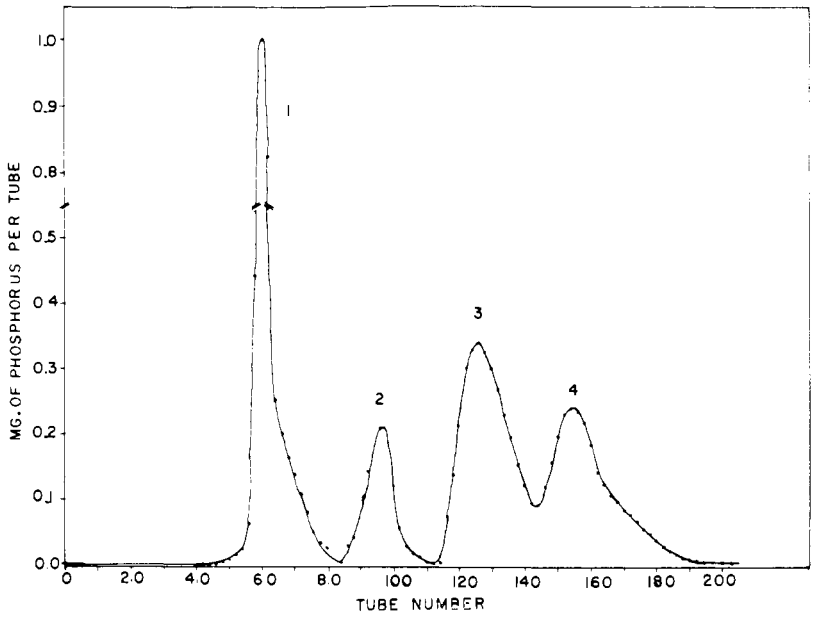

Figure 3. Gradient elution separation of phospholipids of 3 to 1 sterilized milk concentrate on silicic acid column

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.

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RADIONUCLIDES IN MILK

Determination of Picocurie Concentrations of Iodine-131 in Milk

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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

Iodine-131 in milk is measured in the 1 picocurie per liter range after separating the iodide on a strong-base anion exchange resin, eluting it with sodium perchlorate, and precipitating silver iodide. The precipitate is washed to remove chloride and organic impurities, the chemical yield is measured, and the sample is counted in a beta counter. Minimum detectable concentrations for 1-liter samples are 3 pc. per liter with an internal proportional counter and 0.3 pc. per liter with an anticoincidence counter. The decontamination factor from interfering fallout radionuclides in milk exceeds 40,000.

procedure may be low by several per cent (*4, 6, 9, 10, 14*) because a small fraction of the iodine in the milk may not be in equilibrium with the iodide form. Thus, although the metabolized radioiodine which is in equilibrium with the iodide form is retained by the resin, a small fraction may be irreversibly protein-bound and pass through the resin column. Extreme values for the unabsorbed fraction are given as 0 (*14*) and 15% (*9*); in pooled milk, consisting of a mixture from many sources, the fraction is reported to be negligible (*11, 14*). The preservatives hydrogen peroxide and formaldehyde increase the fraction of irreversibly protein-bound iodine (*4, 11*)—the latter has been used to separate radioiodine quantitatively with the protein fraction of milk (*11*)—and must not be added to milk before the anion exchange separation. Other preservatives, such as sodium ethyl mercury thiosalicylate, do not adversely affect the state of the radioiodine (*8*).

Experimental

Reagents and Apparatus. Dowex 1-X8 (50- to 100-mesh) strong-base anion exchange resin in chloride form.

Glass column (20-mm. i.d.) with extra coarse fritted glass disk, to contain 5.0 cc. (wet volume) of the resin.

Standardized 0.085*M* sodium iodide carrier, free of radioactive contamination.

