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Estimation of the Pantothenic Acid Content of Foods Using a Microbiological Assay and a Radioimmunoassay

Joan Howe Walsh

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ESTIMATION OF THE PANTOTHENIC ACID CONTENT
OF FOODS USING A MICROBIOLOGICAL
ASSAY AND A RADIOIMMUNOASSAY

by
Joan Howe Walsh

A dissertation submitted in partial fulfillment
of the requirements for the degree
of
DOCTOR OF PHILOSOPHY
in
Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah
1979
ACKNOWLEDGEMENTS

This research was one part of a large pantothenic acid research project, funded by the Department of Health, Education and Welfare, Public Health Service grant HEW/PHS 5R01AM18746.

I most appreciated the help of my entire committee. Dr. Donald V. Sisson provided valuable assistance on statistical tests for my data, and Dr. Gary H. Richardson offered needed advice on freezing the bacterial cultures.

Drs. R. Gaurth Hansen and Bonita W. Wyse served as my committee co-chairmen and they both gave me excellent guidance. In particular, Dr. Wyse was very generous with her time during the entire research project, and offered comments during the writing of this dissertation that strengthened it significantly. She also offered positive reinforcement and friendship to me throughout this project.

Nancy Walsh, our laboratory technician, gave me a lot of assistance in getting started on the radioimmunoassay and shared many good ideas with me. Kathy Daugherty did an excellent job of typing this dissertation, producing beautiful copy in record time.

I thank my parents, Walter and Nathalie Howe, and my sister, Dr. Barbara Howe for their great encouragement and support.

My husband John deserves very special thanks for inspiring me by his own example of hard work, and for lifting my spirits after the long days at work.

Joan Howe Walsh
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ABSTRACT

Estimation of the Pantothenic Acid Content of Foods Using a Microbiological Assay and a Radioimmunoassay

by

Joan Howe Walsh, Doctor of Philosophy
Utah State University, 1979

Major Professors: Dr. R. Gaurth Hansen and Dr. Bonita W. Wyse
Department: Nutrition and Food Sciences

Seventy-five processed and prepared foods commonly consumed in the United States were analyzed for pantothenic acid. The foods were analyzed using the traditional microbiological assay for the vitamin and using a new radioimmunoassay (RIA).

The preparation of food sample extracts for both assays was modified. After the food samples underwent enzyme hydrolysis, the food-enzyme mixture was dialyzed to obtain a clear extract for use in the assays. Previously, the food-enzyme mixture was poured through filter paper for clarification.

A very high correlation ($r^2 = .937$) between the results from the RIA and the microbiological assay was found. There was a statistically significant difference between the two assay results for all foods and for the subgroups meats, breads and cereals, and fruits and vegetables at $p = .05$. At $p = .01$, all foods and the subgroup of meats had significantly differences between the microbiological assay results.
and RIA results. Breads and cereals and fruits and vegetables did not have significantly different results between the two assay methods at \( p = .01 \). For all foods and all subgroups, the microbiological assay produced a higher mean result than the RIA.

The RIA is an acceptable assay for pantothenic acid in breads and cereals and fruits and vegetables. Further study is needed to determine if the microbiological assay or the RIA provides the truest picture of the pantothenic acid content of animal tissues.

Findings from the assay of 75 foods are reported, with results from both assays expressed as milligrams of pantothenic acid per 100 grams of food, milligrams of pantothenic acid per serving, and milligrams of pantothenic acid per 1,000 kilocalories of food.

(77 pages)
CHAPTER I
REVIEW OF LITERATURE

Pantothenic acid is an essential factor in the human diet. It is required as a component of coenzyme A and acyl carrier protein, which are in turn necessary for several key metabolic reactions in the cell (1). As with all vitamins, researchers felt a need to develop techniques to measure the amount of the vitamin in food so that estimates of dietary intakes could be made.

**Development of the Microbiological Assay**

Skeggs and Wright (2) first identified *Lactobacillus arabinosus* (later named *Lactobacillus plantarum*) as a suitable organism for the assay of pantothenic acid. *L. arabinosus* required free pantothenic acid for growth, so when all required nutrients except the vitamin were supplied, and then increasing amounts of pantothenic acid were added, the organism responded with increasing growth. Skeggs and Wright also reported that the growth of cells could be read as turbidity in a spectrophotometer, as well as by the longer titrimetric procedure.

Food contains bound as well as free pantothenic acid, and the microbiological assay was responsive only to the free vitamin. In 1945, Ives and Strong (3) recommended that Mylase P be used for enzymatic liberation of bound pantothenic acid. Later, Neilands and Strong (4) compared the pantothenic acid releasing activity of Mylase P with a double enzyme system of homogenized pigeon liver and alkaline phosphatase, and found the latter system to be superior. The pigeon liver
preparation, however, contained large amounts of intrinsic pantothenic acid, which meant that most of the pantothenic acid found in the assay was from the enzyme, rather than from the sample. A Dowex anion exchange treatment was developed that sharply reduced the amount of pantothenic acid in the pigeon liver homogenate, yet left the enzyme in an active form (5).

Assays carried out since the early 1950's have not departed much from the procedural steps outlined above. Zook, MacArthur and Toepfer (6) carried out the largest number of assays to date, reporting on the pantothenic acid content of 161 foods assayed microbiologically. Their report provides considerable detail on the procedures for preparing food samples for assay and the assay procedure itself. They also showed that the results from their microbiological assays were largely comparable to the result from rat and chick bioassays for the vitamin.

The Association of Official Analytical Chemists (A.O.A.C.) studies and approves reliable assays for vitamins, and thus took an interest in developing a standard procedure for assaying pantothenic acid. The first step was to carry out a collaborative study, involving eight laboratories, on the microbiological assay for free pantothenic acid (7). Fairly good agreement was obtained between the assay results of the different laboratories on four unknown samples containing free pantothenic acid.

Since large quantities of naturally occurring pantothenic acid is in a bound form, the A.O.A.C. (8) proposed a collaborative study on the double enzyme system for release of pantothenic acid from larger molecules. The study moved slowly, and a problem arose. There was
no stable standard that contained a known amount of bound pantothenic acid that could be used as a check on full liberation of pantothenic acid from the bound form (9). Dried yeast, which has a high but unknown percentage of bound pantothenic acid was assayed by four different laboratories each using five different levels of liver enzyme. The results were quite variable so no statements on optimum enzyme levels for freeing pantothenic acid could be made. The Associate Referee for the procedure recommended "that these collaborative studies be inactivated at this time. When sufficient information on the specific enzyme activity is available, it may be desirable to reactivate collaborative studies" (9). No further reports have been made on the subject of enzyme hydrolysis of samples. The A.O.A.C. does have an Official Final Action procedure for the microbiological assay of free pantothenic acid, but they have published no standardized procedures for the enzymatic liberation of the vitamin (10).

Reports on Pantothenic Acid in Foods

Of the thousands of food items available for consumption in the United States, pantothenic acid content is available for about 700 items. This figure was arrived at by totalling the number of the foods for which there is pantothenic acid data from the new Handbook Number 8-1, (11), from Home Economics Research Report number 36 (12), and a list of additional foods obtained from the Consumer Food and Economics Institute of U.S.D.A. (see Appendix A). Most of the above values are reported for foods in the raw state, such as meat, poultry, fish, fresh produce and frozen vegetables. A number of the items reported in Home Economics Research Report number 36 are foods rarely consumed in this
country, such as acerola juice, walnuts with shells, dates with pits, cabbage juice and raw lungs.

There is a notable lack of information on the pantothenic acid content of cooked foods, such as meats, cereal products, pasta and vegetables. Pantothenic acid is a water soluble labile compound that may not remain stable during the cooking process. Meyer, Mysinger and Wodarski (13) reported that 89% of pantothenic acid was retained in the meat of an oven roasted beef loin, and 56% of the vitamin was retained in the muscle of an oven braised round. In both cases the remaining pantothenic acid was recovered in the meat drippings. Cooking of frozen vegetables can result in a 10 to 40% loss of pantothenic acid, and cooking frozen meats and fish can cause losses of up to 15% of pantothenic acid (14).

There are few reports on the pantothenic acid content of processed foods, which constitute an increasing proportion of the American diet. The refining of grains, and the freezing, canning and dehydration of foods may alter nutritional properties of the foods. Many foods are processed with added fat and sugar, which will increase the caloric density of the food without increasing the vitamin content. There was one attempt to consolidate the reported data on pantothenic acid (and other nutrients) in raw, frozen and canned foods and estimate losses in the preservation process (15). Table 1 was compiled from data presented in that report. The weaknesses in this report are that only a small number of food samples were compared, and the canned or frozen foods may have been analyzed in a different laboratory than the raw food. The latter fact makes comparisons difficult, as the results of any two microbiological assays may vary from 10 to 15%.
Table 1. Estimated losses of pantothenic acid in the freezing and canning of foods.\(^a\)

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Number of samples compared</th>
<th>Freezing Percentage loss of P.A. compared to raw food</th>
<th>Canning Number of samples compared</th>
<th>Percentage loss of P.A. compared to raw food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish and seafood</td>
<td>3</td>
<td>20.8%</td>
<td>10</td>
<td>19.9%</td>
</tr>
<tr>
<td>Meats</td>
<td>3</td>
<td>26.2%</td>
<td>1</td>
<td>36.8%</td>
</tr>
<tr>
<td>Root vegetables</td>
<td>4</td>
<td>46.1%</td>
<td>1</td>
<td>36.8%</td>
</tr>
<tr>
<td>Legumes</td>
<td>5</td>
<td>57.1%</td>
<td>5</td>
<td>77.8%</td>
</tr>
<tr>
<td>Leafy vegetables</td>
<td>12</td>
<td>48.2%</td>
<td>7</td>
<td>56.4%</td>
</tr>
</tbody>
</table>

\(^a\)Adapted from Schroeder (15).
Overall, there is evidence for losses of pantothenic acid during the processing and cooking of foods. Since the food tables are very incomplete with respect to pantothenic acid in processed and prepared foods, estimate of dietary intakes are usually based on the raw food values, which may be spuriously high.

**Dietary Intakes of Pantothenic Acid**

There are very few published estimates of dietary pantothenic acid intakes in the United States. Table 2 summarizes the findings in the literature. In two studies by Chung *et al.* (16) and Ahrens and Boucher (21) aliquots of meals were actually analyzed. The types of diets analyzed by Chung *et al.* varied considerably in composition and calorie level from the majority of diets currently consumed in the United States. Ahrens and Boucher (21) reported on a laboratory analysis of a weekly composite diet derived from the findings of the 1965 U.S.D.A. Household Food Consumption Survey. The mean daily pantothenate intake of 45 milligrams per day is three times higher than the highest previous report (16). The authors did not explain this discrepancy.

**The Need for Pantothenic Acid Research**

The Food and Nutrition Board of the National Academy of Sciences, National Research Council, has been unable to establish a recommended dietary allowance (RDA) for pantothenic acid because the requisite experimental data is lacking (22). A knowledge of the range of dietary intakes and average intakes of pantothenic acid by healthy individuals is necessary to arrive at a recommendation. Because of
Table 2. Pantothenic acid content of American diets

<table>
<thead>
<tr>
<th>Reference</th>
<th>Diet or Group Studied</th>
<th>Method</th>
<th>Meal Level/day (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(16)</td>
<td>high cost diet (3013)</td>
<td>Analyzed</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>low cost diet (2436 kcal)</td>
<td>Analyzed</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>'poverty' diet (1117 kcal)</td>
<td>Analyzed</td>
<td>6.0</td>
</tr>
<tr>
<td>(17)</td>
<td>8 women, ages 18-24</td>
<td>Calculated(^a)</td>
<td>6.7</td>
</tr>
<tr>
<td>(18)</td>
<td>40 preschool children, 3-1/2 to 5-1/2 years</td>
<td>Calculated(^a)</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>lower income</td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>higher income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(19)</td>
<td>17 pregnant teenagers</td>
<td>Calculated(^a)</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>14 postpartum teenagers</td>
<td></td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>5 non-pregnant teenagers</td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>(20)</td>
<td>10 males, 27-33 years (consuming prison meals)</td>
<td>Calculated(^a)</td>
<td>6.4</td>
</tr>
<tr>
<td>(21)</td>
<td>Weekly composite derived from 1965 U.S.D.A. Household Food Consumption Survey</td>
<td>Calculated(^a)</td>
<td>45.0</td>
</tr>
</tbody>
</table>

\(^a\)These calculated values are not entirely reliable because the food composition tables are lacking in values for many cooked foods and processed foods.
gaps in the available data on pantothenic acid in foods, it is presently difficult, if not impossible, to take the information from a dietary interview and accurately estimate the pantothenic acid content of the diet.

Goddard and Matthews (23) of the U. S. Department of Agriculture have stressed the need for new food composition data, especially for processed and cooked foods, for the new edition of Handbook 8. Pennington (24) proposed that pantothenic acid, along with six other "index nutrients" (vitamin B₆, magnesium, vitamin A, folacin, iron and calcium) could be used together for judging overall dietary nutrient adequacy. Such a system is probably not feasible until more complete food composition information is available.

New Developments in Pantothenate Assay
Methodology

One of the reasons for the lack of information on pantothenic acid in foods and diets may be the relative difficulty of the microbiological assay. Each food presents a different array of nutrients and other biomolecules that may stimulate or inhibit the assay organism (25).

Recently, a radioimunoassay (RIA) was developed for the measurement of pantothenic acid in biological tissues (26). The assay is sensitive to free pantothenic acid only, and has been validated as an assay for the vitamin in blood and liver. The RIA may be a workable and more rapid assay for pantothenic acid in foods. If the two assays produce comparable results, an investigator can place more confidence in the results of either assay. A chemical assay is also more advantageous to some investigators who are not equipped and trained for regular microbial assays.
Research Rationale and Goals

There is a lack of data on the pantothenic acid content of foods consumed in the United States. As long as such information is lacking, it is impossible to accurately estimate the pantothenic acid content of a variety of diets and to establish a recommended dietary allowance for the vitamin.

The goals of the research reported herein are as follows:

A. To determine if a radioimmunoassay for pantothenic acid can be profitably applied to the assay of the vitamin in foods.

B. To modify and improve the present system for the preparation of food samples.

C. To report on the pantothenic acid content of seventy-five processed or cooked foods.
CHAPTER II
METHODOLOGY

Selection of Foods to be Analyzed

Foods selected to be analyzed had to meet three criteria: (1) no recent data existed on the pantothenic acid content of the food in the ready-to-eat state, (2) information was available on the kilocalorie content of the food, and (3) the food was commonly consumed in the United States. Not all foods meeting these criteria could be analyzed, but seventy-five foods representing a wide variety of food types were chosen. A check was made with the U.S. Department of Agriculture to confirm that pantothenic acid data on these types of foods were needed for inclusion in a national food composition data bank (27).

Preparation of Food Samples

Three packages, units or pieces of each food were purchased at a Logan, Utah supermarket. Whenever possible more than one brand or variety (such as different flavors of rice-a-roni or pop-tarts) was selected. Food items requiring refrigeration were stored at 5°C and those requiring freezer storage were held at -10°C. Perishable foods were prepared, diluted and frozen as described below within two days of purchase.

Preparation consisted of bringing each food item to a ready-to-eat stage. With some foods such as dry breakfast cereals, no preparation was necessary. For most foods, some cooking, mixing or rehydration was required. In all cases where package directions existed, those
directions were followed exactly. Where package directions did not exist, standard food preparation methods were followed, and the source for preparation directions was noted.

