THE B-VITAMIN CONTENT OF COOKED MEATS

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CHAPTER I

INTRODUCTION

The Functions and Human Requirements for B-Vitamins

Niacin, thiamine, riboflavin, pyridoxine, pantothenic acid, and vitamin B_{12} are classified as B-vitamins. They are biologically related substances which are essential for the metabolic activity of all living cells. All are involved in growth and in all phases of nerve development, and when not present in sufficient amounts lead to abnormal conditions involving the nerves, the skin, and the blood cells.

Niacin:

Niacin was first isolated from natural materials by Funk (17) in 1911. Warburg and Christian (61) isolated nicotinamide from coenzyme II and showed that it functioned as part of a hydrogen transport system, and Euler et al. (14) reported its presence in coenzyme I.

In living animal tissues almost all of the niacin is found as the amide. It is bound into the diphosphopyridine nucleotides, or coenzyme I, and triphosphopyridine nucleotides, or coenzyme II.

Niacin is chemically stable to air, light, heat, acids, and alkalies. Its activity is not destroyed to any great extent in ordinary cooking processes. Certain factors affect the availability of niacin for the body's use. These are: the amount of tryptophan

in relation to the balance of other amino acids (niacin may be formed from tryptophan in the presence of riboflavin and pyridoxine (46)), the type and amount of carbohydrate, the amount of fat, relative availability of niacin in food, and the possibility of intestinal microbial synthesis (54).

The nutritional significance of niacin was demonstrated ~~in 1937~~ by Elvehjem et al. (12) who cured canine black tongue by administering this vitamin. Following this finding, Fouts (16) obtained excellent results in curing the corresponding human deficiency disease, pellagra, by the same means.

The pellagra-preventing activity of niacin is recognized as one of its chief functions. However pellagra is now known to result from a diet which contains poor quality protein, such as corn. Corn is especially low in its content of tryptophan and niacin. A dietary deficiency of niacin is usually accompanied by deficiencies of other members of the B-complex, particularly thiamine, riboflavin, and pyridoxine.

The Food and Nutrition Board of the National Research Council (35) has set the recommended daily allowance for niacin at 13 to 16 milligrams for men, 10 to 12 milligrams for women, and 8 to 12 milligrams for children. A report by the Home Economics Research Branch of the U. S. Department of Agriculture in 1955 (36) indicated that the average U. S. consumption of niacin per day was 19.9 mg. This amount is in excess of the recommended allowance and indicates that the average diet is adequate in this vitamin. The best dietary sources of niacin are whole grain cereals and cereal products, meat, fish, and peanuts (62).

Thiamine:

Thiamine was isolated as a crystalline substance by Williams et al. (67) in 1926 and was shown to have great anti-neuritic activity. Nearly all of the thiamine in blood and in animal tissue is present in the form of thiamine pyrophosphate or cocarboxylase, the coenzyme form of the vitamin. Thiamine activity is associated with this coenzyme function. It participates in all decarboxylations which lead to the formation of carbon dioxide. The principal decarboxylation function in which the vitamin is concerned is the breakdown of pyruvic acid, the end product of carbohydrate metabolism. Thiamine is essential for growth, normal functioning of nervous tissue, and normal digestion and gastrointestinal tonus.

This vitamin is distributed widely in foods, although it is not found in large amounts in any one type of food. The best sources are yeast, cereal germs, pork, and nuts (2). It is synthesized by plants and by a number of microorganisms. In vegetable products, thiamine is present chiefly in its simple form, while in animal tissues, it is present largely in the form of the diphosphate derivative. Ferrebee et al. (15) have reported that the concentrations of thiamine in human tissue can be temporarily increased by thiamine therapy, and reduced by a diet inadequate in thiamine.

Beriberi, the specific deficiency disease prevented by thiamine, is practically unknown in western countries, because the vitamin is widely distributed in the food supply. Although there is a probability of the existence of cases of thiamine deficiency of varying degrees of severity in this country, the detection of early signs of the disease is difficult (55).

The principal factors which influence thiamine requirements

are the carbohydrate and the calorie intake. On a high carbohydrate diet, large amounts of thiamine are needed to perform its specialized function in carbohydrate metabolism. The requirement for thiamine is reduced when fat forms a large part of the diet and carbohydrate a small part. For practical purposes however, the thiamine need may be based on total caloric intake.

Daily dietary allowances for thiamine average 1.5 milligrams for men, 1.2 milligrams for women, and 0.8 to 1.2 milligrams for children (35). These figures are based on the recommendations of the National Research Council of 0.5 milligrams per 1000 calories. An additional intake of 0.2 milligrams of thiamine for each 1000 calories above the 3000 calorie level is advisable (35). The U.S.D.A. food consumption studies (36) gave the average thiamine intake per day as 1.89 milligrams, which is more than adequate to meet body requirements.

Riboflavin:

The discovery of riboflavin, the "yellow enzyme", by Warburg and Christian (61) in 1932 was followed by the isolation of it as a crystalline material by Kuhn in 1933 (25).

This vitamin has a characteristic yellow color and is sensitive to inactivation by light, especially in the presence of alkali. It is heat stable if protected from light (19).

Riboflavin exists naturally in the bound form as a part of the coenzyme, flavin-adenine-dinucleotide, or FAD (55). The main functions of riboflavin are those associated with the coenzyme activity in cellular oxidation. In conjunction with vitamin B_6, it is necessary for the conversion of tryptophan to niacin (46).

The clinical manifestations of riboflavin deficiency are chiefly

those associated with the eye and the skin. Corneal vascularization and involvement of the eyelid have been found to result from a riboflavin deficiency. Eye and skin lesions are not clearly defined as riboflavin deficiencies and appear to overlap to some extent with other deficiency symptoms, particularly those of niacin deficiency (44).

Riboflavin is synthesized by plants, yeasts, lower fungi, and some bacteria. Higher animals are unable to synthesize the vitamin, but bacteria are capable of providing riboflavin for the host. The synthesis of riboflavin in green plants is important in supplying human riboflavin requirements. The method of this synthesis is not known, but there is apparently a greater amount of synthesis in the leaves than in other parts of the plant. The best dietary sources of riboflavin are milk, cheese, cereal products, meats, and fish (62).

The amount of riboflavin required by the human organism is affected by synthesis of riboflavin by the bacterial flora and differences in the availability of the vitamin from different food.

Macrae and co-workers (29) in 1944, found no riboflavin deficiencies in men receiving diets containing 2.0 milligrams per day, and concluded that this intake was sufficient to meet the daily riboflavin requirement of the body. The recommended daily dietary allowances for riboflavin are 1.6 milligrams for men, 1.4 milligrams for women, and around 2.0 milligrams for children. The U.S.D.A. study of 1955 (36) gave the average riboflavin intake per day as 2.36 milligrams, making the average dietary intake sufficient for this nutrient.

Vitamin B_6 or Pyridoxine:

The specific chemical properties of vitamin B_6 (pyridoxine) as present in crude concentrates of fish muscle and wheat germ were established definitely by Birch and Gyorgy in 1936 (6). Within a short time,

Lepkovsky (27) isolated pyridoxine from various natural materials.

Pyridoxine is stable to heat and alkali but its biological activity is destroyed by light, especially ultraviolet light (55). This sensitivity to light is present in both neutral and alkaline solutions.

As a result of microbiological assays on extracts of natural materials, Snell et al. (57) recognized that forms of vitamin B_6 other than pyridoxine existed. At present three forms of the vitamin are known to exist. These are pyridoxine, pyridoxal, and pyridoxamine (56). All three of these are naturally occurring substances with vitamin B_6 activity and are classed together as the vitamin B_6 group.

It has been found that pyridoxal and pyridoxamine are the predominant forms of vitamin B_6 in hydrolyzed animal tissues and pyridoxine the more evident form in plant materials (40).

Vitamin B_6 functions in the form of a coenzyme, pyridoxal-5-phosphate (55). It is concerned with the activity of a wide variety of enzyme systems, all of which are characterized by their action on amino acids. Pyridoxal-5-phosphate is involved in decarboxylation, transamination, and racemization of amino acids.

Vitamin B_6 is essential for complete metabolism of tryptophan and is needed by the human organism for the utilization of the amino acids, glutamic acid, lysine, methionine, histidine, cystine, glycine, and alanine. It is involved in metabolism of fats and fatty acids and functions with pantothenic acid and pteroylglutamic acid in antibody production (55).

Vitamin B_6 is known to be required in the nutrition of all animals and of man. A deficiency of the vitamin manifests itself first in retardation of growth, then changes occur in the skin and the nervous system and the body loses the ability to convert tryptophan to niacin (55).

The daily dietary requirement for man for vitamin B_6 has not been definitely established, nor have the factors which influence requirements been investigated. It has been estimated to approximate 1.5 milligrams per day for adults or about 0.03 milligrams per kilogram per day (65). The critical level of minimal intake in infants seems to lie between 0.01 and 0.02 milligrams per kilogram per day (55).