Procedure. To 1.0 liter of whole milk, stored at 32° to 34° F. and containing no hydrogen peroxide or formaldehyde, add 1 ml. of iodide carrier and stir. Pass milk through the resin column at 20 ml. per minute. Wash milk from resin with water, stirring the resin if necessary.

Elute iodide from resin with 75 ml. of 2*M* sodium perchlorate at 2 ml. per minute. Add 2 ml. of 0.045*M* silver nitrate and 4 ml. of 4*M* nitric acid to the eluate. Stir well and let the precipitate form at room temperature for 30 minutes. Transfer to a glass centrifuge tube with 5 ml. of 0.2*M* nitric acid and centrifuge.

Wash precipitate first with 20 ml. of 0.2*M* nitric acid and then with a mixture of 10 ml. of 0.2*M* nitric acid, 10 ml. of 95% ethyl alcohol, and 10 ml. of diethyl ether. Suspend precipitate in 5 ml. of concentrated ammonium hydroxide, stir well to dissolve silver chloride, and heat below boiling. Centrifuge thoroughly and carefully decant solution to minimize loss of precipitate.

Suspend precipitate in 10 ml. of water, acidify with 1 ml. of 4*M* nitric acid, stir, and filter on tared filter paper. Wash with 0.2*M* nitric acid, and dry first with 95% ethyl alcohol and then with diethyl ether. Weigh filter paper and compute chemical yield. Mount for counting.

Count with anticoincidence beta detector calibrated for the iodine-131 counting efficiency at the 17- to 18-mg. weight of the sample. Compute iodine-131 concentration in milk by dividing count rate by counting efficiency, fractional yield, and radioactive decay factor for period between sampling and counting. Check for purity by counting again after several days and comparing observed half life with the iodine-131 value of 8.08 days.

Results and Discussion

Resin, milk, and elutriant volumes were selected on the basis of tracer experiments performed with iodine-131 in the iodide form added to milk 24 hours before use. As shown in Figure 1, only 1% of the iodide in 1 liter of pasteurized

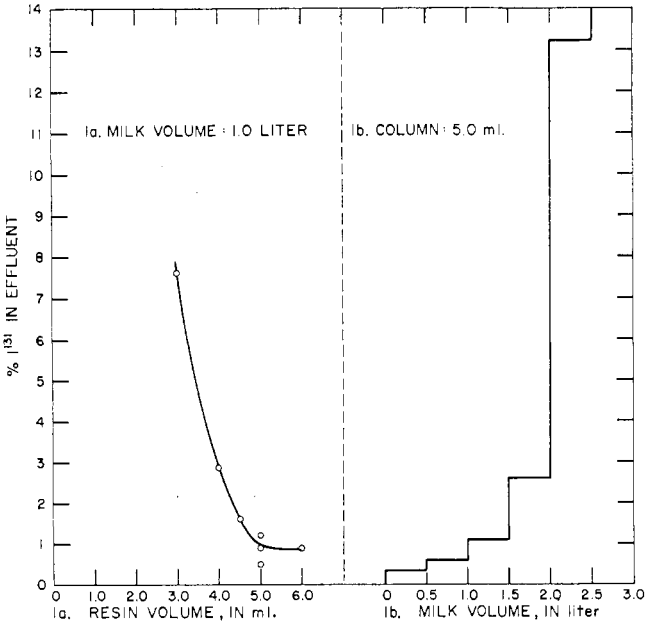

Figure 1. Iodine-131 losses with Dowex 1-X8 (50- to 100-mesh, Cl^- form) anion exchange resin as a function of column and milk volume