The choice of whether to pool the individual samples before or after cooking depended on the characteristics of the foods. Foods with drip loss or distinct formulation differences such as sausages, frankfurters, fish sticks, and foods that were packaged to be prepared individually such as Rice-a-roni, cake mixes and cornbread mixes were prepared individually first and then pooled for analysis. Details on the preparation of samples and unit weights and cooked yields of all food samples are in Appendix B.

Following preparation and pooling, a sample of known weight of the food was homogenized in a blender with sufficient distilled water of known weight to produce a pourable slurry. An aliquot of at least 75 ml was then poured into a screw top jar, frozen, and stored at -10°C until analyzed. Foods for previous analyses were frozen before being assayed (6), however there are reports of losses of pantothenic acid in frozen foods (15). For this reason, a study of the effects of freezing foods before analyses was undertaken.

Release of Pantothenic Acid from Food Samples

To prepare for the enzyme digestion step food samples were thawed at 5°C and adjusted to between pH 6.5 and pH 7.5 with 50% (by weight) NaOH and about 10 grams of each food sample was autoclaved at 121°C for 10 minutes (6). Several trials were run to determine if autoclaving resulted in destruction of pantothenic acid. A pilot study had
previously been run to determine the approximate levels of pantothenic acid in some of the foods. In cases where there was information in the literature on the pantothenic acid content of a raw food, this value could be used to estimate the amount of pantothenic acid in the cooked food. This information was used in determining the exact amount of sample that should be subjected to enzyme treatment and the amount of water to dilute the samples with so that the results fell on the assay standard curves.

Between three and five subsamples were analyzed for each food. A one to two gram subsample of each composite food sample was transferred to each assay tube.

Before either assay could be carried out, an enzymatic digestion step had to be done. Both assays measure only free pantothenic acid, so the vitamin had to be freed from any larger coenzyme molecules in the food. Two enzymes, calf intestinal alkaline phosphatase and a peptidase found in pigeon liver, were used to carry out this release of pantothenic acid.

The alkaline phosphatase was prepared fresh for each assay. Alkaline phosphatase powder (Sigma Chemical, St. Louis, Missouri 63178) was made up to a 6% (by weight) solution with distilled water and stirred at room temperature until dissolved.

The pigeon liver enzyme was obtained from a pigeon liver acetone powder (Sigma Chemical) that has a fairly high content of pantothenic acid. To reduce the level of exogenous pantothenic acid added to the assay system, the pigeon liver was treated with dowex anion exchange resin before adding it to the assay tubes. The dowex treated pigeon liver was prepared as follows: Six grams of pigeon liver acetone powder
was dissolved in 60 ml of ice cold 0.02 M potassium bicarbonate in a Virtis homogenizer for one minute at speed 80. This step and all others were carried out at 5°C to preserve enzyme activity. The liver homogenate was then spun at 15,000 rpm for 15 minutes in a Beckman centrifuge J-21C, rotor JA-14, (Beckman Instruments, Palo Alto, California 94304), and the supernatant was drawn off with a pipette. The supernatant was then added to a beaker containing 60 grams of 50-100 mesh Dowex 1 x 4 anion exchange resin, chloride form (Bio-Rad Laboratories, 32nd and Griffin, Richmond, California). The Dowex had previously been rinsed three times with distilled water and the volume adjusted up to 125 ml with distilled water. The pH of the Dowex solution was adjusted to 8.0 with 1.0 M Tris-HCl, pH 8.3.

The Dowex resin enzyme mixture was stirred in the cold for 5 minutes, and the Dowex resin was then allowed to settle out. The supernatant from this beaker was added to a second beaker of Dowex resin, prepared in the same manner as above. The 5 minute stirring procedure was repeated and the supernatant was again allowed to separate from the resin. The supernatant was pipetted off and stored at -10°C in 5 ml aliquots. For each assay batch, sufficient enzyme for the assay was thawed at room temperature before use.

For the assay, to each tube containing a weighed food sample the following were added: 0.4 ml of 6% alkaline phosphatase, 0.4 ml of dowex-treated pigeon liver, 0.4 ml of 1 M Tris, pH 8.3, and 0.8 ml water. Sample tubes were covered, mixed, and incubated overnight at 37°C.

The turbidity and high protein content of the food-enzyme mixture made it necessary to clarify the mixture by some means at this point. Each sample was therefore quantitatively transferred to 1 cm. dialysis
tubing, (Scientific Products, 2177 W. Custer Road, Salt Lake City, Utah 84104) using a small funnel and two-one milliliter washes with distilled water. The tubing was tied securely at both ends and folded inside an Erlenmeyer flask containing from 60 to 150 ml of distilled water. Each flask was stirred continuously in the cold (5°C) for at least eight hours. Following dialysis, the tubing and its contents were discarded, and the remaining water extract was used for both the RIA and the microbiological assay. Pilot studies have showed that adding 3H-pantothenic acid to the inside of the dialysis tubing along with the food and enzyme established an equilibrium concentration between the inside and outside of the tubing. Therefore, results from both assays were calculated using the total volume of the inside and the outside of the dialysis tubing.

Radioimmunoassay Procedures

For the RIA, the procedure of Wyse et al. (26) was followed. Antibody was prepared by injecting New Zealand rabbits with a pantothenic acid-bovine serum albumin conjugate. The resulting antiserum was diluted 100-fold with a 10 ml/L solution of rabbit albumin. Each assay tube contained 0.5 ml of the diluted antiserum, 0.50 ml of standard or sample, and 50 ul of radiolabeled (15,000 counts per minute) sodium D-pantothenate (New England Nuclear, Boston, Massachusetts 02118). After incubation at room temperature for 15 minutes, neutral saturated ammonium sulfate was added to 50% saturation and the suspension was centrifuged (6,000 x g, 12 min, 4°C). The precipitate was washed with 0.5 ml of half-saturated ammonium sulfate and recentrifuged. The washed precipitate, containing antibody-bound pantothenic acid, was
dissolved in 0.5 ml of Soluene-350 tissue solubilizer (Packard Instrument Co., Inc., Downer's Grove, Illinois 06515) and transferred quantitatively to vials containing 12 ml of Dimilume-30 (Packard Instrument Co.). The radioactivity was counted in a Packard Tri-Carb liquid scintillation counter. Results were read on a 5 to 100 nanogram (per 0.500 ml) standard curve. An enzyme blank value was subtracted from each sample value.

Microbiological Assay Procedures

The general procedure followed for the microbiological assay was that recommended by the Association of Official Analytical Chemists (10). Certain modifications were made in the assay, such as the use of a dried commercially prepared medium for the assay tubes. For the spectrophotometric readings, absorbance rather than transmittance was used.

The same extracts were used for the microbiological assay as for the RIA. In all cases, except where the pantothenic acid concentration of the extract was known or suspected to be very low, the samples were further diluted with distilled water to place the samples in the range of the 10 to 130 nanogram per ml standard curve.

Five assay tubes, containing 1, 2, 3, 4 and 5 ml of diluted extract and 4, 3, 2, 1 and 0 ml of distilled water were prepared for each sample. Difco pantothenate assay medium (Difco Laboratories, P.O. Box 1058A, Detroit, Michigan 48232) was used, with the addition of 0.1% by volume of a 10% solution of polysorbate 80 in absolute ethanol (10).
The medium was prepared by adding 7.3 grams of the powdered mix to 100 ml of distilled water and mixing over medium heat until dissolved. Five ml of media was added to each assay tube. Following autoclaving at 121°C for 10 minutes, the cooled tubes were inoculated with a suspension of *Lactobacillus plantarum* cells (A.T.C.C. 8014). For each assay batch, cells frozen and stored at -20°C in a complete nutrient medium were thawed at room temperature. The cell suspension was prepared by aseptically transferring the thawed cells to a tube containing 10 ml of Difco Lactobacilli MRS Broth. The MRS Broth is a nutritionally complete powder that is mixed with water (55 grams to 1000 ml) and after autoclaving can be used to culture *Lactobacillus plantarum* cells.

The cells in MRS Broth were incubated at 37°C for 6 to 8 hours, and then centrifuged for 10 minutes. The supernatant was aseptically poured off and the cells were resuspended in 0.9% sterile saline. The centrifugation and resuspension steps were repeated twice more. The tubes were then incubated at 37°C for 17 to 20 hours. Turbidity was measured on a Bausch and Lomb Spectronic 21 at 620 nm. Again in this assay, an enzyme blank value was subtracted from each sample value.

### Quality Control and Validation Procedures

A check on enzyme activity and assay precision was carried out for each batch of samples. A 2 gram sample of dried bakers yeast was dissolved in 35 grams of distilled water, and the pH was adjusted to 7.0 with 50% (by weight) NaOH. The sample was autoclaved and then frozen. Along with each batch of food samples one ml of this diluted yeast was subjected to enzyme treatment and dialysis and was assayed by both microbiological and RIA methods.
On five different occasions 1.00 or 2.00 microgram recovery samples of calcium pantothenate were added to duplicates of food samples being assayed, and the recovered pantothenic acid was calculated.

To check on variability within an assay batch, eight samples of each of two types of avocados were subjected to enzyme hydrolysis and assayed by both methods.

Food samples were assayed at different levels to determine if increasing levels of pantothenic acid paralleled the standard curve for each assay technique. For the RIA, samples of wheat germ extract were added undiluted, and in dilutions of 1:2, 1:3, 1:4, 1:8, 1:12, 1:16 and 1:24 to assay tubes containing antisera and $^{3}$HPA and the assay was carried out in the usual manner. For the microbiological assay, yeast, frankfurters and pork sausage were selected to illustrate the parallelism of a food sample assayed at five different levels, relative to the standard curve.

After three assays had been completed on all of the foods for each of the two methods, the preliminary results, calculated as milligrams of pantothenic acid per 100 grams of diluted sample, were examined. In cases where the mean concentration of pantothenic acid was greater than 0.100 milligrams of pantothenic acid, any food sample with a coefficient of variation greater than 15% was reassayed. For food samples with a concentration of pantothenic acid less than or equal to 0.100 milligrams per 100 grams of diluted sample those with a coefficient of variation greater than 20% were reassayed. The decision to carry out one or two repeat assays depended on the degree of variability found between the first three assay results.
Organization and Statistical Testing of Results

The correlation between the two assay methods was computed using the mean values for pantothenic acid in each food. The hypothesis that the two assay methods produced equal results was tested using an F test.

To examine the contribution that these foods make to the pantothenic acid content of the diet, results were calculated as (a) mg of pantothenic acid per 100 grams of food, (b) mg of pantothenic acid per serving, and (c) mg of pantothenic acid per 1,000 kilocalories of the food. The portion sizes of foods were determined either from suggested serving sizes on nutrition labels or package preparation directions; or weights of food items such as crackers, potatoes, or frankfurters; or standard serving sizes suggested in USDA Handbook number 456 (28). Calorie content of foods was again determined from nutrition label information, manufacturer's printed information or USDA Handbooks (11, 28, 29).
CHAPTER III
RESULTS

Pantothenic Acid in Foods Assayed

Table 3 contains the findings on the amount of pantothenic acid in 100 grams of each of the 75 foods analyzed by both assay methods. In all cases, the 100 gram portion used refers to the edible portion of the food only, so no bones or other non-edible wastes were included. All foods were in a prepared or ready-to-eat state. As much as possible, the terms used to describe and alphabetize the foods were the same as those used in U.S.D.A. Handbooks number 8 (29) and 456 (28). All values in Table 3 represent the mean of three observations. The three observations for each food are found in Appendix C.

It is obvious from the standard deviation reported with each result that values have been extended beyond the last significant digit. While this is not in keeping with good data reporting practices, it has been traditional to report pantothenic acid values to .001 milligram in food composition tables (11, 12). These results and those in Tables 4 and 5 should be used only with the realization of their limits.

While nutrients are commonly reported in reference to a 100 gram portion, there is practical value to presenting nutrients per serving of a particular food (Table 4). Serving sizes for foods vary among individuals and families, but there are some suggested or common serving sizes that have evolved from recipe standardization and commercial portioning or packaging of foods. In Table 4, the serving size is
Table 3. Pantothenic acid content per 100 grams of foods analyzed

<table>
<thead>
<tr>
<th>Food</th>
<th>Microbiological Method</th>
<th>Radioimmunoassay (RIA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breads, Cereals and other Grain Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biscuits from mix</td>
<td>.395 (.025)</td>
<td>.319 (.058)</td>
</tr>
<tr>
<td>Biscuits, homemade</td>
<td>.437 (.027)</td>
<td>.382 (.033)</td>
</tr>
<tr>
<td>Biscuits, refrigerated dough</td>
<td>.238 (.037)</td>
<td>.376 (.055)</td>
</tr>
<tr>
<td>Bread &quot;Bran'ola&quot;</td>
<td>.463 (.050)</td>
<td>.458 (.044)</td>
</tr>
<tr>
<td>Cake, yellow, from mix</td>
<td>.274 (.033)</td>
<td>.272 (.011)</td>
</tr>
<tr>
<td>Cereals, ready to eat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Jacks</td>
<td>.225 (.037)</td>
<td>.122 (.009)</td>
</tr>
<tr>
<td>Cheerios</td>
<td>1.359 (.083)</td>
<td>1.341 (.198)</td>
</tr>
<tr>
<td>Corn Chex</td>
<td>.336 (.063)</td>
<td>.193 (.041)</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>.176 (.031)</td>
<td>.284 (.032)</td>
</tr>
<tr>
<td>Froot Loops</td>
<td>.247 (.011)</td>
<td>.234 (.041)</td>
</tr>
<tr>
<td>Granola</td>
<td>.824 (.100)</td>
<td>.536 (.024)</td>
</tr>
<tr>
<td>Grape Nuts</td>
<td>.451 (.071)</td>
<td>.543 (.122)</td>
</tr>
<tr>
<td>Products 19</td>
<td>.432 (.068)</td>
<td>.443 (.136)</td>
</tr>
<tr>
<td>Sugar Smacks</td>
<td>.297 (.049)</td>
<td>.458 (.016)</td>
</tr>
<tr>
<td>Total</td>
<td>.716 (.081)</td>
<td>.546 (.066)</td>
</tr>
<tr>
<td>Wheat Chex</td>
<td>.551 (.044)</td>
<td>.502 (.042)</td>
</tr>
<tr>
<td>Wheaties</td>
<td>.723 (.105)</td>
<td>.689 (.155)</td>
</tr>
<tr>
<td>Cornbread, from mix</td>
<td>.399 (.016)</td>
<td>.374 (.080)</td>
</tr>
<tr>
<td>Crackers, graham</td>
<td>.443 (.042)</td>
<td>.463 (.064)</td>
</tr>
<tr>
<td>Crackers, saltines</td>
<td>.268 (.051)</td>
<td>.297 (.055)</td>
</tr>
<tr>
<td>Crackers, whole wheat</td>
<td>.412 (.044)</td>
<td>.367 (.019)</td>
</tr>
<tr>
<td>English muffins</td>
<td>.516 (.055)</td>
<td>.480 (.016)</td>
</tr>
<tr>
<td>Granola snack bars</td>
<td>.527 (.051)</td>
<td>.545 (.054)</td>
</tr>
<tr>
<td>Macaroni, cooked</td>
<td>.115 (.023)</td>
<td>.107 (.019)</td>
</tr>
<tr>
<td>Noodles, cooked</td>
<td>.216 (.039)</td>
<td>.156 (.020)</td>
</tr>
<tr>
<td>Oatmeal, instant</td>
<td>.325 (.052)</td>
<td>.317 (.026)</td>
</tr>
<tr>
<td>Oatmeal, quick</td>
<td>.304 (.030)</td>
<td>.233 (.064)</td>
</tr>
<tr>
<td>Oatmeal, regular</td>
<td>.292 (.012)</td>
<td>.270 (.027)</td>
</tr>
<tr>
<td>Pancakes, from mix</td>
<td>.353 (.016)</td>
<td>.313 (.032)</td>
</tr>
<tr>
<td>Pop Tarts</td>
<td>.218 (.032)</td>
<td>.212 (.040)</td>
</tr>
<tr>
<td>Pretzels</td>
<td>.300 (.040)</td>
<td>.475 (.157)</td>
</tr>
<tr>
<td>Rice, brown</td>
<td>.447 (.039)</td>
<td>.351 (.006)</td>
</tr>
<tr>
<td>Rice-a-roni</td>
<td>.199 (.021)</td>
<td>.184 (.010)</td>
</tr>
<tr>
<td>Rice, white</td>
<td>.285 (.042)</td>
<td>.261 (.036)</td>
</tr>
<tr>
<td>Rolls, hamburger</td>
<td>.529 (.072)</td>
<td>.471 (.075)</td>
</tr>
<tr>
<td>Stuffing mix, stove top</td>
<td>.137 (.006)</td>
<td>.172 (.003)</td>
</tr>
<tr>
<td>Twinkie</td>
<td>.245 (.077)</td>
<td>.236 (.021)</td>
</tr>
<tr>
<td>Waffles, frozen</td>
<td>.344 (.040)</td>
<td>.323 (.023)</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>1.498 (.202)</td>
<td>1.220 (.052)</td>
</tr>
<tr>
<td>Whole wheat cereal, cooked</td>
<td>.169 (.023)</td>
<td>.115 (.016)</td>
</tr>
</tbody>
</table>
Table 3 (continued)