Data on the occurrence of vitamin B_6 in foods show that it is widely distributed. Muscle meats, liver, vegetables, and whole grain cereals are among the best sources. Its wide distribution may be a reflection of its multiple roles in anabolic and catabolic reactions of the amino acids and proteins. Also this may explain why a deficiency disease caused by the lack of this vitamin has not been found (55).

Pantothenic Acid:

This vitamin was established as the chick antidermatitis factor by Jukes (23) in 1939 and following this, was synthesized in 1940 by Stiller (59). Williams and Major (66) described the structure of pantothenic acid.

Pantothenic acid occurs naturally in a bound form as part of coenzyme A and functions as part of this system in acetyl group transfer (28). Pantothenic acid is universally distributed in all living cells and tissues and its relative abundance as coenzyme A in living cells is associated with the variety of metabolic reactions of which the coenzyme is a part (54). Stanberry et al. (58) have shown that the pantothenic acid, or coenzyme A, content of certain animal tissues is changed as a result of deficiencies of niacin, thiamine, or riboflavin. Pantothenic acid is stable to oxidation and reduction agents, but labile in dry heat, hot alkali, acid, and alcohol (54). It is essential in all living organisms for the development of the central nervous system, for

growth, and for maintaining normal skin conditions.

The nutritional requirement is probably influenced by the presence of other fractions of the vitamin B-complex. Yacowitz et al. (68) have shown that vitamin B_{12} will reduce the amount of pantothenic acid needed for growth, survival, and prevention of dermatosis in chicks. Emerson and Wurtz (13) have demonstrated that biotin protects against pantothenic acid deficiencies in the rat. The best dietary sources of this vitamin are liver and kidney, muscle meats, wheat germ and wheat bran (54).

The approximate requirement of man for pantothenic acid is based on the known requirements of experimental animals. The need of the growing animal for pantothenic acid is much greater than that of the adult, and conditions of stress, pregnancy, and hyperthyroidism increase the requirement (10). It is suggested that the human requirement is probably less than 0.1 milligram per kilogram of body weight, or no more than 6 to 8 milligrams daily for adults (54).

The approximate intake of pantothenic acid in the United States varies from 3 to 12 milligrams per day. From data on daily excretions, this amount appears to be adequate to protect against deficiencies, and suggests that the adult requirement may lie between 3 and 5 milligrams per day. The infant and growing child require about 5 milligrams daily (54).

Vitamin B_{12}:

Rickes et al. (43) in 1948 reported the first results of the successful isolation of vitamin B_{12}, a red crystalline substance showing positive hematological response in Addisonian pernicious anemia. The same workers (42) reported that the red color was a result of a cobalt complex in the vitamin B_{12} molecule.

The structure of the vitamin B_{12} molecule was described fully by

Johnson and Todd (22) in 1956. It was described as a highly complex molecule containing phosphorus and cobalt bound to a cyanide group. The name "cyanocobalamin" has been proposed for the molecule. Other forms of the vitamin in which the cyanide is replaced by groups such as hydroxyl and nitrite, have been found to exist. Many of these "pseudo" forms have shown the same activity for curing pernicious anemia as the vitamin itself (24).

In addition to its role in hemopoesis, vitamin B_{12} has been shown to be effective as a human growth factor (64), and in the treatment of neurologic disorders.

Vitamin B_{12} is present in greatest amounts in animal products, certain molds, and bacteria (11). For this reason the term "animal protein factor" was at first applied to the vitamin. Its presence in animal tissue seems to be dependent on the ability of organisms in the intestinal tract to synthesize the vitamin. It is absent completely in higher plants and in yeast.

Although vitamin B_{12} is an essential nutrient, it appears that normal nutrition and blood cell production can be maintained in almost complete absence of a dietary supply of the material (5). When the diet lacks foods of animal sources, the nutritional needs for vitamin B_{12} are met presumably by the intestinal bacterial synthesis of the vitamin.

A diet which includes ordinary amounts of milk, eggs, and meat probably contains more than an adequate supply of vitamin B_{12}. Because a daily parenteral dose of one microgram of vitamin B_{12} induces complete remission in patients with pernicious anemia, the inference may be made that the usual daily requirement is met by the absorption of one microgram from the alimentary tract. However, no specific requirement has

as yet been determined. It appears probable that only a fraction of the vitamin B_{12} naturally present in food is absorbed even under normal digestive conditions (53).

Vitamin Losses During Food Preparation

Although the B-vitamins as a group are relatively heat stable, the usual household cooking procedures result in vitamin losses. Differences in cooking methods, time of cooking, and temperature have been shown to affect the retention or loss of the vitamins in foods. The extent to which these variables can affect the vitamin content of cooked meats as used in this study will be discussed in the following paragraphs.

Niacin:

Niacin is one of the most stable of the vitamins under ordinary conditions met in food processing, storage, and cooking (54). Some losses in cooking are encountered, particularly when water is used which gives opportunity for leaching out of the vitamin.

The loss of niacin during the cooking of meats by the usual broiling and roasting methods has been reported as 15% and 20% by McIntire and co-workers (31, 33), while Schweigert et al. (52) reported a loss of 21% in roasting. A loss of 48% in stewing was found by McIntire (31).

Cover et al.(8) have reported the effect of cooking at high (205° C)[1] and low (150° C)[2] temperatures. An average loss of 6% in beef and pork was shown at the low temperature but 31% at the high temperature.

Cover, McLaren, and Pearson (9) have shown that length of cooking time seems to have little effect on niacin retention in meats. They obtained a loss of 25% of the niacin in rare beef rib roasts, and 21% when cooked well done.

Thiamine:

Average losses of thiamine during roasting and broiling of meat have been reported as 30% to 43% by McIntire et al. (31, 33) and 36%

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1. 401° F
2. 302° F

to 58% by Mayfield and Hedrick (30). Even greater losses, of 50% to 60%, were reported in braising by McIntire and co-workers (31, 33) who also reported losses as high as 74% in stewing. The least loss of thiamine occurred in frying, a loss of only 14% being reported by Schweigert et al. (52).

Roasting at a high temperature of 205° C resulted in a total average loss of 50% of the thiamine in beef and pork, while a temperature of 150° C resulted in an average loss of only 25% (8).

A comparison of the values obtained by Cover, McLaren, and Pearson (9) in rare and well done roast rib of beef shows a loss of 25% in the rare and 31% in the well done. Thus it appears that greater losses of thiamine occur with increased cooking time.

Riboflavin:

The major loss of riboflavin which occurs during cooking is probably attributable to extraction of the vitamin by the water used in the cooking or blanching (30). Cheldelin, Woods, and Williams (7) have shown losses as large as 48% when eggs, milk, and pork chops were cooked in uncovered dishes. Under similiar conditions, no riboflavin was lost when cooking dishes were covered. They concluded that losses of riboflavin resulting from exposure to light during cooking may be significant.

Losses of riboflavin during roasting of meat were reported as 26% by Schweigert and co-workers (52), and 18% by McIntire et al. (31). Losses of 27% during braising and 33% in stewing were also reported by McIntire et al. (31). Schweigert et al. (52), reported a loss of 23% in frying.

The effect of temperature has been shown by Cover et al. (8) who have reported an average loss of 9% in beef and pork when a low

temperature of 150° C was used, but 32% at a temperature of 205° C. A loss of 17% in rare roast beef and 23% in well done beef was reported by Cover, McLaren, and Pearson (9).

Vitamin B_6:

The loss of vitamin B_6 during cooking is considerable and is difficult to explain because this vitamin is considered one of the heat stable members of the B-group (32). Henderson et al. (18) have attributed losses during cooking to leaching because the vitamin is water soluble.

McIntire et al. (32) have reported average losses of 58% to 86% in meats after cooking by various methods. Losses of 66% to 72% were shown when veal and lamb were roasted, while Henderson et al. (18) reported losses of 20% to 50% in roasting. Stewing seemed to result in the greatest losses, as much as 82% and 84% losses having been reported (32). A loss of 66% in broiling and 82% in braising were found by McIntire (32), while the least losses of the vitamin were found in fried meats, as reported by Henderson (18).

Pantothenic Acid:

In the usual cooking procedures relatively small amounts of pantothenic acid are lost although some inactivation of the vitamin is known to take place (63). One factor which affects the pantothenic acid content of meat is the loss in the drip when frozen meat is defrosted. Pearson et al. (38) have reported a loss of 33% during this process.

Cover, McLaren, and Pearson (9) have reported that the length of cooking time affects the pantothenic acid content. A loss of 9% of the pantothenic acid in rare roast beef was found in contrast to 25% when the roast was well done.

The effect of temperature on pantothenic acid has been shown by Cover et al. (8) who have reported the loss of 7% to 10% in beef and pork at a low cooking temperature of 150° C, but an average loss of 39% at a high cooking temperature of 205° C.