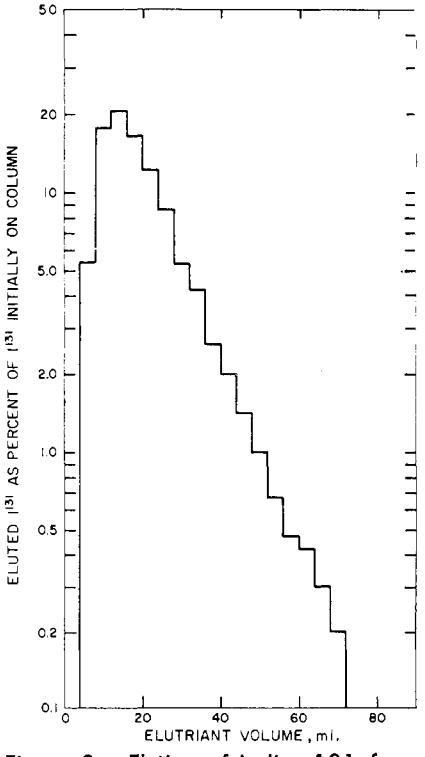

Figure 2. Elution of iodine-131 from 5.0 ml. of Dowex 1-X8 resin with 2M $NaClO_4$

Table I. Measurement of Iodine-131 in Pasteurized Milk

Milk Sample[a]	Iodine-131 Content, Pc./L., Added	γ-Spectroscopy	This procedure	Standard Deviation, Pc./L., This Procedure: Replicates	Counting
1. High-level tracer	2480 ± 50	2590 ± 120	2490	80	14
2. Intermediate-level tracer	45 ± 1	47 ± 4	47	4	2
3. Intermediate-level fallout	0	14 ± 3	17.6	1.5	1.1
4. Low-level fallout	0	1 ± 3	2.4	0.6	0.3
5. No iodine-131 present	0	0 ± 3	0.09	0.15	0.10

[a] Eight replicates analyzed for samples 2, 3, and 4; six for 1 and 5.

homogenized milk passed through 5 cc. of resin. This volume of resin—small compared with the amount used in similar separations (*2, 12*)—was chosen to minimize the required elutriant volume. The loss of iodide in the same volume of raw milk is 2 to 3%. For the first liter, flow rates of 20 and 10 ml. per minute can be maintained for pasteurized and raw milk, respectively. The lower retention and slower flow rate of the raw milk are attributable to the resin being coated by butterfat. Figure 1 indicates that an additional liter of milk can pass through the resin without exhausting its capacity, but the column visibly degenerates as bubbles form, and the flow rate decreases to one half the initial rate. Sodium perchlorate was found to be the most effective elutriant, 75 ml. removing 99.5% of the iodide tracer, as shown in Figure 2. An alternative procedure of oxidizing the iodide on the resin with permanganate to the less strongly held iodate was unsuccessful: A fraction of the radioiodine was rapidly eluted, but the remainder was retained by the column, possibly as molecular iodine.

The iodine-131 is precipitated from the perchlorate eluate as silver iodide. Only a slight excess of silver nitrate precipitant is added to minimize the amount of contaminating silver chloride, and the precipitate is coagulated at room temperature rather than by heating to prevent oxidation of iodide to the volatile iodine by perchloric acid. The iodine-131 loss during precipitation is 3%. If the analytical result is needed as soon as possible, the precipitate may now be filtered, washed, dried, and counted. At this point, the estimated yield for pasteurized homogenized milk is 95% (93% for raw milk), and can be reproduced within a few per cent. If the yield is to be measured rather than estimated, fats are removed by washing with the alcohol-ether mixture, and chlorides are removed with ammonium hydroxide. The purified silver iodide is then filtered, washed, dried, weighed, and counted. The major loss in the entire procedure is approximately 5% during the ammonium hydroxide step, and the average yield is 89%. The entire analysis in quadruplicate, including counting for 50 minutes, can be performed in 4.5 hours by one analyst.