<table>
<thead>
<tr>
<th>Food</th>
<th>Microbiological Method</th>
<th>Radioimmunoassay (RIA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat, Fish, Poultry and Meat Analog</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef, chuck steak-pan broiled</td>
<td>.899 (.101)</td>
<td>.846 (.112)</td>
</tr>
<tr>
<td>Beef, lean ground-pan broiled</td>
<td>.881 (.044)</td>
<td>.741 (.065)</td>
</tr>
<tr>
<td>Beef, regular ground-pan broiled</td>
<td>.844 (.017)</td>
<td>.671 (.048)</td>
</tr>
<tr>
<td>Beef short ribs - braised</td>
<td>.536 (.040)</td>
<td>.433 (.065)</td>
</tr>
<tr>
<td>Breakfast links</td>
<td>.103 (.019)</td>
<td>.091 (.012)</td>
</tr>
<tr>
<td>Chicken breast - baked</td>
<td>1.428 (.169)</td>
<td>1.188 (.049)</td>
</tr>
<tr>
<td>Chicken legs - baked</td>
<td>1.435 (.061)</td>
<td>1.212 (.066)</td>
</tr>
<tr>
<td>Fish filet, frozen - cooked</td>
<td>.339 (.048)</td>
<td>.250 (.016)</td>
</tr>
<tr>
<td>Fish sticks, frozen - cooked</td>
<td>.392 (.047)</td>
<td>.337 (.039)</td>
</tr>
<tr>
<td>Halibut steak - poached</td>
<td>.472 (.073)</td>
<td>.261 (.014)</td>
</tr>
<tr>
<td>Pork loin chops - pan broiled</td>
<td>.746 (.049)</td>
<td>.650 (.051)</td>
</tr>
<tr>
<td><strong>Sausages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bologna</td>
<td>.580 (.022)</td>
<td>.498 (.042)</td>
</tr>
<tr>
<td>Frankfurters</td>
<td>.471 (.040)</td>
<td>.342 (.025)</td>
</tr>
<tr>
<td>Liverwurst</td>
<td>3.128 (.249)</td>
<td>2.244 (.088)</td>
</tr>
<tr>
<td>Ground pork - pan broiled</td>
<td>.749 (.102)</td>
<td>.566 (.039)</td>
</tr>
<tr>
<td>Salami</td>
<td>.955 (.084)</td>
<td>.997 (.086)</td>
</tr>
<tr>
<td><strong>Dairy Products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese, monterey jack</td>
<td>.198 (.027)</td>
<td>.285 (.036)</td>
</tr>
<tr>
<td>Cheese, romano</td>
<td>.253 (.049)</td>
<td>.448 (.022)</td>
</tr>
<tr>
<td><strong>Fruits and Vegetables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applesauce, sweetened</td>
<td>.060 (.007)</td>
<td>.043 (.008)</td>
</tr>
<tr>
<td>Applesauce, unsweetened</td>
<td>.095 (.019)</td>
<td>.087 (.034)</td>
</tr>
<tr>
<td>Cranberry juice cocktail</td>
<td>.067 (.009)</td>
<td>.056 (.009)</td>
</tr>
<tr>
<td>Fruit Cocktail, canned</td>
<td>.060 (.006)</td>
<td>.042 (.006)</td>
</tr>
<tr>
<td>Orange juice, frozen - reconstituted</td>
<td>.148 (.015)</td>
<td>.197 (.029)</td>
</tr>
<tr>
<td>Pickles</td>
<td>.031 (.10)</td>
<td>.048 (.004)</td>
</tr>
<tr>
<td>Potatoes, baked</td>
<td>.555 (.077)</td>
<td>.318 (.045)</td>
</tr>
<tr>
<td>Potatoes, boiled</td>
<td>.409 (.022)</td>
<td>.291 (.018)</td>
</tr>
<tr>
<td>Potatoes, canned</td>
<td>.174 (.027)</td>
<td>.152 (.026)</td>
</tr>
<tr>
<td>Potatoes, instant mashed</td>
<td>.345 (.021)</td>
<td>.248 (.036)</td>
</tr>
<tr>
<td>Potatoes, mashed</td>
<td>.275 (.030)</td>
<td>.237 (.031)</td>
</tr>
<tr>
<td>Potato chips</td>
<td>.796 (.054)</td>
<td>.712 (.057)</td>
</tr>
<tr>
<td>Tomatoes canned</td>
<td>.167 (.010)</td>
<td>.169 (.013)</td>
</tr>
<tr>
<td>Tomato paste, canned</td>
<td>.753 (.067)</td>
<td>.771 (.049)</td>
</tr>
<tr>
<td>Tomato sauce, canned</td>
<td>.309 (.028)</td>
<td>.285 (.035)</td>
</tr>
<tr>
<td>V-8 juice</td>
<td>.297 (.034)</td>
<td>.218 (.027)</td>
</tr>
<tr>
<td>Yams, canned</td>
<td>.426 (.040)</td>
<td>.404 (.045)</td>
</tr>
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</table>
Table 4. Pantothenic acid per serving in foods analyzed

<table>
<thead>
<tr>
<th>Food</th>
<th>Microbiological</th>
<th>RIA</th>
<th>Serving Size</th>
<th>Source of Serv. Size Info.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breads, Cereals and other Grain Products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biscuit from mix</td>
<td>.111</td>
<td>.089</td>
<td>1 bisc=28 gms</td>
<td>T</td>
</tr>
<tr>
<td>Biscuits, homemade</td>
<td>.122</td>
<td>.113</td>
<td>1 bisc=28 gms</td>
<td>T</td>
</tr>
<tr>
<td>Biscuits, refrigerated dough</td>
<td>.044</td>
<td>.070</td>
<td>1 bisc=18.7 gms</td>
<td>W</td>
</tr>
<tr>
<td>Bread, Bran'ola</td>
<td>.313</td>
<td>.310</td>
<td>2 slic=67.7 gms</td>
<td>W</td>
</tr>
<tr>
<td>Cake, yellow from mix</td>
<td>.252</td>
<td>.250</td>
<td>1 slic=92 gms</td>
<td>T</td>
</tr>
<tr>
<td>Cereals, ready to eat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Jacks</td>
<td>.063</td>
<td>.034</td>
<td>1 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Cheerios</td>
<td>.380</td>
<td>.375</td>
<td>1¼ cups=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Corn Chex</td>
<td>.094</td>
<td>.054</td>
<td>1 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>.049</td>
<td>.080</td>
<td>1 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Froot Loops</td>
<td>.069</td>
<td>.066</td>
<td>1 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Granola</td>
<td>.231</td>
<td>.150</td>
<td>¼ cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Grape Nuts</td>
<td>.126</td>
<td>.152</td>
<td>¼ cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Product 19</td>
<td>.121</td>
<td>.124</td>
<td>3/4 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Sugar Smacks</td>
<td>.083</td>
<td>.128</td>
<td>3/4 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Total</td>
<td>.200</td>
<td>.153</td>
<td>1 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Wheat Chex</td>
<td>.154</td>
<td>.140</td>
<td>2/3 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Wheaties</td>
<td>.200</td>
<td>.193</td>
<td>1 cup=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Cornbread, from mix</td>
<td>.219</td>
<td>.206</td>
<td>2½&quot; x 2½&quot; x 1-3/4&quot; piece</td>
<td>T</td>
</tr>
<tr>
<td>Crackers, graham</td>
<td>.138</td>
<td>.144</td>
<td>2 crack=31 gms</td>
<td>W</td>
</tr>
<tr>
<td>Crackers, saltines</td>
<td>.085</td>
<td>.034</td>
<td>10 crack=31.6 gms</td>
<td>W</td>
</tr>
<tr>
<td>Crackers, whole wheat</td>
<td>.082</td>
<td>.073</td>
<td>10 crack=20 gms</td>
<td>W</td>
</tr>
<tr>
<td>English muffins</td>
<td>.270</td>
<td>.251</td>
<td>2 halves=52.3 gms</td>
<td>W</td>
</tr>
<tr>
<td>Granola snack bars</td>
<td>.148</td>
<td>.153</td>
<td>1 bar=28 gms</td>
<td>L</td>
</tr>
<tr>
<td>Macaroni, cooked</td>
<td>.122</td>
<td>.114</td>
<td>½ cup dry, cooked = 106.2 gms</td>
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<tr>
<td>Noodles, cooked</td>
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<td>.187</td>
<td>3/4 cup=120 gms</td>
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<tr>
<td>Oatmeal, instant</td>
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<td>.506</td>
<td>1 prepared packet = 159.6 gms</td>
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<tr>
<td>Oatmeal, quick</td>
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<td>.373</td>
<td>1/3 cup dry, cooked = 160 gms</td>
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<td>Oatmeal, regular</td>
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<td>.432</td>
<td>1/3 cup dry, cooked = 160 gms</td>
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<tr>
<td>Pancakes, from mix</td>
<td>.286</td>
<td>.254</td>
<td>3-4&quot; cakes=81 gms</td>
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<tr>
<td>Pop Tarts</td>
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<td>.109</td>
<td>1 tart=51.6 gms</td>
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<tr>
<td>Pretzels</td>
<td>.010</td>
<td>.016</td>
<td>10 sm. pretzels = 34 gms</td>
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<tr>
<td>Rice, brown</td>
<td>.581</td>
<td>.456</td>
<td>2/3 cup=130 gms</td>
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Table 4 (continued)

<table>
<thead>
<tr>
<th>Food</th>
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<tbody>
<tr>
<td></td>
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<td>Breads, Cereals and other Grain Products (continued)</td>
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<td></td>
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<tr>
<td>Rice-a-Roni</td>
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<td>.245</td>
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<td>Rice, white</td>
<td>.389</td>
<td>.356</td>
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<tr>
<td>Rolls, hamburger</td>
<td>.245</td>
<td>.218</td>
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<td>Stuffing mix, stove top</td>
<td>.149</td>
<td>.187</td>
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<tr>
<td>Twinkie</td>
<td>.120</td>
<td>.115</td>
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<tr>
<td>Waffles, frozen</td>
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<td>.238</td>
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<tr>
<td>Wheat germ</td>
<td>.419</td>
<td>.342</td>
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<tr>
<td>Whole wheat cereal</td>
<td>.308</td>
<td>.210</td>
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<td>Meat, Fish, Poultry and Meat Analogs</td>
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<td>Beef, chuck steak-pan broiled</td>
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<td>.719</td>
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<tr>
<td>Beef, lean ground-pan broiled</td>
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<td>Beef, regular ground-pan broiled</td>
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<td>.570</td>
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<td>Beef short ribs-braised</td>
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<td>Breakfast links</td>
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<td>.060</td>
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<tr>
<td>Chicken breast - baked</td>
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<td>Fish sticks, frozen-cooked</td>
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<td>Halibut steak - poached</td>
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<td>Pork loin chops-pan broiled</td>
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<td>.507</td>
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<td>Sausages</td>
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<td>Bologna</td>
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<td>.139</td>
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<td>Frankfurters</td>
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<td>.150</td>
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<td>Liverwurst</td>
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<td>.628</td>
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<tr>
<td>Ground pork-pan broiled</td>
<td>.202</td>
<td>.153</td>
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<tr>
<td>Salami</td>
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<td>.279</td>
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<tr>
<td>Dairy Products</td>
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<tr>
<td>Cheese, monterey jack</td>
<td>.055</td>
<td>.080</td>
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<td>Cheese, romano</td>
<td>.071</td>
<td>.137</td>
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Table 4 (continued)

<table>
<thead>
<tr>
<th>Food</th>
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<tr>
<td></td>
<td>Microbio-</td>
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<td></td>
<td>logical</td>
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<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Fruits and Vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applesauce, sweetened</td>
<td>.077</td>
<td>.055</td>
<td>1/4 cup=128 gms</td>
<td>L</td>
</tr>
<tr>
<td>Applesauce, unsweetened</td>
<td>.116</td>
<td>.106</td>
<td>1/2 cup=122 gms</td>
<td>L</td>
</tr>
<tr>
<td>Cranberry juice cocktail</td>
<td>.127</td>
<td>.106</td>
<td>6 oz.=190 gms</td>
<td>L</td>
</tr>
<tr>
<td>Fruit cocktail, canned</td>
<td>.154</td>
<td>.108</td>
<td>1 cup=255 gms</td>
<td>L</td>
</tr>
<tr>
<td>Orange juice, frozen - reconstituted</td>
<td>.277</td>
<td>.268</td>
<td>6 oz glass=187 gms</td>
<td>T</td>
</tr>
<tr>
<td>Pickles</td>
<td>.009</td>
<td>.014</td>
<td>2 pickles=30 gms</td>
<td>T</td>
</tr>
<tr>
<td>Potatoes, baked</td>
<td>1.021</td>
<td>.585</td>
<td>1 potato-184 gms</td>
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</tr>
<tr>
<td>Potatoes, boiled</td>
<td>1.002</td>
<td>.713</td>
<td>1 potato=245 gms</td>
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</tr>
<tr>
<td>Potatoes, canned</td>
<td>.183</td>
<td>.160</td>
<td>105 gms</td>
<td>C</td>
</tr>
<tr>
<td>Potatoes, instant mashed</td>
<td>.362</td>
<td>.260</td>
<td>1/2 cup=105 gms</td>
<td>L</td>
</tr>
<tr>
<td>Potatoes, mashed</td>
<td>.289</td>
<td>.249</td>
<td>1/2 cup=105 gms</td>
<td>C</td>
</tr>
<tr>
<td>Potato chips</td>
<td>.159</td>
<td>.142</td>
<td>10 chips=20 gms</td>
<td>T</td>
</tr>
<tr>
<td>Tomatoes, canned</td>
<td>.402</td>
<td>.407</td>
<td>1 cup=241 gms</td>
<td>T</td>
</tr>
<tr>
<td>Tomato paste, canned</td>
<td>.640</td>
<td>.685</td>
<td>1/2 6 oz. can=85 gms</td>
<td>C</td>
</tr>
<tr>
<td>Tomato sauce, canned</td>
<td>.346</td>
<td>.316</td>
<td>112 gms</td>
<td>L</td>
</tr>
<tr>
<td>V-8 juice</td>
<td>.499</td>
<td>.366</td>
<td>6 oz. glass=168 gms</td>
<td>L</td>
</tr>
<tr>
<td>Yams, canned</td>
<td>.381</td>
<td>.361</td>
<td>3 potatoes + syrup 89.4 gms</td>
<td>W</td>
</tr>
</tbody>
</table>

*Refers to the source used to arrive at an average serving size of a food. T = Table of food composition, W = Foods were weighed before analysis, L = Food package label gave a suggested serving size, C = Food weight was set for comparison with similar foods of the same weight.
defined for each item, and the source of information for arriving at
each serving size is indicated. In a few cases where no definite
portion information could be found, serving sizes were chosen to allow
comparison of the pantothenic acid content of a food with equal serving
sizes of similar foods. Again, all serving weights and volumes refer
to the cooked or prepared edible portion of the food.