Vitamin B_{12}:

Vitamin B_{12} is known to be stable to heat in neutral solutions, but is inactivated by heating in dilute acid or alkali solution (26). Little information is available on retention of vitamin B_{12} during the usual cooking practices. Scheid, Andrews, and Schweigert (47) have stated that over 70% of the vitamin is retained during the cooking of pork, beef, and lamb.

Purpose of the Work

The purpose of the work which will be reported here was to determine the niacin, thiamine, riboflavin, vitamin B_6, pantothenic acid, and vitamin B_{12} content of various kinds and cuts of cooked meats.

Meats have been shown to be good sources of the B-complex vitamins and this study was undertaken to extend our knowledge of their contribution toward providing these essential nutrients for the human diet.

Until the present time, complete data have not been available for vitamin B_6, pantothenic acid, or vitamin B_{12} in meats. Therefore, it was desired to bring up to date the previous figures reported on all the vitamins and to provide new and more complete information on these three vitamins in particular.

CHAPTER II

METHODS AND PROCEDURE

The steps taken to accomplish the previously stated purposes of this study involved: 1) selection and preparation of composite samples of the cooked meats to be analyzed, and 2) the determination of the content of several of the B-vitamins in the meat by microbiological assay and by chemical methods.

Selection, Cooking, and Preparation of Composite Samples

The retail cuts of beef, pork, veal, and lamb to be analyzed were suggested by the Research Committee of the National Live Stock and Meat Board. Three carcasses of each kind of animal were selected to supply three each of the cuts to be analyzed.[1] The carcasses chosen were considered to be a "high-good, low-choice" grade. The meats were then cut in the manner specified by the National Live Stock and Meat Board (34a). Each cut was wrapped separately, brought to the laboratory, and cooked within three days of the time the carcass was cut.[2] No cuts were frozen for storage before preparation.

The weight of each fresh cut was taken before cooking. The meat was then cooked according to the recommended methods of the Live Stock

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1. Selection and cutting of the carcasses was done under the direction of Dr. Lowell Walters, of the Animal Husbandry Department of the Oklahoma State University.
2. The meat cuts were cooked by Mrs. Amy Thompson in the Nutrition Research Laboratory.

and Meat Board (34) and the cooked weight was recorded.

The procedure followed in cutting the retail cuts from the original carcasses and the method of cooking are found in Appendix B, pages 73 to 75.

After cooking, each cut was divided into three portions:

1) Obviously lean or "separable lean"

2) Lean and fat intermixed or marble

3) Obviously fat or "separable fat"

After a cut was divided, each separate portion was ground twice in a kitchen type electric grinder with a fine plate. Nitrogen determinations were made by the macro Kjeldahl method, as given in Official and Tentative Methods of Analysis p. 12 (1), on each of the three portions of each cut to give the percent of nitrogen in each.[1] The protein content in each portion was calculated by multiplying the nitrogen content by 6.25. The samples of the individual portions were stored in screw top glass jars and frozen until further analyses could be made.

There were a total of forty-seven cuts of beef, veal, lamb, and pork. In addition to these, the processed pork items, canadian style bacon and link sausage, were also analyzed, using the entire cooked and ground sample. From these, thirty-four of the more common cuts of meat that had been previously ground, were selected for the vitamin analyses. Composites of the same cut from each of the three carcasses were uniformly mixed and a portion of this was used for the analysis. In the case of the processed meats, the sample analyzed was a composite of three packages of the same brand of product. Composites of three different brands were analyzed in each case.

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1. Nitrogen determinations were done by Mrs. Ruby Moore of the Nutrition Research Department.

Analytical Procedures

A microbiological assay method was used to determine the niacin, pantothenic acid, vitamin B_6, and vitamin B_{12} in the meats. The thiamine and riboflavin content was determined by chemical methods, employing the fluorometric principle.[1]

Microbiological methods are based on the observation that certain microorganisms, or yeasts, require specific vitamins for growth. Using a basal medium complete in all respects except for the vitamin under test, growth responses of the organism are compared quantitatively in standard and in unknown solutions. Either the acid or the turbidity produced by the organism is measured to determine the extent of growth and from this the amount of vitamin in the test solution (2).

The fluorometric procedure is based on the fact that some vitamins will fluoresce in a light of specific wave length. Under standard conditions and in the absence of other interfering substances, the intensity of fluorescence is proportional to the concentration of the vitamin present in the dilute sample solution (2).

The complete analytical procedure used to determine each vitamin is given in Appendix A, pages 51 to 72.

Niacin:

The assay method which was used was a modification of that described in Official and Tentative Methods of Analysis, Assn. Off. Agric. Chemists, (1). The niacin was released from the meat samples by hydrolysis with sulfuric acid. The microoganism, Lactobacillus arabinosus 17-5, was employed for the determination.

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1. The thiamine and riboflavin determinations were made by co-workers in the Agricultural Chemistry Department at Oklahoma State University, under the direction of Mr. George Odell.

Thiamine:

The thiamine was liberated from the sample with the enzymes, papain and diastase, in a sodium acetate buffer. A fluorometric procedure, using the Farrand Fluorometer, was followed in determining the thiamine content. The method was adapted from that described in Methods of Vitamin Assay, Assn. of Vitamin Chemists, Inc. (2).

Riboflavin:

Riboflavin was released with the enzymes, papain and diastase, in a sodium acetate buffer, as for thiamine. A fluorometric procedure was then used for quantitative measurements. The source for the method was also Methods of Vitamin Assay (2).

Vitamin B_6:[1]

The data for this vitamin were obtained by a microbiological procedure, and acid hydrolysis of the sample was used for release of the vitamin. The yeast strain, Saccharomyces carlsbergensis, was used as the test organism. A modification of the method of Atkin et al. (3) as described in Methods of Vitamin Assay (2) was used.

Pantothenic Acid:

The pantothenic acid procedure was adapted from Methods of Analysis (1). The method of Ives and Strong (20) was used for enzymatic liberation of the vitamin from the sample. Lactobacillus arabinosus 17-5 was the test organism which was used.

Vitamin B_{12}:

A modified procedure of the method given in the Pharmacopoeia of the United States (39) was used. Vitamin B_{12} was released from the meat samples by autoclaving with an acetate-bisulfite buffer (4). The microorganism, Lactobacillus leichmannii 7830, was used as the test organism.

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1. The vitamin B_6 values were obtained by Dr. B. S. Schweigert and coworkers at the American Meat Institute Foundation, Chicago, Ill.

CHAPTER III

RESULTS

The findings for the vitamin content of each meat cut are presented in Tables I to VI inclusive. The means are presented together with the results of duplicate assays. Results are reported on the basis of the cooked meats as eaten and on the basis of 100 grams of protein. All results, except vitamin B_{12}, are reported as milligrams of vitamin per 100 grams of cooked meat. Vitamin B_{12} is presented as micrograms per 100 grams of cooked meat.

Table I shows the values obtained for niacin. The findings show that meat from young animals, veal and lamb, generally contained greater amounts of niacin that from the older type animals, beef and pork. The greatest amount of niacin was found in lean ground beef, veal sirloin roast, veal rump roast, and lamb loin chop. In general, when lean and marbled portions of the same sample were analyzed, the niacin content of the lean sample was higher than for the marbled portion.

Values for thiamine are presented in Table II. The meats which contained the greatest amount of thiamine were the pork and lamb cuts. The amounts for pork showed a total average yield of 0.998 milligrams per 100 grams of meat, while lamb cuts gave a total average amount of 0.217 milligrams.

Less thiamine was found in veal and beef cuts. The veal showed 0.166 milligrams as the total average, while beef yielded the smallest amounts, or a total average of 0.101 milligrams per 100 grams of meat.