As shown in Table I, the procedure was tested for accuracy, reproducibility, and sensitivity with pasteurized homogenized milk containing various concentrations of iodine-131. For samples 3 and 4, consisting of milk containing metabolized iodine-131 from fallout, values agreed within the error of measurement for gamma spectral analysis of the milk sample and analysis by the procedure reported here. Because higher iodine-131 concentrations were not available for precisely measuring accuracy and reproducibility, samples 1 and 2 were spiked with tracer, 24 hours before analysis. Values obtained by both procedures are in agreement with each other and with the amount of tracer added. The standard deviation of sample 1 replicates, which have a small counting error, is approximately 3%; at lesser concentrations, the counting error becomes the limiting factor for reproducibility, and determines the sensitivity of the procedure.

The minimum detectable concentration of iodine-131 is indicated for 1 hour and overnight (15-hour) counting by the standard deviation values of samples 4 and 5, which were counted for these respective periods of time. If the minimum detectable level is considered to be that concentration which is equal to its 2-standard deviation value, then this level is 1 and 0.3 pc. per liter for 1-hour and overnight counting, respectively. The counting error for these samples is computed for a background of 1 count per minute, counting efficiency of 32%, yield of 89%, and decay factor of 0.84 (for 2 days of decay). Using the more generally available internal proportional counter (without an anticoincidence system), the minimum detectable concentration is 3 pc. per liter, as computed for an iodine-131 counting efficiency of 45%, a background of 45 counts per minute, a 30-minute counting period, and the above yield and decay factor. If necessary, the sensitivity can be almost doubled by using a 2-liter sample.

The decontamination provided by this procedure was tested for the fission products found in Public Health Service milk-network samples—strontium-89, strontium-90 plus yttrium-90, cesium-137, and barium-140 plus lanthanum-140. Strontium-85 tracer was used to represent the strontium radioisotopes, and lanthanum-140 to represent itself as well as yttrium-90. The tracers were added to the milk 24 hours before analysis, and were measured in the final silver iodide precipitate by gamma spectral analysis. The initial concentration of the tracers was 200,000 pc. per liter for cesium-137 and between 300,000 and 400,000 pc. per liter for strontium-85, barium-140, and lanthanum-140. Less than 5 pc. of the tracers remained in the precipitate, demonstrating a decontamination factor of 40,000 or greater. As reported, effective decontamination is provided by the anion exchange resin (*2*). Since these fission products are rarely found in concentrations above 1000 pc. per liter, this decontamination is sufficient. Satisfactory separation from the 1300 pc. of naturally occurring potassium-40 per liter of milk is indicated by the absence of significant radioactivity in the precipitate of sample 5 of Table I.

In addition to its application in detecting iodine-131 in the 1 pc. per liter range, this procedure has been used for simplifying the routine milk-network analysis of iodine-131 and for rapidly estimating iodine-131 in milk at concentrations significant for radiological health action (above 100 pc. per liter). Milk-network analysis is simplified by passing the milk through the resin at the sampling station and then shipping the small resin column instead of the bulky milk sample (*13*). Higher iodine-131 concentrations in milk can be determined in less than 2 hours for multiple samples by proportionately reducing milk sample, resin, and elutriant volume, and counting the silver iodide for a short period (*3*).

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SEED MEAL DETOXICATION

Seed Meal from *Crambe abyssinica*

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Compositional characteristics of crambe seed meal are reported which are significant to its utilization. The hexane-extracted meal contains 9 to 11% thioglucosides, determined by a sulfate procedure described in detail. These thioglucosides are extractable with acetone or methanol containing 20 to 25% water, which removes one fifth to one fourth of the meal solids. Extraction of defatted meal with aqueous acetone removed much of the toxic or unpalatable material which was present in both the whole and the hexane-extracted seed meal as determined by rat feeding experiments. Nitrogen solubility of the hexane-extracted meal plotted as a function of pH gave two minima at which 40 and 42% of the nitrogen are in solution, only 12% of which was accounted for as nonprotein nitrogen.