Expressing the nutrients in a food relative to the kilocalories is
another useful way of comparing the nutritive value of foods. The
nutrient density of pantothenic acid relative to kilocalories is
shown in Table 5. The food items are ranked from highest to lowest
pantothenic acid content per 1,000 kilocalories based on the results
of the microbiological assay. The exact ranking of the foods based on
the RIA results would be different, however the trend is the same. In
Table 5, the source of information for the kilocalorie data is indicated,
including the code number of the equivalent or most similar food item
to be found in the U.S.D.A. food composition table. Nutrition labels,
if present, were the preferred source of kilocalorie content information
because they represent current values for the food as it is sold.

Comparison of Assay Results

One objective of the present study was to determine if the micro-
biological assay and the radioimmunoassay produce comparable results
in measuring pantothenic acid in a variety of foods.

The correlation between the two assay methods was computed using
the mean pantothenic acid value for each food, and the correlation
coefficient was $r^2 = .937$. 
<table>
<thead>
<tr>
<th>Food</th>
<th>Pantothenic acid (mg)</th>
<th>Source of Kilocalorie Information*</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-8 juice</td>
<td>13.131</td>
<td>456 - #2294c</td>
</tr>
<tr>
<td>Chicken legs, baked</td>
<td>10.565</td>
<td>456 - #685a</td>
</tr>
<tr>
<td>Chicken breast, baked</td>
<td>10.514</td>
<td>456 - #685a</td>
</tr>
<tr>
<td>Liverwurst</td>
<td>10.189</td>
<td>456 - #2003</td>
</tr>
<tr>
<td>Tomato paste, canned</td>
<td>9.204</td>
<td>456 - #2295</td>
</tr>
<tr>
<td>Tomatoes, canned</td>
<td>7.882</td>
<td>456 - #2284d</td>
</tr>
<tr>
<td>Potato, baked</td>
<td>7.732</td>
<td>456 - #1786a</td>
</tr>
<tr>
<td>Potato, boiled</td>
<td>5.378</td>
<td>456 - #1787a</td>
</tr>
<tr>
<td>Oatmeal, quick</td>
<td>4.418</td>
<td>Label</td>
</tr>
<tr>
<td>Oatmeal, regular</td>
<td>4.245</td>
<td>Label</td>
</tr>
<tr>
<td>Beef, lean ground</td>
<td>4.027</td>
<td>456 - #368c</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>3.953</td>
<td>Label</td>
</tr>
<tr>
<td>Rice, brown</td>
<td>3.756</td>
<td>456 - #1870a</td>
</tr>
<tr>
<td>Yams, canned</td>
<td>3.741</td>
<td>456 - #2252c</td>
</tr>
<tr>
<td>Potatoes, instant mashed</td>
<td>3.715</td>
<td>456 - #1798a</td>
</tr>
<tr>
<td>Cheerios cereal</td>
<td>3.454</td>
<td>Label</td>
</tr>
<tr>
<td>Oatmeal, instant</td>
<td>3.244</td>
<td>Label</td>
</tr>
<tr>
<td>Beef chuck steak</td>
<td>3.109</td>
<td>456 - #234b</td>
</tr>
<tr>
<td>Tomato sauce, canned</td>
<td>3.089</td>
<td>Label</td>
</tr>
<tr>
<td>Salami</td>
<td>3.034</td>
<td>456 - #2018c</td>
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<td>Orange juice, frozen</td>
<td>3.011</td>
<td>456 - #1437c</td>
</tr>
<tr>
<td>Beef, regular ground</td>
<td>2.945</td>
<td>456 - #370c</td>
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<td>Whole wheat cereal</td>
<td>2.933</td>
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<tr>
<td>Potatoes, mashed</td>
<td>2.931</td>
<td>456 - #1793a</td>
</tr>
<tr>
<td>Halibut, poached</td>
<td>2.757</td>
<td>456 - #1104b</td>
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<tr>
<td>Rice, white</td>
<td>2.620</td>
<td>456 - #1872a</td>
</tr>
<tr>
<td>Applesauce, unsweetened</td>
<td>2.320</td>
<td>456 - #28e</td>
</tr>
<tr>
<td>Fish sticks, frozen</td>
<td>2.225</td>
<td>456 - #1017b</td>
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<tr>
<td>English muffins</td>
<td>2.077</td>
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<td>Pork chops</td>
<td>1.908</td>
<td>456 - 1717c</td>
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<td>Bologna</td>
<td>1.884</td>
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<td>Wheaties cereal</td>
<td>1.836</td>
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<td>Total cereal</td>
<td>1.818</td>
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<td>Hamburger rolls</td>
<td>1.778</td>
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<td>Granola cereal</td>
<td>1.777</td>
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<tr>
<td>Fish filet, frozen</td>
<td>1.732</td>
<td>Label</td>
</tr>
<tr>
<td>Noodles</td>
<td>1.728</td>
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<td>Rice-a-roni</td>
<td>1.658</td>
<td>Label</td>
</tr>
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<td>Bread, bran'ola</td>
<td>1.605</td>
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</tr>
<tr>
<td>Pork sausage</td>
<td>1.566</td>
<td>456 - #2014d</td>
</tr>
<tr>
<td>Pancakes from mix</td>
<td>1.562</td>
<td>456 - #1457b</td>
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<td>Frankfurters</td>
<td>1.545</td>
<td>456 - #1999c</td>
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Table 5 (continued)

<table>
<thead>
<tr>
<th>Food</th>
<th>Microbiological</th>
<th>RIA</th>
<th>Source of Kilocalorie Information*</th>
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</thead>
<tbody>
<tr>
<td>Wheat Chex cereal</td>
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<td>Potato chips</td>
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<td>Frozen waffles</td>
<td>1.337</td>
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<td>Grape nuts cereal</td>
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<td>Label</td>
</tr>
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<td>Beef short ribs, braised</td>
<td>1.255</td>
<td>1.013</td>
<td>456 - #224c</td>
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<td>Cornbread from mix</td>
<td>1.230</td>
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<td>Biscuits from mix</td>
<td>1.220</td>
<td>.978</td>
<td>456 - #416a</td>
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<td>Biscuits, homemade</td>
<td>1.184</td>
<td>1.035</td>
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<td>Graham crackers</td>
<td>1.154</td>
<td>1.206</td>
<td>8 - #914</td>
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<td>Product 19 cereal</td>
<td>1.100</td>
<td>1.127</td>
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<td>Cranberry juice cocktail</td>
<td>1.024</td>
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<td>Whole wheat crackers</td>
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<td>Granola snack bars</td>
<td>.987</td>
<td>1.020</td>
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<td>Stove top stuffing</td>
<td>.876</td>
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<td>Potatoes, canned</td>
<td>.870</td>
<td>.760</td>
<td>8 - #1796</td>
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<tr>
<td>Biscuits from refrigerated dough</td>
<td>.859</td>
<td>1.357</td>
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<td>Macaroni, cooked</td>
<td>.813</td>
<td>.760</td>
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<td>Yellow cake from mix</td>
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<td>456 - #1023d</td>
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<td>Pretzels</td>
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<td>.662</td>
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<td>.645</td>
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<td>Pop tarts</td>
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<td>.537</td>
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<td>Monterey Jack cheese</td>
<td>.518</td>
<td>.755</td>
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<td>Corn flakes cereal</td>
<td>.445</td>
<td>.727</td>
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</tr>
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<td>Breakfast links</td>
<td>.340</td>
<td>.300</td>
<td>Label</td>
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<tr>
<td>Pickles</td>
<td>.211</td>
<td>.318</td>
<td>456 - #1561b</td>
</tr>
</tbody>
</table>

*456 refers to Agriculture Handbook No. 456, Nutritive Value of American Foods in Common Units, 1975

8 refers to Agriculture Handbook No. 8, Composition of Foods, 1963

8-1 refers to Agriculture Handbook No. 8-1, Composition of Foods - Dairy and Egg Products, 1976.
The hypothesis $H_0: \mu_1 = \mu_2$ was tested, where $\mu_1$ was the mean of the assay results by the microbiological method, and $\mu_2$ was the mean of the RIA results. An $F$ test was performed assuming a mixed model, where the methods were fixed and the foods were random. The mathematical model used was

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + E_{ijk},$$

where $\mu$ is the overall mean of all the observations, $M_i$ is the effect of the method, $F_j$ is the effect of the food, $MF_{ij}$ is the interaction effect of methods with foods, and $E_{ijk}$ is the unexplained error in each observation.

The results of the $F$ test on all foods are presented in Table 6. At $p = .05$ the hypothesis of equality of the means of the two assay methods is rejected. There was a statistically significant difference between the results produced by the two assays. Overall the microbiological assay results were 13.4% higher than the results from the RIA. Not surprisingly, the individual foods were statistically different from one another in pantothenic acid content.

Assay results from three of the four different food groups were compared using the same mathematical model as for the above $F$ test, to see if the assays behaved differently with different types of foods. The results are presented in Tables 7, 8 and 9. The dairy group was not considered separately because only two foods from this group were analyzed. For each of the three food groups, the two assay methods produced significantly different results at $p = .05$. At $p = .01$, only meats were significantly different. For the bread and cereal group, the microbiological assay produced answers that were an average
Table 6. F-test for equality of assay results in all foods by two assay methods

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>EMS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>1</td>
<td>0.3666089</td>
<td>0.3666089</td>
<td>0</td>
<td>3.666089</td>
</tr>
<tr>
<td>Food</td>
<td>74</td>
<td>68.211404</td>
<td>0.9217757</td>
<td>0</td>
<td>6.82114</td>
</tr>
<tr>
<td>Method x Food</td>
<td>74</td>
<td>1.985642</td>
<td>0.026833</td>
<td>0</td>
<td>0.01986</td>
</tr>
<tr>
<td>Error</td>
<td>300</td>
<td>1.98859</td>
<td>0.0066286</td>
<td>0</td>
<td>0.00198</td>
</tr>
<tr>
<td>Total</td>
<td>449</td>
<td>72.55224</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different at p = .05
**Significantly different at p = .01

Table 7. Test for equality of assay results in breads and cereals by two assay methods

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>1</td>
<td>0.057296</td>
<td>0.057296</td>
<td>4.95*</td>
</tr>
<tr>
<td>Food</td>
<td>39</td>
<td>16.238539</td>
<td>0.4163727</td>
<td>99.32**</td>
</tr>
<tr>
<td>Method x Food</td>
<td>39</td>
<td>0.45143</td>
<td>0.0115751</td>
<td>2.76**</td>
</tr>
<tr>
<td>Error</td>
<td>160</td>
<td>0.670773</td>
<td>0.0041923</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>17.418038</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different at p = .05
**Significantly different at p = .01
Table 8. Test for equality of assay results in fruits and vegetables by two assay methods

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>1</td>
<td>.04209</td>
<td>.04209</td>
<td>6.24*</td>
</tr>
<tr>
<td>Food</td>
<td>16</td>
<td>4.7312141</td>
<td>.2957008</td>
<td>269.70**</td>
</tr>
<tr>
<td>Method x Food</td>
<td>16</td>
<td>.107872</td>
<td>.006742</td>
<td>6.15**</td>
</tr>
<tr>
<td>Error</td>
<td>68</td>
<td>.074556</td>
<td>.0010964</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>4.9557321</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different at p = .05  
**Significantly different at p = .01

Table 9. Test for equality of assay results in meats by two assay methods

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>1</td>
<td>.6853927</td>
<td>.6853927</td>
<td>10.92**</td>
</tr>
<tr>
<td>Food</td>
<td>14</td>
<td>29.730666</td>
<td>2.123619</td>
<td>38.83**</td>
</tr>
<tr>
<td>Method x Food</td>
<td>14</td>
<td>.878889</td>
<td>.0627777</td>
<td>2.96**</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>1.273816</td>
<td>.212302</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>32.568763</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different at p = .05  
**Significantly different at p = .01
of 6.6% higher than the RIA. For fruits and vegetables, the microbiological assay results were 11.6% higher, and for meats the results were 23.2% higher.

Preparing Food Samples for Assay and Assay Procedures

The methods used for preparing extracts from food samples are described in detail in only one previous report (6). Some parts of these procedures had to be validated for use in this experiment, and some modifications were made.

After the food samples were brought to a ready to eat state, they were homogenized in a blender with water and the homogenate was frozen until analysis. Zook and colleagues (6) also froze their food samples until analysis, but some reports indicate losses of pantothenic acid during freezing of foods (14). Table 10 contains results from an experiment to determine the effects of freezing on pantothenic acid content of food samples. The samples were assayed microbiologically.

Table 10. Effects of freezing on pantothenic acid content of food samples

<table>
<thead>
<tr>
<th>Food</th>
<th>n</th>
<th>mg P.A. per 100 grams of food</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unfrozen</td>
<td>Frozen and Thawed</td>
<td></td>
</tr>
<tr>
<td>Cooked ground beef</td>
<td>1</td>
<td>.540</td>
<td>.934</td>
<td></td>
</tr>
<tr>
<td>Whole wheat bread</td>
<td>1</td>
<td>.580</td>
<td>.842</td>
<td></td>
</tr>
<tr>
<td>Yeast (+ s.d.)</td>
<td>4</td>
<td>9.90±1.66</td>
<td>11.50±1.05</td>
<td></td>
</tr>
</tbody>
</table>
Since the higher values for the frozen samples may be a result of rupture of cell membranes during freezing and thawing, sonication of the foods was tried to determine if further disruption of the cell contents could be obtained. Food homogenates were sonicated for 5 minutes, with the sample tubes packed in ice during sonication. Results of this experiment are presented in Table 11.

Table 11. Effects of freezing and sonication on pantothenic acid content of food samples

<table>
<thead>
<tr>
<th>Food</th>
<th>n</th>
<th>Frozen</th>
<th>Sonicated</th>
<th>Frozen and Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>1</td>
<td>.412</td>
<td>.400</td>
<td>.420</td>
</tr>
<tr>
<td>Yeast</td>
<td>3</td>
<td>11.20±1.10</td>
<td>10.93±1.41</td>
<td>11.07±1.12</td>
</tr>
<tr>
<td>Cabbage (+ s.d.)</td>
<td>1</td>
<td>.378</td>
<td>.370</td>
<td>.366</td>
</tr>
</tbody>
</table>

Freezing is an acceptable method for storing food samples until analysis. When samples were repeatedly frozen and thawed for repeat analysis, however, some losses of pantothenic acid were observed. For this reason, it is best to carry out all assays on a food after the first thawing.