Table I. Niacin Content of Cooked Meats

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
BEEF				
Steak				
bottom round - lean	5.75	5.62	5.69	15.6
top round - lean	5.45	5.78	5.61	14.3
- marble	6.10	5.85	5.97	15.8
sirloin - lean	2.90	2.88	2.89	10.7
- marble	3.70	3.99	3.85	16.6
T-bone - lean	6.47	6.98	6.73	25.4
- marble	4.97	5.27	5.12	22.1
Roast				
standing rib - lean	5.15	5.85	5.50	20.6
- marble	3.35	2.99	3.17	16.2
standing rump - lean	4.25	4.60	4.43	13.7
- marble	4.35	4.19	4.27	13.5
Ground beef - 75% lean	5.55	5.65	5.60	21.9
- 85% lean	8.61	7.86	8.23	27.3
Stew meat - entire	4.85	5.28	5.07	15.6
Corned beef - lean	4.85	5.27	5.06	16.6
- marble	2.70	2.88	2.79	14.5
LAMB				
Leg roast - lean	7.38	7.24	7.31	24.9
Loin chop - lean	7.74	8.17	7.95	28.7

TABLE I. (Continued)

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
PORK				
Cured ham shank - lean	4.54	4.67	4.61	16.4
- marble	3.31	3.52	3.41	14.9
Fresh center slice ham - lean	5.36	5.32	5.34	13.8
Loin chop - lean	5.37	5.73	5.55	16.0
Sirloin roast - lean	4.39	4.78	4.59	14.7
PROCESSED PORK				
Canadian style bacon - packer C	3.16	3.48	3.32	12.2
- packer A	5.21	5.38	5.29	17.3
- packer B	5.01	5.51	5.26	15.9
Link sausage - packer C	4.27	4.11	4.19	19.3
- packer A	4.18	3.77	3.97	21.1
- packer E	4.16	3.50	3.82	17.6
VEAL				
Roast				
standing rump - lean	8.67	9.17	8.92	28.0
- marble	7.13	7.34	7.23	25.2
sirloin - lean	8.32	9.04	8.68	30.0
Cutlet - lean	7.13	7.56	7.35	19.3
Loin chop - lean	6.92	7.39	7.15	21.0

TABLE II. Thiamine Content of Cooked Meats

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
BEEF				
Steak				
bottom round - lean	0.130	0.133	0.132	0.363
top round - lean	0.093	0.118	0.106	0.270
- marble	0.074	0.081	0.078	0.196
sirloin - lean	0.124	0.099	0.111	0.411
- marble	0.081	0.087	0.084	0.362
T-bone - lean	0.128	--	0.128	0.483
- marble	0.045	--	0.045	0.194
Roast				
standing rib - lean	0.081	0.087	0.084	0.315
- marble	0.049	0.043	0.046	0.236
standing rump - lean	0.114	0.105	0.110	0.342
- marble	0.087	0.105	0.096	0.303
Ground beef - 75% lean	0.167	0.155	0.161	0.629
- 85% lean	0.229	0.208	0.218	0.724
Stew meat - entire	0.108	0.087	0.098	0.301
Corned beef - lean	0.060	0.056	0.058	0.191
- marble	0.049	0.043	0.046	0.239
LAMB				
Leg roast - lean	0.223	0.228	0.226	0.771
Loin chop - lean	0.198	0.220	0.209	0.755

TABLE II. (Continued)

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
PORK				
Cured ham shank - lean	0.916	0.892	0.904	3.217
- marble	0.619	0.637	0.629	2.747
Fresh center slice ham - lean	0.684	0.669	0.677	1.754
Loin chop - lean	1.176	1.188	1.182	3.416
Sirloin roast - lean	1.254	1.288	1.271	4.087
PROCESSED PORK				
Canadian style bacon - packer C	0.867	0.860	0.864	3.165
- packer A	0.768	0.792	0.780	2.557
- packer B	1.331	1.316	1.324	4.012
Link sausage - packer C	0.913	0.933	0.923	4.253
- packer A	0.724	0.693	0.708	3.766
- packer E	0.644	0.667	0.656	3.023
VEAL				
Roast				
standing rump - lean	0.179	0.193	0.186	0.583
- marble	0.117	0.114	0.115	0.401
sirloin - lean	0.186	0.173	0.180	0.623
Cutlet - lean	0.136	0.142	0.139	0.365
Loin chop - lean	0.223	0.201	0.212	0.620

TABLE III. Riboflavin Content of Cooked Meats

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
BEEF				
Steak				
bottom round - lean	0.325	0.333	0.329	0.904
top round - lean	0.324	0.330	0.327	0.832
- marble	0.264	0.266	0.265	0.703
sirloin - lean	0.540	0.656	0.598	2.215
- marble	0.252	0.232	0.242	1.043
T-bone - lean	0.092	--	0.092	0.347
- marble	0.162	--	0.162	0.698
Roast				
standing rib - lean	0.241	0.211	0.226	0.846
- marble	0.196	0.181	0.189	0.969
standing rump - lean	0.238	0.237	0.238	0.739
- marble	0.217	0.217	0.217	0.685
Ground beef - 75% lean	0.183	0.178	0.181	0.707
- 85% lean	0.133	0.140	0.137	0.455
Stew meat - entire	0.249	0.249	0.249	0.766
Corned beef - lean	0.326	0.303	0.315	1.036
- marble	0.154	0.159	0.157	0.818
LAMB				
Leg roast - lean	0.326	0.302	0.314	1.072
Loin chop - lean	0.342	0.312	0.327	1.181

TABLE III. (Continued)

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
PORK				
Cured ham shank - lean	0.288	0.286	0.287	1.021
- marble	0.186	0.170	0.178	0.777
Fresh center slice ham - lean	0.317	0.325	0.321	0.832
Loin chop - lean	0.201	0.189	0.195	0.563
Sirloin roast - lean	0.341	0.342	0.341	1.096
PROCESSED PORK				
Canadian style bacon - packer C	0.135	0.129	0.132	0.483
- packer A	0.174	0.143	0.159	0.521
- packer B	0.153	0.158	0.156	0.473
Link sausage - packer C	0.241	0.254	0.248	1.143
- packer A	0.162	0.145	0.154	0.819
- packer E	0.207	0.205	0.206	0.949
VEAL				
Roast				
standing rump - lean	0.210	0.229	0.220	0.690
- marble	0.188	0.168	0.178	0.620
sirloin - lean	0.271	0.243	0.257	0.889
Cutlet - lean	0.345	0.385	0.365	0.958
Loin chop - lean	0.334	0.299	0.317	0.927

TABLE IV. Vitamin B_6 Content of Cooked Meats

Cut and portion	Milligrams per 100 gm cooked meat	Milligrams per 100 gm protein calculated from the mean
	Mean	
BEEF		
Steak		
bottom round - lean	0.45	1.24
top round - lean	0.54	1.37
- marble	0.42	1.11
sirloin - lean	0.42	1.55
- marble	0.40	1.80
T-bone - lean	--	--
- marble	0.28	1.21
Roast		
standing rib - lean	0.48	1.80
- marble	0.25	1.28
standing rump - lean	0.41	1.27
- marble	0.35	1.10
Ground beef - 75% lean	0.46	1.80
- 85% lean	0.65	2.16
Stew meat - entire	0.22	0.68
Corned beef - lean	0.34	1.12
- marble	0.20	1.04
LAMB		
Leg roast - lean	0.32	1.09
Loin chop - lean	0.33	1.19

TABLE IV. (Continued)

Cut and portion	Milligrams per 100 gm cooked meat	Milligrams per 100 gm protein calculated from the mean
	Mean	
PORK		
Cured ham shank - lean	0.44	1.57
- marble	0.22	0.96
Fresh center slice ham - lean	0.44	1.14
Loin chop - lean	0.48	1.39
Sirloin roast - lean	0.56	1.80
PROCESSED PORK		
Canadian style bacon - packer C	0.48	1.76
- packer A	0.63	2.07
- packer B	0.55	1.67
Link sausage - packer C	0.16	0.74
- packer A	0.13	0.69
- packer E	0.27	1.24
VEAL		
Roast		
standing rump - lean	0.48	1.50
- marble	0.48	1.67
sirloin - lean	0.52	1.80
Cutlet - lean	0.50	1.31
Loin chop - lean	0.43	1.26

TABLE V. Pantothenic Acid Content of Cooked Meats

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
BEEF				
Steak				
bottom round - lean	0.588	0.665	0.627	1.72
top round - lean	0.338	0.485	0.411	1.05
- marble	0.575	0.670	0.623	1.65
sirloin - lean	2.440	2.671	2.555	9.46
- marble	0.693	0.817	0.755	3.25
T-bone - lean	0.617	0.597	0.607	2.29
- marble	1.259	1.291	1.275	5.49
Roast				
standing rib - lean	0.579	0.617	0.598	2.24
- marble	0.529	0.486	0.507	2.60
standing rump - lean	0.806	0.657	0.731	2.27
- marble	0.484	0.496	0.490	1.55
Ground beef - 75% lean	0.460	0.420	0.440	1.72
- 85% lean	0.452	0.451	0.451	1.50
Stew meat - entire	0.478	0.493	0.485	1.49
Corned beef - lean	0.673	0.786	0.729	2.40
- marble	0.301	0.324	0.313	1.63
LAMB				
Leg roast - lean	0.615	0.606	0.611	2.08
Loin chop - lean	0.643	0.542	0.593	2.14

TABLE V. (Continued)

Cut and portion	Milligrams per 100 gm cooked meat			Milligrams per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
PORK				
Cured ham shank - lean	0.649	0.639	0.644	2.29
- marble	0.462	0.518	0.490	2.14
Fresh center slice ham - lean	0.484	0.497	0.491	1.27
Loin chop - lean	0.389	0.415	0.402	1.16
Sirloin roast - lean	0.831	0.930	0.881	2.83
PROCESSED PORK				
Canadian style bacon - packer C	0.430	0.416	0.423	1.55
- packer A	0.493	0.657	0.575	1.89
- packer B	0.289	0.377	0.333	1.09
Link sausage - packer C	0.617	0.552	0.585	2.69
- packer A	0.597	0.550	0.573	3.05
- packer E	0.591	0.472	0.531	2.45
VEAL				
Roast				
standing rump - lean	0.751	0.673	0.712	2.23
- marble	0.701	0.733	0.717	2.50
sirloin - lean	0.799	0.880	0.839	2.90
Cutlet - lean	0.439	0.554	0.497	1.30
Loin chop - lean	0.513	0.486	0.499	1.46