ABYSSINIAN kale (*Crambe abyssinica* Hochst ex R. E. Fries) is a member of the Cruciferae family, related to rape and mustard. It is of interest as a possible farm crop grown as an industrial raw material because the acids from the seed oil glycerides contain 54 to 61% erucic acid (*19*). The crude protein content of the hexane-extracted seed meal without pericarp (pod) ranges from 49 to 55% and has an amino acid composition indicative of good nutritional quality (*8*). However, the seed also contains thioglucosides (*3*) that yield on enzymatic hydrolysis isothiocyanates, oxazolidinethiones, and possibly other similar products which, if present in sufficiently large amounts, can cause meals to be unpalatable, toxic, or both to farm animals (*2*).

Information is given here on the total thioglucoside content of defatted crambe seed meal and the extent of thioglucoside removal from the meal by selected solvents. Rat feeding tests are used as a guide for evaluating the presence of toxic or growth-inhibiting materials in seed, pericarp, and selected solvent-extracted meals. Solubilities of nitrogenous meal components in different solvents and amino acid compositional data are also included.

Experimental

Seed Origin and Meal Preparation. Mature seed grown in Texas (1961 planting) was selected for the experimental work. The sample as received contained 22% pericarp (pod). After removal of the pericarp the seed contained 46% oil which was removed by cold hexane (b.p. 30° to 60° C.) extraction of the flaked seed, leaving a seed meal, ground to pass a 100-mesh screen, containing 0.2 to 0.8% residual oil and 49% crude protein. The product had a yellow color, pleasant odor, and bitter taste. Additional crambe accessions from Montana, Nebraska, and Texas (1962 plantings) were also used to obtain some measure of variability in composition.

Estimation of Thioglucosides. Total thioglucosides were estimated by measuring the sulfate ion formed by myrosinase hydrolysis of the thioglucosides in water extracts. One gram of air-dry meal in a 125-ml. Erlenmeyer flask was placed in a boiling water bath. After 5 minutes, 30 ml. of boiling water were put in the flask, which was held at above 90° C. for 10 minutes. After gentle shaking for 10 minutes the solids were removed by centrifuging. The supernatants, including three thorough 10-ml. washes of the solids, were made to 50-ml. volume. Two 20-ml. samples of the extract were pipetted into 50-ml. flasks and 4.0 ml. of 0.1*M*, pH 6.0, citrate buffer were added to each. The thioglucosides in one flask were enzymatically hydrolyzed by addition of 15 mg. of white mustard myrosinase prepared according to Schwimmer (*13*). After standing overnight at room temperature both samples were analyzed for sulfate ion by a method similar to that reported by Fritz and Yamamura (*6*). Each solution was passed through a 5- to 6-cm. × 0.9-cm. diameter column of IR 120-H, 20- to 50-mesh ion exchange resin followed by distilled water washes to make a volume of 50 ml. Twenty-milliliter aliquots from each were made to about 100-ml. volume with absolute alcohol. Five milliliters of standard 0.005*M* sulfuric acid in 80% ethanol were added to the aliquot without myrosinase. After addition of 6 drops of 0.2% aqueous thorin indicator, each solution was titrated to matched end points with 0.005*M* barium perchlorate in 80% ethanol.

The method may be carried out without using a comparison sample to which no myrosinase has been added. However, titration of the sulfate in the comparison sample provides information on thioglucoside hydrolysis in the seed meal which may occur before analysis. Normally this titration was low. Calculation of total thioglucosides was based on the total sulfate titrated from the aliquot to which myrosinase was added. To estimate oxazolidinethione-forming thioglucosides an aliquot of the water extract was buffered to pH 7 and the thioglucosides were converted by mustard myrosinase. After 3 hours at 38° to 40° C. the oxazolidinethione formed was extracted into peroxide-free ether and estimated according to the method of Ettlinger and Thompson (*4*).

Prior to development of the above method oxazolidinethiones and volatile isothiocyanates formed from the thioglucosides were estimated by the methods of Wetter (*22, 23*) for measurement of these substances in rapeseed. Some of the data in the tables were obtained by these earlier methods.