Zook et al. (6) autoclaved their food samples before enzyme hydrolysis. Since pantothenic acid is a water soluble vitamin there might be some danger of pantothenic acid destruction during this procedure. Hamm and Lund (30) showed that when food purees in the pH range 5.4 to 7.0 were autoclaved at 118°C, very little destruction of pantothenic acid took place. A similar experiment conducted as part of this research project (Table 12) showed no losses of pantothenic
acid in autoclaved yeast and milk samples. Autoclaving is advantageous because it destroys food bacteria that could produce or degrade pantothenic acid during the subsequent 37°C enzyme incubation period.

Table 12. Effects of autoclaving on pantothenic acid content of food samples

<table>
<thead>
<tr>
<th>Food</th>
<th>mg P.A. per 100 grams of food (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not Autoclaved</td>
</tr>
<tr>
<td>Milk</td>
<td>4</td>
</tr>
<tr>
<td>Yeast</td>
<td>1</td>
</tr>
</tbody>
</table>

Zook et al. (6) used 0.40 ml of a 2% alkaline phosphatase solution and 0.20 ml of the dowex treated pigeon liver homogenate per sample. From experimentation with different levels of alkaline phosphatase, and pigeon liver enzyme, it was determined that 0.40 ml of a 6% alkaline phosphatase solution and 0.4 ml of a 10% solution of dowex treated pigeon liver powder per sample provided maximal liberation of bound forms of pantothenic acid. The experiments were conducted with samples of dried bakers yeast, and the results are shown in Table 13. Appropriate enzyme blank values have been subtracted from the results.

A clear, non-turbid food extract is desirable for the microbiological assay, as organism growth is measured turbidometrically. An extract with similar properties was needed for the RIA. Zook et al. (6) clarified and diluted the food-enzyme mixture by pouring the sample through Whatman #40 filter paper. This filtration method, however, is slow and laborious. It often takes two or more hours for the sample
Table 13. Effects of varying levels of enzyme on yeast assay results

<table>
<thead>
<tr>
<th>ml 0.6% alkaline phosphatase</th>
<th>ml 10% pigeon liver enzyme</th>
<th>mg P.A. per 100 gm yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>3.6</td>
</tr>
<tr>
<td>0.20</td>
<td>0</td>
<td>7.0</td>
</tr>
<tr>
<td>0.40</td>
<td>0</td>
<td>6.2</td>
</tr>
<tr>
<td>0</td>
<td>0.40</td>
<td>5.8</td>
</tr>
<tr>
<td>0</td>
<td>0.60</td>
<td>5.2</td>
</tr>
<tr>
<td>0.20</td>
<td>0.40</td>
<td>5.9</td>
</tr>
<tr>
<td>0.40</td>
<td>0.40</td>
<td>9.4, 9.6</td>
</tr>
<tr>
<td>0.20</td>
<td>0.60</td>
<td>6.0</td>
</tr>
<tr>
<td>0.40</td>
<td>0.60</td>
<td>9.5, 9.1</td>
</tr>
</tbody>
</table>

and added water to pass through the filter paper. Use of a Buchner funnel in this laboratory proved unsatisfactory as particulate matter was difficult to exclude. Additionally, the filtrate produced is often turbid. Zook, et al. reported having to repeat the filtration process for many food samples. This turbidity, which may be invisible upon inspection, makes it necessary to preread the absorbance of each sample before adding the assay organism.

The dialysis method developed during this research project eliminated the above problems and a very clear extract is produced that can be used in the RIA as well as the microbiological assay. The dialysis system was tested by adding $^3$H-pantothenic acid to a food enzyme mixture and dialyzing as usual for 8 hours. The contents of the dialysis tubing were then centrifuged at 8,000 rpm for 10 minutes to clear the mixture as much as possible. Three 1 ml samples each
from the supernatant and the outside of the dialysis tubing were added to Aquasol scintillation cocktail and counted. The extract from outside the tubing had $14,799 \pm 200$ cpm and the extract from inside the tubing had $13,334 \pm 19$ cpm. The counting efficiency of the extract from inside the tubing was only 93% of the counting efficiency of the extract from outside, so the concentration of $^{3}$H-pantothenic acid in the two compartments was essentially equal.

The results of a series of experiments on dialysis and filtration of food samples are shown in Table 14.

**Table 14. Pantothenic acid in food samples filtered or dialyzed before analysis**

<table>
<thead>
<tr>
<th>Food</th>
<th>Number of samples</th>
<th>Dialyzed</th>
<th>Filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground beef</td>
<td>1</td>
<td>.540</td>
<td>.680</td>
</tr>
<tr>
<td>Apple</td>
<td>1</td>
<td>.140</td>
<td>.100</td>
</tr>
<tr>
<td>Whole wheat bread</td>
<td>1</td>
<td>.580</td>
<td>.630</td>
</tr>
<tr>
<td>Cabbage</td>
<td>1</td>
<td>.378</td>
<td>.275</td>
</tr>
<tr>
<td>Composite meal sample</td>
<td>4</td>
<td>.367 (.030)</td>
<td>.320 (.092)</td>
</tr>
<tr>
<td>Milk</td>
<td>1</td>
<td>.350</td>
<td>.320</td>
</tr>
<tr>
<td>Baker's yeast</td>
<td>1</td>
<td>11.1</td>
<td>11.0</td>
</tr>
</tbody>
</table>

**Quality Control and Validation Procedures**

The results from the assay of a dried baker's yeast sample during each of thirteen separate assay runs are presented in Table 15. The mean pantothenic acid content of the samples measured by the RIA was 12.2% below the mean pantothenic acid content of the yeast samples measured microbiologically.
Between three and five replicates of each food were analyzed by each method. As described in Chapter II, more than three replicates of a food were analyzed if the coefficient of variation (standard deviation ÷ mean x 100) was unacceptably high. After all assays were completed, extreme values were excluded, and the three replicates that resulted in the smallest coefficient of variation were used to arrive at the mean value for each food assayed by each method (see Appendix C).

Known amounts of calcium pantothenate were added to food samples and assayed by both methods. One hundred and five percent (± 7.6) of added calcium pantothenate spike was recovered in the microbiological assays. A mean of 89.3% (± 7.9) of the spike was recovered in the RIA.

Table 16 contains the results of the experiment on variability of results within the same assay batch. The coefficient of variation within a batch is smaller than the variation shown for yeast assayed in 13 different batches (Table 15). For the Fuerte variety of avocado, the RIA produced an answer 4.2% higher than the microbiological assay. For the Hass variety, the RIA measured 6.5% more pantothenic acid than the microbiological assay.

Figure 1 shows the parallelism of three different foods assayed at different levels to the standard curve for the microbiological assay. The three foods were assayed in the same batch and are compared to the actual standard curve used for that batch. Figure 2 shows the parallelism of a sample of wheat germ assayed at 8 different dilutions to the standard curve for the RIA.
Table 15. Yeast assay results from thirteen weekly assays

<table>
<thead>
<tr>
<th>Method</th>
<th>mg P.A. per 100 gm yeast (S.D.)</th>
<th>Coefficient of Variation (s.d. x 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiological assay</td>
<td>12.98 (1.62)</td>
<td>12.5%</td>
</tr>
<tr>
<td>Radioimmunoassay</td>
<td>11.39 (1.45)</td>
<td>12.7%</td>
</tr>
</tbody>
</table>

Table 16. Pantothenic acid content avocados measured in the same assay batch

<table>
<thead>
<tr>
<th>Avocado variety</th>
<th>Fuerte</th>
<th>Hass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microbiological</td>
<td>RIA</td>
</tr>
<tr>
<td>Assay method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.805</td>
<td>.891</td>
<td>.924</td>
</tr>
<tr>
<td>.738</td>
<td>.797</td>
<td>.833</td>
</tr>
<tr>
<td>.740</td>
<td>.775</td>
<td>.845</td>
</tr>
<tr>
<td>.703</td>
<td>.769</td>
<td>.854</td>
</tr>
<tr>
<td>.734</td>
<td>.848</td>
<td>.962</td>
</tr>
<tr>
<td>.726</td>
<td>.838</td>
<td>.845</td>
</tr>
<tr>
<td>.984</td>
<td>.766</td>
<td>.876</td>
</tr>
<tr>
<td>.726</td>
<td>.728</td>
<td>.894</td>
</tr>
</tbody>
</table>

\[ \bar{X} \text{ P.A. + s.d.} \quad .770+.0915 \quad .802+.0534 \quad .879+.0450 \quad .936+.0864 \]

Coefficient of Variation 11.8% 6.6% 5.1% 9.2%
Figure 1. Parallelism of three food samples to microbiological assay standard curve.
Figure 2. Parallelism of wheat germ sample to RIA standard curve.
CHAPTER IV
DISCUSSION AND CONCLUSIONS

As stated at the end of Chapter I, the goals of this research report were (1) to determine if a radioimmunoassay (RIA) for pantothenic acid could be successfully applied to the assay of the vitamin in foods, (2) to modify and improve the present system for the preparation of food samples, and (3) to report on the pantothenic acid content of 75 foods that had been hitherto unreported.

The Use of an RIA to Measure Pantothenic Acid in Foods

A very high correlation \( r^2 = .937 \) between the results from the RIA and the microbiological assay for pantothenic acid in foods has been found. The methods do not produce identical results, however. At \( p = .05 \), the methods produce significantly different results for all foods studied and for the subgroups of meats, fruits and vegetables, and breads and cereals. At \( p = .01 \) the methods are different for all foods, and for meats alone, and the methods are not significantly different for fruits and vegetables and breads and cereals. In all cases, the microbiological assay produced a higher average result than the RIA. Interestingly, when a large number of avocado samples were assayed, the RIA gave results that were 4.2 to 6.5% higher than the microbiological assay.

When comparing the microbiological assay results with the RIA results, and in using either of these assays to draw conclusions about pantothenic acid in foods, it is important to remember the fairly large
variability in results within a given method. Assay results for the same food by the same method have a coefficient of variation of between 5.1 and 11.8% within the same assay batch (Table 16) and about 12.6% among different batches (Table 15). For many foods, the range of results for the two assays were overlapping (see Appendix C).

Looking at the assay data for fruits and vegetables and breads and cereals, it is difficult to find a pattern in the type of foods that are likely to have higher or lower results from the methods. Only one meat product had an RIA result higher than the microbiological result, and the meat group had the largest difference between results from the two assays. Hamm and Lund (30) reported that meat purees assayed microbiologically for pantothenic acid showed increasing levels of the vitamin per milliliter of sample as the total sample volume was increased. This did not occur in the present experiment. As the volume of sample added to the assay tubes increased from 1 to 5 milliliters, the concentration of pantothenic acid per milliliter remained constant. At present no explanation can be given for the especially marked differences in the meat results except that foods of plant and animal origin are complex mixtures of organic and inorganic molecules that may react with components of either assay system.

When considering dietary intakes of the vitamin, a range of 5 to 10 milligrams a day is suggested (22). In this context, the differences between the assay results, usually considerably less than 0.1 milligram per serving of food (see Table 4), may not be significant in the overall calculation of daily dietary intake of pantothenic acid. Table 17 illustrates this point. In light of the approximate dietary requirement for pantothenic acid of 5 to 10 milligrams per
Table 17. Calculation of daily dietary intake of pantothenic acid using two assay method results

<table>
<thead>
<tr>
<th>Food</th>
<th>Microbiological</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 oz. orange juice</td>
<td>.277</td>
<td>.268</td>
</tr>
<tr>
<td>2/3 cup oatmeal</td>
<td>.467</td>
<td>.432</td>
</tr>
<tr>
<td>1 piece cornbread</td>
<td>.219</td>
<td>.209</td>
</tr>
<tr>
<td>2 oz. bologna</td>
<td>.324</td>
<td>.278</td>
</tr>
<tr>
<td>2 slices bread</td>
<td>.318</td>
<td>.310</td>
</tr>
<tr>
<td>2 pickles</td>
<td>.018</td>
<td>.028</td>
</tr>
<tr>
<td>10 potato chips</td>
<td>.159</td>
<td>.142</td>
</tr>
<tr>
<td>2/3 cup rice</td>
<td>.389</td>
<td>.356</td>
</tr>
<tr>
<td>1 baked chicken breast</td>
<td>1.342</td>
<td>1.117</td>
</tr>
<tr>
<td>1/2 cup unsw. applesauce</td>
<td>.116</td>
<td>.106</td>
</tr>
<tr>
<td>1 refrigerator biscuit</td>
<td>.044</td>
<td>.070</td>
</tr>
<tr>
<td>1 cup fruit cocktail</td>
<td>.154</td>
<td>.108</td>
</tr>
<tr>
<td><strong>Total mg of pantothenic acid per day</strong></td>
<td><strong>3.827</strong></td>
<td><strong>3.424</strong></td>
</tr>
</tbody>
</table>
day, the differences in assay results for breads and cereals and fruits and vegetables do not seem critical. Because only two samples of dairy food were analyzed, no conclusions can be drawn about the relative merits of the two assays when applied to this group. The assay differences for meats are marked and further study is needed to determine which assay gives a realistic estimate of the pantothenic acid content of meats.

The acceptance and use of more than one method for nutrient assay is not unprecedented. There are several acceptable methods for the assay of lipids in foods (31). The choice of an assay method depends on factors such as physical properties of the food, equipment and expertise available, and time available for conducting the assay. The results of this study suggest that a similar situation may exist regarding the two assays for pantothenic acid. The microbiological assay requires aseptic working conditions, continuous culture of the assay organism and considerable practice on the part of the technician. It is also a longer assay to perform than the RIA. The RIA requires access to a scintillation counter and scrupulous care in the handling of radioactive materials. The research institution and society are burdened with the problems inherent in the disposal of radioactive wastes.

Assay Procedures

The alkaline phosphatase and pigeon liver enzyme system for release of pantothenic acid is the most commonly used for food assays. As reported in Chapter I, there is no standard food that can be used as a indicator that all bound forms of pantothenic acid have been released by an enzyme treatment. A weakness in this research project
is that there may be incomplete liberation of bound pantothenic acid in foods. While excess enzyme was used, there may be bound forms of the vitamin that are not cleaved by these two enzymes. There is a possibility that bound forms of the vitamin could be broken down by bacterial enzymes in *L. plantarum* or break down without enzyme assistance during the long microbiological incubation period. This could explain the higher microbiological results if one assumes incomplete release of bound forms of the vitamin during the enzyme treatment. Differences in enzymatic release of pantothenic acid between different subsamples of the same food may be responsible for a good deal of the variability observed in both assays. The mean coefficient of variation of three replicates of a standard curve point in the microbiological assay is 3.69. For the RIA the mean coefficient of variation is 2.12. This accounts for only part of the variation in samples shown in Table 1. There is ongoing research in this laboratory on improved enzymatic, chemical and physical methods for complete release of bound forms of pantothenic acid.

The microbiological assay was carried out according to the method of A.O.A.C. (10). A powdered commerical assay medium (Difco Pantothenate Assay Medium) was tested and found to produce a standard growth curve that was comparable to the growth curve obtained from a medium made according to the A.O.A.C. instructions.