TABLE VI. Vitamin B_{12} Content of Cooked Meats

Cut and portion	Micrograms per 100 gm cooked meat			Micrograms per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
BEEF				
Steak				
bottom round - lean	1.63	1.67	1.65	4.53
top round - lean	2.01	2.31	2.16	5.50
- marble	1.67	1.91	1.79	4.75
sirloin - lean	3.96	3.67	3.81	14.11
- marble	1.96	1.57	1.77	7.63
T-bone - lean	1.00	1.10	1.05	3.96
- marble	1.80	1.80	1.80	7.76
Roast				
standing rib - lean	2.99	2.73	2.86	10.71
- marble	1.51	2.26	1.89	9.69
standing rump - lean	1.76	1.46	1.61	5.00
- marble	2.69	1.84	2.27	7.16
Ground beef - 75% lean	1.37	1.27	1.32	5.16
- 85% lean	0.90	0.89	0.89	2.96
Stew meat - entire	2.63	3.38	3.01	9.26
Corned beef - lean	1.56	1.66	1.61	5.30
- marble	1.49	1.42	1.45	7.55
LAMB				
Leg roast - lean	2.84	3.28	3.06	10.44
Loin chop - lean	2.44	2.39	2.41	8.70

TABLE VI. (Continued)

Cut and portion	Micrograms per 100 gm cooked meat			Micrograms per 100 gm protein calculated from the mean
	Assay 1	Assay 2	Mean	
PORK				
Cured ham shank - lean	1.50	1.10	1.30	4.62
- marble	1.20	0.95	1.07	4.67
Fresh center slice ham - lean	1.35	1.45	1.40	3.63
Loin chop - lean	1.06	1.09	1.07	3.09
Sirloin roast - lean	1.16	1.20	1.18	3.79
PROCESSED PORK				
Canadian style bacon - packer C	0.97	0.74	0.85	3.11
- packer A	1.67	1.66	1.67	5.47
- packer B	1.18	1.03	1.11	3.36
Link sausage - packer C	1.43	1.41	1.42	6.54
- packer A	1.35	1.62	1.49	7.93
- packer E	0.96	1.42	1.19	5.48
VEAL				
Roast				
standing rump - lean	2.64	2.73	2.69	8.43
- marble	1.98	2.46	2.22	7.73
sirloin - lean	2.54	2.94	2.74	9.48
Cutlet - lean	2.31	2.17	2.24	5.88
Loin chop - lean	2.73	2.72	2.73	7.98

Table III shows the results for riboflavin determinations. The figures show little variation in the riboflavin content of meat from the different kinds of animals. In general lamb cuts contained the largest amount, or a total average of 0.321 milligrams per 100 grams of meat. The pork cuts yielded the smallest amount, averaging 0.232 milligrams for all cuts.

The vitamin B_6 content of cooked meats is shown in Table IV. These assays were performed on two independent hydrolysates of each meat sample, and each hydrolysate was tested at two levels. The results given in this table represent the averages calculated from these results. In general, no appreciable differences in vitamin B_6 content between the various kinds of meats were found. The highest values were found for ground beef, beef top round, veal sirloin roast, veal cutlet, pork sirloin roast, and two of the samples of canadian style bacon. The lowest values were reported for link sausage.

Table V presents the results of the pantothenic acid content of the various samples. In contrast with the results for niacin, there were no noticeable differences in the pantothenic acid content of meat from different animals. However, there were some cuts which contained large amounts of pantothenic acid. Among these were: beef T-bone steak, sirloin steak, veal sirloin steak, and pork sirloin roast. There were also some samples which were low in pantothenic acid content. Among these were: marbled corned beef, top round steak, and some of the pork cuts.

The values for vitamin B_{12} are presented in Table VI. Veal and lamb cuts contained the largest amounts of this vitamin. There was a consistent pattern of greater B_{12} content in the lean cuts of all kinds of meats. The samples which contained the least amount of vitamin B_{12} were the processed pork samples.

CHAPTER IV

DISCUSSION

The results reported in the previous chapter yield current information on the niacin, thiamine, riboflavin, vitamin B_6, pantothenic acid, and vitamin B_{12} content of meats which are representative of the cuts of meat on the market today.

As reported in the literature discussion, there is some change in composition of meat when various cooking methods are used, so results are only significant for the method of cooking used here. Since the main purpose of this study was to determine the vitamins in cooked meats only, exact comparisons cannot be made when previous analyses were done on fresh meats.

In general, lean portions of any cut of meat contain greater amounts of the B-vitamins, since they contain larger amounts of protein and the vitamins are associated with the protein part. However, the marbled portion of every sample also contained appreciable quantities of the vitamins. When calculated on a basis of the vitamin content in 100 grams of protein, it is expected that results would be comparable in lean and marble. This figure has been calculated for each of the vitamins and was shown in each of the tables of results.

Comparative figures between the values reported here and those found in the literature are shown in Tables VII to IX inclusive. Values are not always available for identical cuts and where differences

occur as to raw or cooked meats, or lean and partly fat cuts, they are indicated.

The U.S.D.A. Handbook Number 8, Composition of Foods (62), was the source for the niacin, thiamine, and riboflavin figures in Table VII. The U.S.D.A. Handbook Number 97, Pantothenic Acid in Foods (69), was the reference source for pantothenic acid values. The few figures which are available for comparison to the vitamin B_6 results being reported in this study are indicated in Table VIII. Since no single extensive analyses of vitamin B_{12} on cooked meats have been completed, the individual sources are given for previous values in Table IX.

Niacin:

Niacin figures as shown in Table VII reveal that, in general, values secured in the present study are higher than the ones reported in the U.S.D.A. Handbook 8. Exceptions to this are: sirloin steak, beef rib roast, and marbled cured ham. Differences in regard to meat samples used should be noted, i.e., the previous values were reported on "total edible portion" of the meat. Since this study reports separate analyses on lean and on marbled portions, the average of these findings for the same cut of meat are used for comparison.

Cooking methods as reported in the U.S.D.A. Handbook 8 were specified as braised or pot roasted for beef rump roast and veal leg and all the other meats were cooked to medium doneness at moderate temperatures by common methods suitable for the particular cut. All of the U.S.D.A. values were for cooked meats with the exception of link sausage. Data for the cooked meats were estimated from studies relating to changes in the composition of meat during cooking. Because the results in this study are higher for most of the cooked meats, it may be that these estimates were too low.

TABLE VII

Comparison of Values Obtained in the Present Study with Those Reported in the Literature
(Milligrams per 100 grams cooked meat)

Cuts	Niacin		Thiamine		Riboflavin		Pant. Acid	
	USDA (62)	Present Study	USDA (62)	Present Study	USDA (62)	Present Study	USDA (69)	Present Study
Beef								
Ground beef - 75% lean	4.8	5.60	0.08	0.16	0.19	0.18	0.68	0.44
Rump roast	3.1	4.35	0.04	0.10	0.15	0.23	--	0.61
Sirloin steak	4.8	3.37	0.06	0.10	0.19	0.42	--	1.65
Rib roast- lean	--	5.50	--	0.08	--	0.23	0.54	0.60
- marble	4.3	3.17	0.06	0.05	0.18	0.19	0.33	0.51
Stew meat	--	5.07	--	0.10	--	0.25	0.57	0.49
Top round steak	5.5	5.79	0.08	0.09	0.22	0.30	--	0.52
Corned beef- lean	3.5	5.06	0.02	0.60	0.25	0.31	--	0.73
- marble	3.4	2.79	0.02	0.50	0.24	0.16	--	0.31
Lamb								
Leg roast	5.1	7.31	0.14	0.23	0.25	0.31	0.88	0.61
Loin chop	5.6	7.95	0.14	0.21	0.26	0.33	--	0.59
Pork								
Fresh ham slice	4.7	5.34	0.53	0.68	0.24	0.32	--	0.49
Sirloin roast	3.8	4.59	0.70	1.27	0.17	0.34	--	0.88
Loin chop	5.0	5.55	0.83	1.18	0.24	0.19	0.85	0.40
Cured ham- lean	--	4.61	--	0.90	--	0.29	0.69	0.64
- marble	4.2	3.41	0.54	0.63	0.21	0.18	0.53	0.49
Link sausage	2.3	3.66	0.43	0.76	0.17	0.30	0.68	0.63
Veal								
Rump roast	7.9	8.07	0.13	0.15	0.31	0.20	--	0.71
Cutlet or leg	6.1	7.35	0.08	0.14	0.28	0.37	0.91	0.50

TABLE VIII

Comparison of Vitamin B_6 Values Obtained in the Present Study with Those Reported in the Literature

Cuts	McIntire[1] (32)	Henderson[2] (18)	Present[3] Study
	Milligrams per 100 gm cooked meat		
Beef round	0.37 (raw)	0.38-0.40	0.47
Veal leg	0.20	0.40-0.44	0.50 (cutlet)
Veal sirloin chop	0.11	--	0.43
Lamb leg	0.12	0.30	0.32
Lamb sirloin chop	0.11	--	0.33
Pork ham	0.33	0.59	0.44 (fresh)
Cured ham	0.19	0.29	0.33
Pork loin	--	0.45-0.65	0.48 (chop)

1. Determined by the same yeast microbiological method as in the present study. All meats were cooked except when otherwise indicated.

