

BERND KAHN

Radiological Health Research Activities, Division of Radiological Health, Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio

IODINE-131 is measured in milk to study iodine metabolism in animals, to estimate the intake of that radionuclide by humans, and to detect evidence of recently produced fission products in the environment. For animal metabolism and human intake studies, gamma spectral analysis of a gallon of milk (*7*) with a sensitivity of 10 picocuries (pc.) per liter is satisfactory; for environmental detection at the 1 pc. per liter level, beta counting, with its lower background and higher counting efficiency, appears to be more appropriate. In preparing for beta counting, the bulk of the milk is now removed from the iodine by ashing (*5*) or solvent extraction (*1*). This report describes a rapid anion exchange procedure for sample preparation.

Anion exchange resins are used to separate iodine-131 from the other medium- and long-lived fission products in milk so that spectral analysis may be replaced by simply gamma-counting the resin (*2, 12, 14*). The resin can also be beta-counted, but the count rate of the relatively weak beta particles of iodine-131 varies appreciably with the amount of resin and its moisture content (*3*). In the procedure presented here, the iodine is made available for beta counting after separation from the resin; it is eluted from the resin, precipitated as silver iodide, washed to remove silver chloride and organic impurities, and then beta-counted.

Results obtained for specific milks by an anion exchange or solvent extraction