For the RIA, the procedure used for the assay of pantothenic acid in blood was used (26) except that 0.50 ml of sample or standard was used per assay tube. Smaller amounts of sample produced results that had to be read on the low end of the standard curve. With 0.50 ml of sample, most results fell in the 10 ng to 40 ng portion of the standard curve.
Comparison of Assay Methods

The microbiological assay and the RIA can be compared in several ways to determine which method is best for a particular laboratory.

The RIA is more expensive to use than the microbiological assay. An assay of 25 food samples, one enzyme blank and one yeast standard following the present study design would cost approximately $6.32 for the microbiological assay and $18.02 for the RIA. These costs were arrived at using June 1979 prices for major supplies and reagents used for each assay. Glassware, the scintillation counter, the incubator and the autoclave were not included in cost calculation. Low cost reagents used in very small quantities were also not considered. The cost of enzyme digestion, required for both assays was not included. For the microbiological assay, the only cost was for the prepared assay medium. For the RIA, the prices of rabbit albumin, disposable pipette tips, scintillation cocktail and tissue solubilizer were used to calculate the cost.

Using the microbiological assay and allowing for an 18 hour incubation one would wait 25 hours for final results. Total labor time for an experienced technician to prepare the 25 samples on the first day and read results from the spectrophotometer on the second day would be about 6 hours and 20 minutes. The same number of samples would require only 13 hours and 20 minutes for results using the RIA. This allows for a six hour period for vials to sit in the dark in the scintillation counter before being read. This assumes that the counter will begin reading the samples in the evening. Since this does not coincide with the regular hours of the laboratory, an overall
time estimate of 23 hours might be more realistic. In either case, about 4 hours and 50 minutes of a technician's time would be used. If a technician was paid $5.50 an hour, the labor cost difference for assaying 25 food samples would be $8.24, but considering material costs, the RIA would still cost $3.43 more than the microbiological assay. As labor becomes more expensive or limited, the RIA becomes the more economical method, but for the conditions described here, the microbiological assay is less expensive.

Both assay methods require great attention to detail and painstaking care in pipetting. The RIA requires fewer operations than the microbiological assay and is easier to teach to a new technician.

Overall, the available equipment, labor costs, and the expertise of the technician are all determining factors in choosing the RIA or the microbiological assay for pantothenic acid in foods.

Pantothenic Acid in Foods

One of the best ways to compare the pantothenic acid content of different foods is to look at the amount of the vitamin in a serving of each food (Table 4). Among ready to eat breakfast cereals, absolute amounts of pantothenic acid per serving are not high. The oat based cereals, Cheerios and Granola, have the most pantothenic acid per serving. The presweetened cereals, Froot Loops and Sugar Smacks and the corn based cereals, Corn Chex and Corn Flakes have the least pantothenic acid. Interestingly, Product 19 and Total, which are promoted as high vitamin cereals, do not have a great deal of pantothenic acid, because that particular vitamin is not added during fortification.
Among the other bread and cereal products analyzed, oatmeal from the regular, quick or instan dry forms, brown rice and wheat germ are highest in pantothenic acid per serving. Most products using white flour as a primary ingredient, such as biscuits, saltine crackers, pretzels and twinkies were low in pantothenic acid.

Meat, poultry and fresh products contained fairly high amounts of pantothenic acid per serving. Cooked chicken and beef were highest in the vitamin. Liverwurst, made from vitamin-rich liver, was also very high. Beef short ribs which were braised in water for two hours, had considerably less pantothenic acid than the broiled beef dishes.

All of the fruits assayed had less than 0.200 milligrams of pantothenic acid per serving except for frozen reconstituted orange juice. Boiled and baked potatoes and canned tomato products were high in the vitamin.

Energy intake must be limited in the diets of many Americans, and expression of a nutrient per 1,000 kilocalories of a food can show how rich a food is in a nutrient relative to the kcal it supplies (32). Using the microbiological assay results in Table 5, only 26 of the 75 foods would supply at least 5 milligrams of pantothenic acid if the food comprised an entire daily diet of 2,000 kcal. This group of 26 foods is dominated by chicken, beef, potatoes, oat cereals, tomato products and whole grains. The 21 foods that would supply less than 2 milligrams per 2,000 kcal include corn based and pre-sweetened cereals, sweetened fruits products, a vegetable based meat analog (Breakfast Links), cake and Twinkies.
The effect of nutrient dilution on nutrient density is evident in some of the foods analyzed. Using the microbiological results, unsweetened applesauce has 2.3 mg of pantothenic acid per 1,000 kcal, while sweetened applesauce has 0.7 mg. The nutrient density was greatly reduced when sugar, a source of calories but no vitamins, is added to the fruit. Baked and boiled potatoes have 7.7 and 5.4 mg of pantothenic acid per 1,000 kcal, respectively. The addition of fat and milk to make mashed potatoes results in a nutrient density of 2.9 mg per 1,000 kcal. Potato chips, processed with vegetable oils, have 1.4 mg of pantothenic acid per 1,000 kcal. Canned potatoes, with no added calories, are subjected to a long cooking period and unknown storage conditions. They contain only .9 mg of pantothenic acid per 1,000 kcal.

The Food and Nutrition Board of the National Research Council/National Academy of Sciences has not established a recommended dietary allowance for pantothenic acid (22). Five to 10 milligrams per day has been suggested for the daily intake until further evidence is available. The findings in this study suggest two courses of action if this intake is to be met on a 2,000 to 2,500 kcal diet. Either the diets must include a high proportion of meats, whole grains, milk (11) potatoes and lightly processed foods, or more processed foods, especially refined grain products, should be fortified with the vitamin. A wiser course of action would be to conduct further investigations on pantothenic acid nutritional status of Americans to determine what dietary levels of the vitamin are compatible with good health.

Comparison With Previous Reports

Pantothenic acid analyses have previously been performed on a few of the foods analyzed in this research project. Comparison with
these previously reported (12) values is made in Table 18. Most of the results from this project are similar to the older results, except for grape nuts, hamburger rolls, tomato paste and Wheaties. Since many of these foods were assayed more than 20 years ago (6) some of the differences may be due to changes in product formulation. The rest of the difference is probably due to differences in assay technique and unexplained variation in assay results.

Conclusions

The results obtained from this research project on pantothenic acid in foods suggest the following conclusions:

1. The RIA is an acceptable assay for pantothenic acid in breads and cereals and fruits and vegetables. Further study is needed to determine whether the microbiological assay or the RIA provides the truest picture of the pantothenic acid content of animal tissues. For the other foods, the needs and resources of the investigator can determine which assay to use.

2. Dialysis of food samples is more effective than filtration in producing a clear extract for use in both assays.

3. Further study is needed on the pantothenic acid content of American foods, and on optimum dietary levels of pantothenic acid to produce good health.
<table>
<thead>
<tr>
<th>Food</th>
<th>mg P.A. per 100 grams</th>
<th>Descriptive term for food in reference</th>
<th>mg P.A. per 100 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applesauce, sweetened</td>
<td>.060</td>
<td>same</td>
<td>.085</td>
</tr>
<tr>
<td>Grape nuts cereal</td>
<td>.451</td>
<td>wheat and malted barley cereal</td>
<td>.714</td>
</tr>
<tr>
<td>Orange juice, frozen, reconstituted</td>
<td>.148</td>
<td>same</td>
<td>.164</td>
</tr>
<tr>
<td>Potatoes, mashed</td>
<td>.275</td>
<td>frozen mashed potatoes</td>
<td>.240</td>
</tr>
<tr>
<td>Pretzels</td>
<td>.300</td>
<td>same</td>
<td>.540</td>
</tr>
<tr>
<td>Rolls, hamburger</td>
<td>.529</td>
<td>rolls</td>
<td>.310</td>
</tr>
<tr>
<td>Tomatoes, canned</td>
<td>.167</td>
<td>same</td>
<td>.230</td>
</tr>
<tr>
<td>Tomato paste, canned</td>
<td>.753</td>
<td>same</td>
<td>.440</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>1.498</td>
<td>same</td>
<td>1.200</td>
</tr>
<tr>
<td>Wheaties cereal</td>
<td>.723</td>
<td>wheat flakes breakfast cereal</td>
<td>.469</td>
</tr>
<tr>
<td>Yams, canned</td>
<td>.426</td>
<td>canned sweet potatoes</td>
<td>.430</td>
</tr>
</tbody>
</table>

*Source: Home Economics Research Report No. 36 (12).*
REFERENCES


### Table 19

**Supplementary List of Foods Which Have Been Analyzed for Pantothenic Acid (from U.S.D.A.)**

#### Canned vegetables, unprepared

<table>
<thead>
<tr>
<th>Food</th>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagus</td>
<td>Pea, field</td>
</tr>
<tr>
<td>Broadbeans</td>
<td>Snap beans</td>
</tr>
<tr>
<td>Carrots</td>
<td>Spinach</td>
</tr>
<tr>
<td>Endive</td>
<td>Tomato puree</td>
</tr>
<tr>
<td>Mushrooms</td>
<td>Peas and carrots</td>
</tr>
<tr>
<td>Peas, green</td>
<td></td>
</tr>
</tbody>
</table>

#### Frozen vegetables, unprepared

<table>
<thead>
<tr>
<th>Food</th>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichoke</td>
<td>Kale</td>
</tr>
<tr>
<td>Asparagus</td>
<td>Lima beans</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Mustard greens</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>Okra</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Onions</td>
</tr>
<tr>
<td>Collards</td>
<td>Peppers</td>
</tr>
<tr>
<td>Corn, yellow and white</td>
<td>Snap beans</td>
</tr>
<tr>
<td>Cowpeas</td>
<td>Wax beans</td>
</tr>
<tr>
<td>Kale</td>
<td>Italian beans</td>
</tr>
<tr>
<td>Lima beans</td>
<td>Spinach</td>
</tr>
<tr>
<td>Mustard greens</td>
<td>Summer squash</td>
</tr>
<tr>
<td>Okra</td>
<td>Winter squash</td>
</tr>
<tr>
<td>Onions</td>
<td>Zucchini</td>
</tr>
<tr>
<td>Peppers</td>
<td>Turnip greens</td>
</tr>
<tr>
<td>Snap beans</td>
<td>Peas and carrots</td>
</tr>
<tr>
<td>Wax beans</td>
<td>Succotash</td>
</tr>
</tbody>
</table>

#### Dry soups, unprepared

<table>
<thead>
<tr>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentil</td>
</tr>
<tr>
<td>Cream of mushroom</td>
</tr>
<tr>
<td>Potato</td>
</tr>
<tr>
<td>Split pea</td>
</tr>
<tr>
<td>Italian vegetable</td>
</tr>
</tbody>
</table>

#### Canned soups or sauces, not condensed, unprepared

<table>
<thead>
<tr>
<th>Food</th>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentil with ham</td>
<td>Potato</td>
</tr>
<tr>
<td>Clam chowder, Manhattan</td>
<td>Gaspacho</td>
</tr>
<tr>
<td>Clam chowder, New England</td>
<td>Vegetable consomme</td>
</tr>
<tr>
<td>Crab</td>
<td>Soy sauce</td>
</tr>
<tr>
<td>Cream of Shrimp</td>
<td>Teriyaki sauce</td>
</tr>
<tr>
<td>Blackbean</td>
<td>Beef consomme</td>
</tr>
<tr>
<td>Minestrone</td>
<td></td>
</tr>
<tr>
<td>Cream of Mushroom</td>
<td></td>
</tr>
</tbody>
</table>

#### Fresh fruit - Avocado


#### Legumes - Peanut butter
APPENDIX B
Information on Foods Purchased and Prepared

Applesauce, sweetened

Items as purchased and label information
1. Del Monte Gravensteen applesauce, 170 kcal per cup
2. Janet Lee applesauce, 170 kcal per cup
3. Good Day applesauce

Applesauce, unsweetened

Items as purchased and label information
1. Seneca 100% natural unsweetened applesauce, 50 kcal per 1/2 cup

Beef chuck steak

Items as purchased and label information
3 boneless swissed beef chuck steaks

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 269.7 g</td>
<td>185.6 g</td>
</tr>
<tr>
<td>2. 351.5 g</td>
<td>253.4 g</td>
</tr>
<tr>
<td>3. 257.5 g</td>
<td>176.8 g</td>
</tr>
</tbody>
</table>

Preparation method
Steaks were pan broiled over medium high heat for 7 minutes on each side. Drippings were drained and discarded during and after cooking (33).

Beef, lean ground

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 100.0 g</td>
<td>77.2 g</td>
</tr>
<tr>
<td>2. 100.0 g</td>
<td>75.4 g</td>
</tr>
<tr>
<td>3. 100.0 g</td>
<td>76.0 g</td>
</tr>
</tbody>
</table>

Preparation method
Patties were cooked over medium heat for 7 minutes on each side. Drippings were drained and discarded during and after cooking (33).
APPENDIX B
(continued)

Beef, regular ground

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 100.0 g</td>
<td>73.9</td>
</tr>
<tr>
<td>2. 100.0 g</td>
<td>73.0</td>
</tr>
<tr>
<td>3. 100.0 g</td>
<td>72.4</td>
</tr>
</tbody>
</table>

Preparation Method
Patties were cooked over medium heat for 7 minutes on each side. Drippings were drained and discarded during and after cooking (33).

Beef short ribs

Beef short ribs were combined from three packages before cooking.

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
<th>Boneless Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>653.0 g</td>
<td>440.0 g</td>
<td>312.2 g</td>
</tr>
</tbody>
</table>

Preparation Method (Braising)
Ribs were simmered in 1 liter of boiling water for two hours (34).

Biscuits from mix

Items as purchased and label information
1. Jiffy Baking Mix
   2 cups dry mix - 256.1 g
   12 cooked biscuits - 356.2 g

Preparation Method
Combine 2 cups mix with 2/3 cup milk. Roll out and cut into 12 biscuits. Bake at 450° for 10 to 12 minutes.

Biscuits, homemade

Preparation Method
Combine 2 cups sifted flour, 1 T baking powder and 1 t salt. Cut in 1/4 cup vegetable shortening. Mix in 2/3 cup milk and roll out. Cut into biscuits and bake 12 minutes at 425°. Yield - 9 biscuits weighing 403.9 g (33).
APPENDIX B
(continued)

Biscuits, refrigerated dough

Items as purchased and label information

1. Pillsbury Country Style Biscuits.
2. Pillsbury Buttermilk Biscuits.

Preparation Method

1. Preheat oven to 450°. Bake biscuits 8 to 10 minutes in greased round cake pan.
   Uncooked weight 132.0 g
   Cooked weight (6 biscuits) 118.4 g
2. Preheat oven to 450°. Bake biscuits 8 to 10 minutes in greased round cake pan.
   Uncooked weight 127.9 g
   Cooked weight (6 biscuits) 113.2
3. Preheat oven to 425°. Bake biscuits 11 to 13 minutes in ungreased round cake pan.
   Uncooked weight 224.5 g
   Cooked weight (10 biscuits) 203.0 g

Bread, Bran'ola

Items as purchased and label information

1. Bran'ola bread, 2 slices = 195 kcal

Breakfast Links (sausage-like product made from vegetable protein)

Items as purchased and label information

1. Morningstar Farms Breakfast Links
   3 links = 200 kcal

Preparation Method

Place links in cold pan. Cook over medium heat for 5 to 7 minutes.
   raw weight (3 links) 70.6 g
   cooked weight 65.8 g
APPENDIX B
(continued)

Cake, yellow - made from mix

Items as purchased and label information

1. Jiffy Golden Yellow Cake Mix
2. Betty Crocker Super Moist Yellow Cake Mix, 1/12 of 2 layer cake has 270 kcal
3. Albertson's Yellow Cake Mix, 1/12 of 2 layer cake has 190 kcal

Preparation Method

1. Mix 1 box of cake mix with 1 egg, and 1 cup water for 4 minutes. Pour into 8" x 8" pan and bake at 350° for 20 to 25 minutes.
   Cooked weight 334.0 g

2. Combine mix with 1 cup water, 3 eggs and 1/3 cup vegetable oil and beat with electric mixer for 2 minutes. Turn into 2-9" round pans and bake at 350° for 32 to 37 minutes.
   Cooked weight 851.7 g

3. Combine mix with 2 eggs and 1-1/3 cups water. Mix for two minutes. Turn batter into 2 9" round pans and bake at 350° for 35 to 40 minutes.
   Cooked weight 804.14 g

Cereals - no preparation was needed for any of the following ready to eat cereals. Milk was not added to the cereals.