2. Rat growth assay method used. All meats were raw.

3. Figures for lean and marble results averaged to give "total edible" portion.

TABLE IX

Comparison of Vitamin B_{12} Values Obtained in the Present Study with Those Reported in the Literature

Cuts	Thompson[1] (60)	Scheid[2] (48)	Elvehjem[3] (11)	Scheid[4] (47)	Present[5] Study
	Micrograms per 100 gm meat				
Beef round	5.0	1.5-2.5	5.0	--	1.97 (top round)
Beef rib	--	--	--	2.7	2.37
Beef loin	3.0	--	--	--	2.79 (sirloin)
Pork loin	3.0	1.0-2.0	--	--	1.07
Pork ham	2.9	1.0-2.0	2.9	2.1	1.40 (fresh)
Pork shoulder	5.0	--	0.7	--	1.18 (sirloin)
Veal	2.0	--	3.0	--	2.45 (rump roast)
Lamb leg	8.0	2.0-4.0	--	2.3	3.06

1. Cooked meats on a dry weight basis.
2. Fresh meats on fresh weight basis.
3. Cooked meats analyzed on dry weight.
4. Cooked meats.
5. Average of lean and marbled portions or "total edible" portion.

Thiamine:

The thiamine in cooked meats is shown to be more abundant in lean portions than in the marbled portions of every meat sample analyzed in the present study.

The results in Table II show that in the present study, pork cuts contained greater amounts of thiamine than any of the other meats. On the basis of this finding, an increase in pork in the diet would result in increased thiamine intake. A correlation between pork intake and increased thiamine intake was shown in a survey by the U. S. Department of Agriculture Marketing Service in 1955 (36). This report showed that the national average daily intake of thiamine increased 2% from 1954 to 1955 and the increase was attributed to a greater consumption of pork by the public during this period.

Comparisons between the figures obtained in the present study and those reported previously are shown in Table VII. Both the previous figures and the ones being reported here show the greatest amount of thiamine in pork and lamb and the smallest amount in veal and beef. The comparative figures show that in general higher values were obtained for all the meats in the present study than in the previous studies.

Riboflavin:

In general, the lean cuts of all meats showed higher riboflavin content than did the marbled cuts. One exception in which the riboflavin in the marbled portion was greater was beef T-bone steak.

Comparative figures between the results reported in this study and those reported in the U.S.D.A. Handbook 8 (62) are presented in Table VII. Similar results are shown between this and the previous studies. A few meats, such as beef sirloin steak, fresh ham, and link sausage, contained 45%, 75%, and 56% more riboflavin, respectively, than in the previously reported study.

Pantothenic Acid:

The values for pantothenic acid as reported in the U.S.D.A. Handbook Number 97 (69) are based on fresh, uncooked meats and for most of the samples represent total edible portion. Comparisons were therefore made to the average of the lean and the marbled cuts in this study.

Values reported in the present study would be expected to be less than the U.S.D.A. figures because cooking losses have occurred. In general, this is true for the meat samples compared. These losses, however, are relatively low. Under ordinary cooking conditions, losses may range from 10% to 39% (8). In comparing the present study to the U.S.D.A. study (69), losses of 7% to 35% were found.

Cuts which were found to have large amounts of pantothenic acid in the present study were T-bone steak, lean beef rump roast, lean corned beef, veal sirloin, veal rump roast, and pork sirloin roast. In contrast to the niacin results, no marked differences in pantothenic acid between types of meat were found. This was also reported earlier by Schweigert and Guthneck (50) who found close values between beef, pork, and lamb.

Vitamin B_6:

As shown in Table VIII, vitamin B_6 values were determined by McIntire et al. (32) for a few cooked meats using the same yeast microbiological assay method as was used in the present study. The results of the present study show higher values, ranging from 12% to 74% over these and the indication may be that the previous figures of McIntire and co-workers (32) were too low.

The lowest values for vitamin B_6 were found in link sausage, a range of 0.13 to 0.26 milligrams per 100 grams of meat. These low

figures may be assumed to be attributable to their higher fat content.

Vitamin B_{12}:

All of the results shown in Table IX are given as micrograms per 100 grams of meat and the microbiological assay method, employing Lactobacillus leichmannii as the test organism, was used in each case.

The high values reported by Thompson et al. (60) of 5.0, 3.0, and 8.0 micrograms of vitamin B_{12} respectively for beef, pork, and lamb, and by Elvehjem (11), 5.0 and 2.9 micrograms for beef and pork, may be a result of using the dry weight of the sample. They contrast sharply to the results of the present study in which samples were not dried, and showed values of 1.79, 1.07, and 3.60 micrograms of vitamin B_{12} for beef, pork, and lamb.

The results in this study are similar to those of Scheid et al. (47) whose work most nearly approximates the same conditions underwhich the present analyses were done. That is, both analyses were done on cooked, undried meats. Values for beef rib and ham are approximately the same, 2.7 and 2.1 micrograms (47) respectively, and 2.37 and 1.40 micrograms per 100 grams respectively in the present study. A value of 2.3 micrograms of vitamin B_{12} in lamb was reported by Scheid et al. (47) while 3.06 micrograms was obtained in this study. Results from assays by the rat growth methods were reported by Register et al. (41) who found 2.0 micrograms of vitamin B_{12} per 100 grams of beef round and 1.0 micrograms per 100 grams of pork shoulder, compared to 1.79 micrograms in beef and 1.18 micrograms in pork sirloin in the present study.

Register et al. (41) have also reported that beef samples contain twice as much vitamin B_{12} as pork samples and that this may be a result of the synthesis of vitamin B_{12} in ruminants. The pig, a monogastric animal,does not synthesize the vitamin as ruminants do. The results

in this study did not show that all beef cuts were twice as high in vitamin B_{12} content as pork cuts, but the highest values are reported for beef, veal, and lamb cuts, and the lowest for pork.

The effect of cooking may be judged by comparing the results of Scheid et al. (48) on fresh meats with figures from the cooked meats in the present study. The values in the present study ranged from 23% to 45% less than in that study and agree with the earlier work of Scheid et al. (47) which indicated that about 70% of the vitamin B_{12} is retained during cooking of pork, beef, and lamb.

In the following table, one of the meat samples used in the study has been selected to show what percentage of the daily allowance of each of these vitamins it provides. A usual size serving, of approximately 3.5 ounces or 90 grams, is used. Niacin, thiamine, and riboflavin allowances are the National Research Council (35) recommendations. Allowances for vitamin B_6, pantothenic acid, and vitamin B_{12} are from The Vitamins (53, 54, 55).

TABLE X

Percent of Daily Recommended Allowances Supplied by a Serving of One Meat

One serving (90 gm) of ground beef, 75% lean and 25% fat			
Vitamin	Daily Allowance for a Man	Amount in 90 gm this study	Percent of Daily Allowance
Niacin	15.0 mg	5.04 mg	33.6
Thiamine	1.5 mg	0.145 mg	9.7
Riboflavin	1.6 mg	0.162 mg	10.1
Vitamin B_6	1.5 mg	0.41 mg	27.3
Pantothenic acid	4.0 mg	0.39 mg	10.1
Vitamin B_{12}	1.0 mcg	1.19 mcg	119.0

Suggestions for Future Study

Future studies which are indicated on the basis of these data would be further standardization of vitamin assay procedures so that results from different sources will give comparable results. Currently, microbiological assay, chemical assay, and biological growth methods are used. At the present time, results reported are significant only for the assay procedure being used. Procedures may also vary as to the method of sample release of the vitamin, in the use of wet and dry samples, and in fat extraction of the sample.

The organ meats have been shown to contain large amounts of several of these vitamins. Similiar comprehensive studies on the organ meats, and on poultry and fish are needed to complete our information of the B-vitamin content of foods of animal source.

An even more extensive study might include analyses on fresh meats followed by analyses on paired cooked cuts. This would eliminate any variables such as storage and the effect of freezing and thawing, and would show the vitamin content of paired cuts fresh and cooked. This information is needed especially for vitamin B_6 and vitamin B_{12}, since insufficient information is now available on the losses of these vitamins encountered during cooking.