Apple Jacks

Item as purchased and label information

1. Apple Jacks. 110 kcal per 1 ounce (1 cup) serving.

Cheerios

Item as purchased and label information

1. Cheerios. 110 kcal per 1 ounce (1-1/4 cups) serving.

Corn Chex

Item as purchased and label information

1. Corn Chex. 110 kcal per 1 ounce (1 cup) serving.

Corn Flakes

Item as purchased and label information

1. Kelloggs Corn Flakes. 110 kcal per 1 ounce (1 cup) serving.

Froot Loops

Item as purchased and label information

1. Kelloggs Froot Loops. 110 kcal per 1 ounce (1 cup) serving.
APPENDIX B
(continued)

Granola

Items as purchased and label information
1. Nature Valley Granola. 130 kcal per 1 ounce (1/3 cup) serving.
2. Quaker 100% Natural Cereal with Raisins and Dates. 130 kcal per 1 ounce (1/4 cup) serving.
3. Clinton's Crunchy Granola.

Grape Nuts

Item as purchased and label information
1. Grape Nuts. 100 kcal per 1 ounce (1/4 cup) serving.

Product 19

Item as purchased and label information

Sugar Smacks

Item as purchased and label information
1. Sugar Smacks. 110 kcal per 1 ounce (3/4 cup) serving.

Total

Item as purchased and label information
1. Total. 110 kcal per 1 ounce (1 cup) serving.

Wheat Chex

Item as purchased and label information
1. Wheat Chex. 110 kcal per 1 ounce (2/3 cup) serving.

Wheaties

Item as purchased and label information
1. Wheaties. 110 kcal per 1 ounce (1 cup) serving.

Cheese, Monterey Jack

Item as purchased and label information
1. Albertson's Monterey Jack Cheese
2. Albertson's Monterey Jack Cheese
3. Kraft Monterey Jack Cheese - sliced. 100 kcal per 1 ounce serving.
APPENDIX B
(continued)

Cheese, Romano - grated

Items as purchased and label information
1. Kraft Romano Cheese

Chicken breasts - baked

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
<th>Boneless Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 190.2 g</td>
<td>151.1 g</td>
<td>130.0 g</td>
</tr>
<tr>
<td>2. 201.1 g</td>
<td>159.0 g</td>
<td>132.2 g</td>
</tr>
<tr>
<td>3. 268.5 g</td>
<td>215.9 g</td>
<td>165.8 g</td>
</tr>
</tbody>
</table>

Preparation Method
Brush each breast lightly with oil before cooking. Bake at 375° for 30 minutes on each side (34).

Chicken drumsticks - baked

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
<th>Boneless Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 119.8 g</td>
<td>90.3 g</td>
<td>62.5 g</td>
</tr>
<tr>
<td>2. 122.6 g</td>
<td>95.9 g</td>
<td>58.3 g</td>
</tr>
<tr>
<td>3. 130.1 g</td>
<td>99.2 g</td>
<td>67.5 g</td>
</tr>
</tbody>
</table>

Preparation Method
Brush drumsticks lightly with oil before cooking. Bake at 375° for 30 minutes on each side (34).

Cornbread from mix

Items as purchased and label information
1. Martha White Complete Yellow Corn Muffin Mix
2. Aunt Jemima Easy Mix Cornbread. 220 kcal per serving. 1 serving equals 1/6 of pan.
3. Aunt Jemima Easy Mix Cornbread

Preparation Method
1. Combine mix with 1/2 cup water. Transfer to 8" x 8" pan and bake at 400° for 20 to 25 minutes. Cooked weight 255.4 g.
2&3. Combine mix with 1 egg and 1 cup milk. Transfer to pan provided with mix and bake at 400° for 18 to 20 minutes. Cooked weights 376.4 g and 367.1 g.
APPENDIX B
(continued)

Crackers, graham

Items as purchased and label information
1. Keebler Honey Grahams
2. Nabisco Honey Maid Graham Crackers
3. Keebler Cinnamon Crisp Graham Crackers

Weight of one whole cracker
1. 15.5 g
2. 14.5 g
3. 16.6 g

Crackers, saltine

Items as purchased and label information
1. Fireside Saltine Crackers
2. Keebler Club Crackers
3. Nabisco Premium Saltine Crackers

Crackers, whole wheat

Items as purchased and label information
1. Keebler Heary Wheat Snack Crackers
2. Nabisco Wheat Thins
3. Keebler Wheat Toast

Cranberry juice cocktail

Items as purchased and label information
1. Ocean Spray Cranberry Juice Cocktail

English Muffins

Items as purchased and label information
1. Orowheat Extra Crisp English Muffins
2. Wonder English Muffins
3. Janet Lee English Muffins

Preparation Method
Muffin halves were toasted until browned.
Fish filets, frozen

Items as purchased and label information

1. Van de Kamp's Fish Filets
2. Albertson's Fish Filets
3. Mrs. Paul's Fish Filets

Preparation Method

1. Bake at 400\(^\circ\)C for 25 to 30 minutes
   - raw weight: 90.9 g
   - cooked weight: 86.2 g
2. Bake at 425\(^\circ\)C for 30 to 35 minutes
   - raw weight: 85.7 g
   - cooked weight: 79.2 g
3. Bake at 400\(^\circ\)C for 25 minutes
   - raw weight: 62.9 g
   - cooked weight: 56.2 g

(all weights for one filet)

Fish sticks, frozen

Items as purchased and label information

1. Van de Kamp's batter dipped Fish Sticks
2. Gorton's Fish Sticks
3. Mrs. Paul's Fish Sticks

Preparation Method

1. Bake at 375\(^\circ\)C for 25 minutes
   - raw weight: 59.3 g
   - cooked weight: 56.2 g
2. Bake at 425\(^\circ\)C for 13 to 15 minutes
   - raw weight: 48.2 g
   - cooked weight: 44.7 g
3. Bake at 375\(^\circ\)C for 15 minutes
   - raw weight: 48.4 g
   - cooked weight: 46.0 g

(all weights for 2 sticks)

Fruit cocktail, canned

Items as purchased and label information

1. Libby's Fruit Cocktail in heavy syrup
   - 1 cup = 170 kcal
2. Albertson's Fruit Cocktail in light syrup
3. Del Monte's Fruit Cocktail in heavy syrup
   - 1 cup = 170 kcal

Preparation Method

Solids and liquids were used for the assay.
Granola snack bars
Items as purchased and label information
1. Nature Valley Granola Bars, cinnamon. 1 bar = 110 kcal
2. Crunchola Snack Bars - peanut butter with cinnamon and raisins
   1 bar = 150 kcal
3. Crunchola Snack Bars - peanut butter with apple crunch
   1 bar = 150 kcal
1. Nature Valley - 1 bar = 23.4 g
2. Crunchola - 1 bar = 29.5 g
3. Crunchola - 1 bar = 28.6 g

Halibut
Preparation Method
Poach steaks for 10 minutes in boiling water (34).

Macaroni
Items as purchased and label information
1. Janet Lee Small Sea Shells. 1/3 cup dry = 150 kcal
2. American Beauty Elbo-Roni. 2 oz. dry = 210 kcal

Preparation Method
1. Boil in salted water for 10 minutes. 75.0 grams dry yields
   202.5 grams cooked
2. Boil in salted water until tender. 75.0 grams dry yields
   180.2 grams cooked
3. Boil in salted water for 8 minutes. 75.0 grams dry yields
   185.7 grams cooked

Noodles
Items as purchased and label information
1. Janet Lee Extra Wide Enriched Egg Noodles. 1-1/2 oz. dry = 150 kcal
2. Janet Lee Extra Wide Enriched Egg Noodles. 1-1/2 oz. dry = 150 kcal
3. American Beauty Enriched Wide Egg Noodles. 2 oz. dry = 220 kcal
Preparation Method

1. Boil in salted water for 8 minutes. 40.0 grams dry yields 105.5 grams cooked.
2. Boil in salted water for 8 minutes. 40.0 grams dry yields 111.5 grams cooked.
3. Boil in salted water until tender. 40.0 grams dry yields 114.4 grams cooked.

Oatmeal, instant
Items as purchased and label information
1. Instant Quaker Oatmeal - maple and brown sugar. 1 packet = 160 kcal.
2. Instant Quaker Oatmeal - regular flavor. 1 packet = 110 kcal.
3. Instant Quaker Oatmeal - raisins and spice. 1 packet = 160 kcal.

Preparation Method
1. Add 1/2 cup boiling water to packet contents and stir.
2. Add 2/3 cup boiling water to packet contents and stir.
3. Add 1/2 cup boiling water to packet contents and stir.

Oatmeal, quick
Items as purchased and label information
1. Janet Lee Quick Oats. 1 oz. uncooked - 110 kcal.

Preparation Method
Cook 2/3 cup oats in 1-1/3 cups boiling water for 1 minute. Allow to stand a few minutes.

Oatmeal, regular
Items as purchased and label information
1. Old Fashioned Quaker Oats. 1 oz. uncooked = 110 kcal.

Preparation Method
Cook 2/3 cup oats in 1-1/2 cups boiling water. Cook for 5 minutes. Allow to stand a few minutes.
APPENDIX B
(continued)

Orange juice, frozen
Items as purchased and label information
1. Good Day Orange Juice
2. Minute Maid Orange Juice
3. Whole Sun Orange Juice

Preparation Method
All samples were reconstituted with 3 parts water to 1 part of juice.

Pancakes, made from mix
Items as purchased and label information
1. Martha White Buttermilk Pancake Mix
2. Betty Crocker Buttermilk Pancake
3. Krusteaz complete buttermilk pancake mix

Preparation Method
2. Combine 1 cup of mix with 2/3 cup of water. Bake on greased griddle.

Pickles
Items as purchased and label information
1. Vlasic Kosher Dill Pickles
2. Vlasic Sweet Gherkins Pickles
3. Janet Lee Sweet Pickles

Pop-tarts
Items as purchased and label information
1. Nabisco Apple Toastettes. 1 pastry = 190 kcal.
2. Kelloggs Strawberry Pop-tarts. 1 pastry = 210 kcal.

Preparation Method
Toast until brown.
### Pork loin chops

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
<th>Boneless Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>141.2 g</td>
<td>101.2 g</td>
<td>79.1 g</td>
</tr>
<tr>
<td>166.0 g</td>
<td>113.0 g</td>
<td>90.2 g</td>
</tr>
<tr>
<td>151.6 g</td>
<td>108.7 g</td>
<td>85.8 g</td>
</tr>
</tbody>
</table>

**Preparation Method**

Brown chop in 1 tsp. oil on both sides. Reduce heat and cook slowly until done (34).

### Potato, baked

- **Raw Weight of 6 potatoes** - 1346.3 g
- **Cooked weight of 6 potatoes** = 1105.5 g

**Preparation Method**

Potatoes were baked at 425° for 1 hour.

### Potato, boiled

- **Raw Weight of 3 potatoes** 752.6 g
- **Cooked Weight of 3 potatoes** 736.4 g

**Preparation Method**

Boil whole unpeeled potatoes until tender.

### Potatoes, canned

**Items as purchased and label information**

1. Janet Lee Whole New Potatoes
2. Janet Lee Sliced New Potatoes
3. Hunt's Whole New Potatoes

**Preparation Method**

Samples were not heated. Solid and liquids were used.

### Potato chips

**Items as purchased and label information**

1. Ruffles Potato Chips
2. Frito-Lay's Natural Style Potato Chips
3. Clover Club Golden Potato Chips
APPENDIX B
(continued)

Potatoes, instant mashed

Items as purchased and label information
- 1. Pride-Pak Mashed Potatoes
- 2. Albertson's Instant Mashed Potatoes
- 3. Betty Crocker Potato Buds

Preparation Method
1. Boil 1/2 cup of water. Add 2 tsp. butter, 1/3 cup cold milk and 1/2 cup potato flakes. Stir and fluff with fork.
2. Boil 1/2 cup of water. Add 2 tsp. butter, 1/4 cup milk and 1/2 cup of potato flakes. Stir and fluff with fork.
3. Heat together 2/3 cup of water, 2 Tbs. milk, and 1 Tbs. butter until boiling. Remove from heat and add 2/3 cup potato buds.

Pretzels

Items as purchased and label information
- 1. Country Club Bavarian Pretzels
- 2. Clover Club Mini Pretzels
- 3. Albertson's Pretzel Twists

Rice, brown

Items as purchased and label information
- 1. S and W Natural Long Grain Brown Rice

Preparation Method
Bring 2-1/2 cups of salted water to a boil. Add 1 cup rice. Simmer, covered, for 45 minutes. Allow to stand, covered, for 5 minutes.

Rice-a-roni

Items as purchased and label information
- 1. Rice-a-roni savory Rice Pilaf
- 2. Rice-a-roni chicken flavor, 3/4 cup = 160 kcal
- 3. Rice-a-roni beef flavor, 1/2 cup = 130 kcal

Preparation Method
2. Same as #1 except use 2-3/4 cups water.
3. Same as #1 except use 2-3/4 cups water.
Rice, white

Items as purchased and label information
1. Uncle Ben's Converted Rice, 2/3 cup = 120 kcal
2. MJB Long Grain White Rice, 1/2 cup = 100 kcal
3. Golden Grain Enriched Long Grain Rice, 1/6 cup dry = 120 kcal

Preparation Method
1. Bring 2-1/2 cups of water to a boil. Add 1 cup of rice, cover and simmer for 20 minutes. Let stand for 5 minutes covered.
2. Bring 2 cups of water to a boil. Add 1 cup of rice, cover and simmer for 20 minutes.
3. Bring 2 cups of water to a boil. Add 1 cup of rice, cover and simmer for 15 minutes.

Rolls, hamburger

Items as purchased and label information
1. Janet Lee Enriched Buns
2. Wonder Hamburger Enriched Buns
3. Albertson's Enriched Buns

Sausage

Bologna

Items as purchased and label information
1. Janet Lee Sliced Bologna
2. Oscar Mayer Bologna
3. Cudahy Bar S Bologna

Frankfurters

Items as purchased and label information
1. A and R Big Dog Franks
2. Janet Lee Weiners
3. Oscar Mayer Fully Cooked Weiners. 1 link = 140 kcal

Preparation Method
Cook in a frying pan over medium heat until browned.

<table>
<thead>
<tr>
<th>Raw Weight</th>
<th>Cooked Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 57.4 g</td>
<td>54.8 g</td>
</tr>
<tr>
<td>2. 35.6 g</td>
<td>34.5 g</td>
</tr>
<tr>
<td>3. 47.1 g</td>
<td>45.5 g</td>
</tr>
</tbody>
</table>

(all weights are for 1 link)

Liverwurst

Items as purchased and label information
1. Janet Lee Liverwurst
APPENDIX B
(continued)

Pork Sausage

Items as purchased and label information

1. Jimmy Dean Bulk Pork Sausage
2. Albertson's Bulk Sausage
3. Albertson's Link Sausage

Preparation Method
Pan broil until browned. Drain off fat as it accumulates.

<table>
<thead>
<tr>
<th>Uncooked Weight</th>
<th>Cooked Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 84.1 g (1 pattie)</td>
<td>63.5 g</td>
</tr>
<tr>
<td>2. 84.0 g (1 pattie)</td>
<td>56.2 g</td>
</tr>
<tr>
<td>3. 83.6 g (3 links)</td>
<td>66.2 g</td>
</tr>
</tbody>
</table>

Salami

Items as purchased and label information

1. Oscar Mayer Cotto Salami
2. Janet Lee Cotto Salami
3. Janet Lee Cooked Bulk Beef Salami

Stuffing mix, stove-top

Items as purchased and label information

1. Stove-top Cornbread Stuffing Mix. 1/2 cup = 170 kcal
2. Stove-top Pork Stuffing Mix. 1/2 cup = 170 kcal
3. Stove-top Chicken Flavor Stuffing Mix. 1/2 cup = 170 kcal

Preparation Method
Combine 1-3/4 cup water and 1/4 cup butter with contents of seasoning packet. Simmer for 6 minutes. Add bread crumbs, mix, and let stand for 5 minutes.

<table>
<thead>
<tr>
<th>Cooked Weight - 1/2 cup</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 111.8 g</td>
</tr>
<tr>
<td>2. 103.1 g</td>
</tr>
<tr>
<td>3. 110.6 g</td>
</tr>
</tbody>
</table>

Tomatoes, canned

Items as purchased and label information

1. Albertson's Whole Peeled Tomatoes
2. Hunt's Whole Tomatoes. 4 oz = 20 kcal
3. Del Monte Whole Peeled Tomatoes. 1 cup = 50 kcal

Preparation Method
Liquid and solids were used. Samples were not heated.
Tomato paste, canned
Items as purchased and label information
1. Progresso Tomato Paste
2. Janet Lee Tomato Paste. 6 oz = 150 kcal
3. Hunt's Tomato Paste

Tomato sauce, canned
Items as purchased and label information
1. Hunt's Tomato Sauce. 4 oz = 30 kcal
2. Janet Lee Tomato Sauce. 8 oz = 80 kcal
3. Progresso Tomato Sauce

Twinkie
Items as purchased and label information
1. Hostess Twinkies

V-8 juice
Items as purchased and label information
1. V-8 Juice. 6 oz = 35 kcal

Waffles, frozen
Items as purchased and label information
1. Kellogg's Eggo Frozen Waffles. 1 waffle = 120 kcal
2. Aunt Jemima Jumbo Buttermilk Waffles. 1 waffle = 80 kcal
3. Downyflake Jumbo Size Waffles. 2 waffles = 170 kcal

Preparation Method
1. Thaw waffles slightly, preheat toaster, and then brown waffles on low heat.
2. Toast waffles until brown.
3. Toast waffles until brown.

Cooked Weight (2 waffles)
1. 75.2 g
2. 76.6 g
3. 74.1 g

Wheat germ
Items as purchased and label information
1. Kretschmer Regular Wheat Germ - toasted. 1/4 cup = 110 kcal, 4% of U.S. RDA for pantothenic acid
3. Fisher Wheat Germ - untoasted. 1 oz = 102 kcal
Whole wheat cereal

Items as purchased and label information
1. Instant Ralston. 1/4 cup (uncooked) = 110 kcal, 2% of U.S. RDA for pantothenic acid.
2. Fisher "Zoom". 1/3 cup (uncooked) = 100 kcal.
3. All-o-Wheat Breakfast Cereal

Preparation Method
1. 1/2 cup dry cereal to 1-1/2 cups boiling water. Cook for 10 seconds. Remove from heat and allow to stand.
2. Add 2/3 cup Zoom to 1-1/3 cups boiling water. Cook for 1 minute. Allow to stand 1 minute.
3. Add 1 cup cereal to 4 cups boiling water. Cook for 3 to 5 minutes.

Yams, canned

Items as purchased and label information
1. Royal Prince Yams

Preparation Method
Solids and liquids were used. Samples were not heated.

Notes on Appendix B
1. All cooking temperatures are in degrees farenheit (°F).
2. Where only one item is listed, three packages of units of the item were used.
# APPENDIX C

## Table 20

Raw Values of Pantothenic Acid Content of Analyzed Foods

<table>
<thead>
<tr>
<th>mg P.A. per 100 grams of food</th>
<th>Microbiological Assay</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microbiological Assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RIA</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food</th>
<th>Microbiological Assay</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applesauce, sweetened</td>
<td>.056 .056 .068</td>
<td>.052 .035 .043</td>
</tr>
<tr>
<td>Applesauce, unsweetened</td>
<td>.074 .100 .110</td>
<td>.094 .050 .117</td>
</tr>
<tr>
<td>Beef chuck steak</td>
<td>.782 .962 .952</td>
<td>.735 .844 .959</td>
</tr>
<tr>
<td>Beef, lean ground</td>
<td>.836 .881 .925</td>
<td>.746 .804 .674</td>
</tr>
<tr>
<td>Beef, regular ground</td>
<td>.835 .833 .863</td>
<td>.714 .679 .620</td>
</tr>
<tr>
<td>Beef short ribs</td>
<td>.521 .505 .581</td>
<td>.366 .439 .495</td>
</tr>
<tr>
<td>Biscuits from mix</td>
<td>.367 .416 .402 .503*</td>
<td>.262 .378 .318 .077*</td>
</tr>
<tr>
<td>Biscuits, homemade</td>
<td>.412 .466 .432</td>
<td>.347 .412 .388</td>
</tr>
<tr>
<td>Biscuits, refrigerated</td>
<td>.281 .201 .219 .580*</td>
<td>.368 .435 .326</td>
</tr>
<tr>
<td>Bread, bran'ola</td>
<td>.432 .437 .521 1.033*</td>
<td>.479 .408 .488</td>
</tr>
<tr>
<td>Breakfast links</td>
<td>.096 .125 .089 .288**</td>
<td>.103 .091 .079 .392**</td>
</tr>
<tr>
<td>Cake, yellow</td>
<td>.250 .312 .261 .155*</td>
<td>.288 .261 .272</td>
</tr>
<tr>
<td>Cereal, ready to eat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Jacks</td>
<td>.259 .231 .185 .409**</td>
<td>.115 .119 .133 .203**</td>
</tr>
<tr>
<td>Cheerios</td>
<td>1.288 1.450 1.388</td>
<td>1.203 1.252 1.568</td>
</tr>
<tr>
<td>Corn Chex</td>
<td>.390 .351 .266</td>
<td>.169 .169 .240 .545*</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>.209 .148 .170</td>
<td>.258 .275 .319</td>
</tr>
<tr>
<td>Froot Loops</td>
<td>.234 .252 .255</td>
<td>.273 .238 .192 .468*</td>
</tr>
<tr>
<td>Granola</td>
<td>.733 .808 .931</td>
<td>.555 .544 .509 .867*</td>
</tr>
<tr>
<td>Grape Nuts</td>
<td>.475 .507 .371 .224**</td>
<td>.664 .545 .420 .308**</td>
</tr>
<tr>
<td>Product 19</td>
<td>.500 .434 .363</td>
<td>.588 .423 .319 .835*</td>
</tr>
<tr>
<td>Sugar Smacks</td>
<td>.248 .346 .297</td>
<td>.454 .476 .444</td>
</tr>
<tr>
<td>Total</td>
<td>.797 .635 .716 .396*</td>
<td>.486 .617 .536 .932*</td>
</tr>
<tr>
<td>Wheat Chex</td>
<td>.577 .500 .577 .399*</td>
<td>.528 .454 .524 .990*</td>
</tr>
<tr>
<td>Wheaties</td>
<td>.620 .736 .612</td>
<td>.652 .860 .556</td>
</tr>
<tr>
<td>Cheese, monterey jack</td>
<td>.184 .181 .229</td>
<td>.323 .280 .251</td>
</tr>
<tr>
<td>Cheese, romano</td>
<td>.299 .201 .259</td>
<td>.488 .509 .466 .184*</td>
</tr>
<tr>
<td>Chicken breast</td>
<td>1.239 1.481 1.564</td>
<td>1.244 1.132 1.208</td>
</tr>
</tbody>
</table>
### APPENDIX C (continued)

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Microbiological Assay</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken legs</td>
<td>1.413 1.504 1.389</td>
<td>1.182 1.287 1.166</td>
</tr>
<tr>
<td>Cornbread from mix</td>
<td>.417 .393 .387</td>
<td>.423 .417 .282</td>
</tr>
<tr>
<td>Crackers, graham</td>
<td>.409 .431 .490</td>
<td>.478 .393 .519</td>
</tr>
<tr>
<td>Crackers, saltine</td>
<td>.211 .284 .309</td>
<td>.356 .287 .247</td>
</tr>
<tr>
<td>Crackers, whole wheat</td>
<td>.457 .408 .370</td>
<td>.347 .370 .385</td>
</tr>
<tr>
<td>Cranberry juice cocktail</td>
<td>.073 .056 .071</td>
<td>.065 .056 .047</td>
</tr>
<tr>
<td>English muffins</td>
<td>.520 .569 .459</td>
<td>.480 .496 .463</td>
</tr>
<tr>
<td>Fish filet, frozen</td>
<td>.293 .328 .391</td>
<td>.240 .268 .242</td>
</tr>
<tr>
<td>Fish sticks, frozen</td>
<td>.340 .430 .408</td>
<td>.308 .381 .322</td>
</tr>
<tr>
<td>Fruit cocktail, canned</td>
<td>.054 .066 .060</td>
<td>.040 .037 .048</td>
</tr>
<tr>
<td>Granola snack bars</td>
<td>.052 .502 .586</td>
<td>.607 .517 .510</td>
</tr>
<tr>
<td>Halibut, poached</td>
<td>.440 .420 .555</td>
<td>.275 .260 .247</td>
</tr>
<tr>
<td>Macaroni, cooked</td>
<td>.141 .098 .107</td>
<td>.090 .128 .102</td>
</tr>
<tr>
<td>Noodles, cooked</td>
<td>.219 .176 .253</td>
<td>.165 .171 .133</td>
</tr>
<tr>
<td>Oatmeal, instant</td>
<td>.381 .313 .280</td>
<td>.345 .310 .295</td>
</tr>
<tr>
<td>Oatmeal, quick</td>
<td>.279 .297 .333</td>
<td>.205* .257 .282</td>
</tr>
<tr>
<td>Oatmeal, regular</td>
<td>.278 .300 .298</td>
<td>.165* .270 .243</td>
</tr>
<tr>
<td>Orange juice, frozen</td>
<td>.131 .151 .161</td>
<td>.180 .180 .231</td>
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<tr>
<td>Pancakes from mix</td>
<td>.342 .345 .372</td>
<td>.159** .318 .342</td>
</tr>
<tr>
<td>Pickles</td>
<td>.038 .020 .036</td>
<td>.052 .043 .048</td>
</tr>
<tr>
<td>Pop tarts</td>
<td>.237 .237 .181</td>
<td>.239 .232 .166</td>
</tr>
<tr>
<td>Pork loin chops</td>
<td>.704 .738 .799</td>
<td>.670 .592 .687</td>
</tr>
<tr>
<td>Potatoes, baked</td>
<td>.468 .614 .584</td>
<td>.364 .314 .275</td>
</tr>
<tr>
<td>Potatoes, boiled</td>
<td>.420 .384 .424</td>
<td>.298* .312 .280</td>
</tr>
<tr>
<td>Potatoes, canned</td>
<td>.157 .167 .198</td>
<td>.298* .125 .153</td>
</tr>
<tr>
<td>Potato chips</td>
<td>.786 .854 .747</td>
<td>.708 .708 .275</td>
</tr>
<tr>
<td>Potatoes, instant mashed</td>
<td>.240 .291 .294</td>
<td>.204 .264 .244</td>
</tr>
<tr>
<td>Potatoes, mashed</td>
<td>.330 .255 .315</td>
<td>.610* .545 .585</td>
</tr>
<tr>
<td>Pretzels</td>
<td>.330 .255 .315</td>
<td>.610* .545 .585</td>
</tr>
</tbody>
</table>
APPENDIX C  
(continued)

mg P.A. per 100 grams of food

<table>
<thead>
<tr>
<th>Microbiological Assay</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice, brown</td>
<td>.403</td>
</tr>
<tr>
<td>Rice-a-roni</td>
<td>.184</td>
</tr>
<tr>
<td>Rice, white</td>
<td>.314</td>
</tr>
<tr>
<td>Rolls, hamburger</td>
<td>.612</td>
</tr>
<tr>
<td>Sausage</td>
<td></td>
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<tr>
<td>Bologna</td>
<td>.606</td>
</tr>
<tr>
<td>Frankfurters</td>
<td>.434</td>
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<tr>
<td>Liverwurst</td>
<td>2.843</td>
</tr>
<tr>
<td>Ground pork</td>
<td>.383</td>
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<tr>
<td>Salami</td>
<td>.920</td>
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<tr>
<td>Stuffing, stove top</td>
<td>.140</td>
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<tr>
<td>Tomatoes, canned</td>
<td>.156</td>
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<tr>
<td>Tomato paste, canned</td>
<td>.811</td>
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<tr>
<td>Tomato sauce, canned</td>
<td>.336</td>
</tr>
<tr>
<td>Twinkies</td>
<td>.331</td>
</tr>
<tr>
<td>V-8 juice</td>
<td>.262</td>
</tr>
<tr>
<td>Waffles, frozen</td>
<td>.373</td>
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<tr>
<td>Wheat germ</td>
<td>1.623</td>
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<tr>
<td>Whole wheat cereal</td>
<td>.171</td>
</tr>
<tr>
<td>Yams, canned</td>
<td>.472</td>
</tr>
</tbody>
</table>

*Dropped from calculation of mean

**Dropped from calculation of mean. These samples came from the same extract as the paired food sample. These aberrant assay results are probably the result of weighing errors or problems in the enzyme digestion stage of the assay.
VITA
Joan Howe Walsh
Candidate for the Degree of
Doctor of Philosophy

Dissertation: Estimation of the Pantothenic Acid Content of Foods
Using a Microbiological Assay and a Radioimmunoassay

Major Field: Nutrition and Food Sciences

Biographical Information:


Education: Attended elementary school in Arlington, Virginia, graduated from Wilton High School, Wilton, Connecticut in 1968, received the Bachelor of Science degree from the University of Connecticut, Storrs, with a major in foods and nutrition in 1972; 1973 completed the requirements for a Master of Public Health degree at the University of California at Berkeley, with a major in nutrition, and completed a dietetic internship; 1979 completed the requirements for the Doctor of Philosophy degree at Utah State University, Logan, with a major in Nutrition and Food Sciences.

Professional Experience: 1974-76, Instructor in Foods and Nutrition, Central Washington University, Ellensburg; 1974-76, Consulting dietitian for Kittitas Valley Community Hospital, Ellensburg, Washington; 1976-79, Teaching assistant and research assistant (summers), Department of Nutrition and Food Sciences, Utah State University; 1977-78 Nutritionist, Bear River District Health Department, Logan, Utah.