The methods and types of feeding of animals today are different than they were when the previously available figures were determined. Much discussion has been centered around whether the method of feeding animals affects the nutritive content of meat. Long term studies which would show the effects of different types of feeding, and of different breeds of animals, would give needed facts. Studies on variances in grades of meats would add more to our knowledge of the vitamin content of meats.

CHAPTER V

SUMMARY

The purpose of the work reported here was to determine the niacin, thiamine, riboflavin, pyridoxine, pantothenic acid, and vitamin B_{12} content of various kinds and cuts of cooked meats. Meats have been shown to be a good source of the B-vitamins and this study was undertaken to increase the extent of our knowledge as to their role in providing these essential nutrients.

The methods used to accomplish these purposes involved: 1) the selection and preparation of composite samples of 34 cuts of cooked meat, and 2) determination of several of the B-vitamins by the microbiological assay and by chemical methods.

The results are presented in chart form. Niacin, thiamine, riboflavin, pyridoxine, and pantothenic acid are reported in milligrams per 100 grams of cooked meat. Vitamin B_{12} is reported as micrograms per 100 grams of cooked meat. The amount of each vitamin per 100 grams of protein is included in each of the tables of results. The results obtained here show, in general, values comparable to the ones previously reported in the literature. They also show that lean meats generally contain more of the vitamins than marbled cuts. Because they contain larger amounts of protein, the lean meats would be expected to contain larger amounts of the vitamins.

A 90 gram serving of ground beef is shown to supply 33.6% of the

daily niacin allowance of a man, 9.66 % of the daily thiamine allowance, 10.1 % of the daily riboflavin allowance, 27.3% of the daily vitamin B_6 allowance, 10.1% of the daily pantothenic acid allowance, and 119% of the daily vitamin B_{12} allowance.

More complete information is needed on meats fresh and cooked by the appropriate assay procedure for each vitamin. Many studies have shown that losses of the vitamins occur during cooking, as well as from storing meats for varying lengths of time and by thawing frozen meats. More information is needed on losses of vitamin B_6 and of vitamin B_{12} during ordinary cooking procedures. Further studies to show the effects of different types of feeding, and of different breeds of animals would give many facts which are still unknown.

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APPENDIX A

NIACIN ASSAY PROCEDURE

Preparation of Inoculum

1. A stock culture of Lactobacillus arabinosus 17-5 (ATCC 8014)[1] was used. The organism was carried on agar stab cultures under refrigeration.

2. Culture medium was prepared as follows:

Yeast extract	2.0 gm
Na acetate, anhydrous	0.5 gm
Dextrose, anhydrous	0.5 gm
Agar	1.5 gm

The medium was made to 100 ml with distilled water, then heated with stirring until the agar dissolved. About 10 ml was put into each test tube and the tubes plugged with cotton, then autoclaved at 121° C for 15 minutes. The tubes were then cooled in an upright position and refrigerated.

Stabs were maintained by weekly transfer, incubated for 48 hours at 37° C, and then kept in the refrigerator.

3. Inoculum: The organisms were transferred from the stab culture to tubes containing approximately 5 ml of the liquid culture medium, prepared without agar. This was incubated for 24 hours at 37° C and the growth suspension obtained was the inoculum. Before inoculation, the organisms were centrifuged and the liquid decanted. After this, the organisms were resuspended in 0.9% KCl solution. This was repeated and the final suspension was used for inoculation.

Solutions Used

1. Niacin Stock Solution I:

Niacin	20.0 mg

U.S.P. Niacin Reference Standard was dried at 90° C overnight, then stored in a desiccator over $CaCl_2$. It was then weighed out and dissolved in ethyl alcohol; then made to a volume of 100 ml in the alcohol. This provided 0.2 mg of niacin per ml.

2. Niacin Stock Solution II:

For this solution, 5.0 ml of stock solution I was made to 100 ml with distilled water. This amount provided 10.0 mcg of niacin per ml.

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1. Now named Lactobacillus plantarum.

3. Niacin Stock Solution III:

Two ml of the stock solution II was made to 100 ml with distilled water. This amount provided 0.2 mcg per ml which was used as the standard for assays. This was prepared fresh for each assay.

4. Acid hydrolyzed Casein Solution:

Vitamin-free casein	10.0 gm
3 N HCl	15.0 ml

The casein was covered with the HCl and autoclaved at 121° C for 4 hours. It was brought immediately to pH 3.5 with 1 N KOH after autoclaving, then was filtered and diluted to 100 ml with distilled water.

5. Cystine-Tryptophan Solution:

L-Cystine	2.0 gm
DL-Tryptophan	1.0 gm

The cystine was put into solution first, using conc. HCl as needed to dissolve. Total volume was then made to 500 ml with distilled water.

6. Adenine-Guanine-Uracil Solution:

Adenine. SO_4	200.0 mg
Guanine. HCl	200.0 mg
Uracil	200.0 mg

These were dissolved with heat in conc. HCl and made to 200 ml with distilled water. This amount provided 0.1% of each in acid.

7. Riboflavin-Thiamine HCl-Biotin Solution:

Riboflavin	10.0 mg
Thiamine. HCl	5.0 mg
Biotin	*0.2 ml (20 mcg)

These were dissolved in 5 N HCl, then diluted to 100 ml with distilled water and kept under toluene.

*Prepared by diluting 10.0 mg Biotin to 100 ml.

8. p-Aminobenzoic-Calcium Pantothenate-Pyridoxine.HCl Solution:

PABA	5.0 mg
Ca pantothenate	10.0 mg
Pyridoxine. HCl	20.0 mg

The vitamins were dissolved in 25% ethyl alcohol and the volume brought to 100 ml with the alcohol.

9. Salt Solution A:

$KH_2 PO_4$	25.0 gm
$K_2H PO_4$	25.0 gm

This solution was made to 500 ml with distilled water.

10. Salt Solution B:

$Mg SO_4 . 7 H_2O$	10.0 gm
Na Cl	0.5 gm
$Fe SO_4 . 7 H_2O$	0.5 gm
$Mn SO_4 . 4 H_2O$	0.5 gm

The solution was made to 500 ml with distilled water.

Note: All solutions were stored in the refrigerator.

Basal Medium

Acid hydrolyzed casein soln	25.0 ml
Cystine-tryptophan soln	25.0 ml
Adenine-guanine-uracil soln	5.0 ml
Riboflavin-Thiamine-biotin soln	1.0 ml
PABA-Ca pant.-pyridoxine soln	1.0 ml
Salt A soln	5.0 ml
Salt B soln	5.0 ml
Dextrose, anhydrous	10.0 gm
Na acetate, anhydrous	5.0 gm

The dextrose and Na acetate were dissolved in the liquid ingredients. The pH was adjusted to 6.8 with 5 N KOH and the final volume made to 250 ml with distilled water.

Preparation of Sample Solution

A 0.5 gm meat sample was weighed on an analytical balance. A volume of 1 N $H_2 SO_4$ equal to 10 times the weight of the sample was added. It was then autoclaved at 121° C for 30 minutes; cooled and brought to pH 6.8 with 1 N KOH. The sample was then made to 100 ml with distilled water and filtered. For dispensing into the tubes, 40 ml of the filtrate was made to 100 ml with distilled water. This was a dilution of 1 to 500.

Assay Procedure

Standard niacin tubes were prepared by adding 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the standard niacin solution III to duplicate test tubes in two rows in each rack. The prepared meat samples were dispensed in duplicate in the same amounts and the volume brought to 1.0 ml with distilled water in all the tubes. Finally, 1.0 ml of the basal medium was dispensed into each tube.

The racks were autoclaved for 5 minutes at 121° C. At the same time, a syringe and approximately 50 ml of 0.9% KCl solution were

sterilized. Racks were then cooled and inoculated aseptically with 1 drop of the inoculum. After shaking, the racks were incubated at 37° C for 72 hours and then titrated with 0.05 N KOH to pH 7.3.

This assay procedure is a modification of the method outlined in Official Methods of Analysis of the Association of Official Agricultural Chemists. 8th Edition, 1955. (1)

THIAMINE ASSAY PROCEDURE

Solutions Used

1. Thiamine Stock Solution:

Thiamine. HCl	25.0 mg
Alcoholic HCl	250.0 ml

The thiamine. HCl was dried over P_2O_5 in a desiccator for 24 hours. It was then dissolved in the alcoholic HCl to provide 100 mcg of thiamine. HCl per ml.

2. Thiamine Solution B:

Thiamine stock solution	2.5 ml

This was diluted to 100 ml with 0.1 N H_2SO_4 and yielded 2.5 mcg thiamine. HCl per ml.

3. Thiamine Working Solution C:

Thiamine solution B	2.0 ml
0.1 N H_2SO_4	365.0 ml
2.5 M Na acetate	25.0 ml
Acid KCl	100.0 ml

The last three solutions were mixed together and a portion of the mix was used to dilute the thiamine solution up to 100 ml. This amount yielded 0.05 mcg thiamine per ml, or 0.25 mcg per 5 ml.

4. Thiamine, Recovery, for checking Decalso:

Thiamine solution B	2.0 ml
0.15 N H_2SO_4	50.0 ml
Na acetate	5.0 ml

These were combined and diluted with distilled water to 100 ml, then 25 ml of the solution was absorbed on the Decalso.

5. Potassium Ferricyanide 1%:

Potassium ferricyanide	1.0 gm

This was dissolved in distilled water and diluted to 100 ml. This reagent is stable indefinitely if kept cool and in the dark in a brown bottle.

6. Alkaline Potassium Ferricyanide:

1% potassium ferricyanide	3.0 ml

This was diluted to 100 ml, using cool 15% NaOH solution. It was prepared fresh for each usage and kept out of sunlight.

7. Sodium Acetate 0.1 M: (Buffer solution)

Na acetate, anhydrous	8.204 gm

This was dissolved in distilled water and made to 1 liter.

8. Acid Potassium Chloride:

Conc. HCl	17.0 ml
25% KCl solution	

The HCl was diluted to 2 liters with the 25% KCl solution.

9. Activated Decalso:

One hundred and sixty grams of 60 to 80 mesh Decalso were shaken in a 2 liter Erlenmeyer flask 4 times with 10 volume portions of 3% acetic acid for 10 minutes each. Between the second and third acid washings, a 20 minute treatment with 5 volumes of 25% KCl was introduced. Thorough washing with distilled water was the last step. This was stored under water.

10. Alcoholic HCl:

Conc. HCl	34.0 ml

This was diluted to 1 liter with distilled water. One ml of this solution was diluted to 1 liter with 25% ethyl alcohol.

11. Quinine Sulfate Stock Solution:

U.S.P. quinine sulfate	10.0 mg

This was dissolved in 0.1 N H_2SO_4 and diluted to 1 liter with the same solvent. It was stored in a brown bottle in the refrigerator.

12. Quinine Sulfate Working Solution:

Quinine sulfate stock soln	1.0 ml

This was diluted to 100 ml with 0.1 N H_2SO_4. The solution was made fresh for each assay.

Note: All solutions were stored in the refrigerator.

Preparation of Sample Solution

Approximately 4 gm of the ground meat sample was weighed and placed in a 250 ml Erlenmeyer flask with 100 ml of the 0.1 M Na acetate buffer and 4 drops of conc. HCl. This provided a buffer medium at pH 4.0. Then 0.1 gm each of the enzymes, papain and diastase, were added.

At the same time, 2.0 ml of thiamine soln B were measured into a 100 ml volumetric flask and treated in the same manner as the unknown. Enzyme blanks were also run with each assay.

The flasks were stoppered and the contents mechanically stirred at approximately 300 r.p.m. for 2 hours. A few drops of toluene were added and the flasks placed in an incubator at 40° C for about 15 hours. After removal from the incubator, 3 ml of chloroform were added, the flasks shaken vigorously for 2 minutes, then allowed to stand till the chloroform settled to the bottom. The sample layer was then decanted from the chloroform, diluted to 250 ml, mixed, and filtered. The chloroform was washed twice in the buffer soln.

Procedure

1. Adsorption and Elution:

Adsorption (or base-exchange) tubes using wet Decalso were prepared and 25 ml of the sample filtrate allowed to drip through the tube. This was then washed 3 times with hot distilled water and the filtrate and washings discarded. The reservoir of the adsorption tube was filled with acid KCl at room temperature and the eluate collected in test tubes which were then diluted to 25 ml. The eluate was mixed well before the next step.

2. Oxidation:

Standards. Thiamine working solution C in 5 ml portions was pipetted into four reaction tubes. Three ml of alkaline ferricyanide was added to 2 of the tubes and 3 ml of 15% NaOH was added to the other two. (The NaOH tubes were the standard "blanks" since they do not show the thiochrome reaction). Twelve ml of isobutyl alcohol were added to all the tubes from a burette. Not more than 3 minutes were allowed to elapse from the time the ferricyanide and the alcohol were added to the tubes until the time of shaking, therefore, only four tubes were handled at one time.

The solution was shaken for 1½ minutes, centrifuged for 1½ minutes if the solution was cloudy, and the water layer removed. About 2 gm of Na sulfate was then added through a small funnel. The tubes were then shaken for half a minute, again centrifuged for 1 minute, and the isobutyl alcohol layer decanted into tubes.

Unknown. Five ml aliquots of the unknown meat sample eluate from step 1 were treated in the same manner as the standards.

Enzyme Blanks. Five ml aliquots of the enzyme blanks were also treated in the same manner as above except the 15% NaOH was substituted for the alkaline ferricyanide solution.

3. Fluorometry and Calculation:

The galvanometer of the Farrand Fluorometer, Model A, was set at 50 with quinine sulfate working solution and the samples read. The "standard" readings and "blank" readings were averaged, but each "unknown" was calculated separately. Recovery of a known solution

should be about 95%.

This thiochrome procedure depends upon oxidation of thiamine to thiochrome, which fluoresces in ultra violet light. The intensity of fluorescence is proportional to the thiochrome present, and hence to thiamine originally in the solution.

This assay procedure is a modification of the method outlined in Methods of Vitamin Assay. 1951 (2)

RIBOFLAVIN ASSAY PROCEDURE

Solutions Used

1. Riboflavin Stock Solution:

Riboflavin	50.0 mg
Glacial acetic acid	1.2 ml

U.S.P. Reference Standard riboflavin was dried in a vacuum desiccator over conc H_2SO_4 for 24 hours. The riboflavin was weighed and transferred to a liter volumetric flask. About 750 ml of distilled water and the glacial acetic acid were added and the solution heated. After cooling to room temperature, it was made to 1 liter with distilled water. This provided 50 mcg of riboflavin per ml in 0.02 N acetic acid. The solution was stored under toluene and protected from light in the refrigerator.

2. Riboflavin Solution B:

Ten ml of stock solution were diluted to 200 ml with 0.02 N acetic acid. This amount provided 2.5 mcg per ml in 0.02 N acetic acid.

3. Riboflavin Working Solution C:

One ml of riboflavin stock solution was diluted to 250 ml with distilled water. This was prepared fresh for each assay and contained 0.2 mcg per ml.

4. Hydrogen Peroxide 3%:

A one to ten dilution of 30% hydrogen peroxide was used. It was kept in the refrigerator and prepared fresh each two weeks.

5. Potassium Permanganate 4%:

Four gm of potassium permanganate were dissolved in distilled water and diluted to 100 ml. The solution was prepared fresh for each assay.

6. Sodium Fluorescein Stock Solution:

Fifty mg of sodium fluorescein was dissolved in 1 liter of distilled water.

7. Sodium Fluorescein Solution B:

One ml of the stock solution was diluted to 1 liter with distilled water. This was made fresh weekly.

8. Sodium Acetate 0.1 M:

Na acetate, anhydrous	8.204 gm

This was dissolved in distilled water and made to 1 liter.

9. Sodium Hydrosulfite:

Na bicarbonate	1.0 gm
Na hydrosulfite	1.0 gm

These were weighed into an Erlenmeyer flask and 40 ml of ice cold distilled water was added. The solution was kept in an ice bath and was not filtered.

Note: All solutions were stored in the refrigerator.

Preparation of Sample Solution

Approximately 4 gm of the ground meat sample was weighed and placed in a 250 ml Erlenmeyer flask with 100 ml of the 0.1 M sodium acetate buffer and 4 drops of conc HCl added. Then 0.1 gm each of the enzymes, papain and diastase, were added.

At the same time, 10 ml of riboflavin working solution B were measured into a 100 ml volumetric flask and treated in the same manner as the unknown. Enzyme blanks were run with each assay.

The flasks were stoppered and the contents mechanically stirred at 300 r.p.m. for 2 hours. A few drops of toluene were added and the flasks placed in an incubator at 40° C for approximately 15 hours. After removal from the incubator, 3 ml of chloroform were added, the flasks shaken vigorously for 2 minutes, then allowed to stand until the chloroform settled to the bottom. The sample layer was then decanted from the chloroform, diluted to 250 ml, and mixed. The chloroform was washed twice in the buffer solution.

Procedure

1. Oxidation:

Twenty-five ml of the filtrate were measured and brought to pH 6.0 with 0.1 N H_2SO_4 and placed in a 100 ml volumetric flask. Then 2 ml of 4% potassium permanganate were added and the solution allowed to stand for exactly 1 minute. The mixture was decolorized with 3% hydrogen peroxide. One drop of caprylic alcohol was added to prevent foaming and the solution was made to 100 ml with water.

2. Fluorometry and Calculation:

The galvanometer of the Farrand Fluorometer, Model A, was set at 50 with sodium fluorescein working solution C. One ml of the sample was measured into the tube and read. The 0.1 ml of riboflavin working solution C was added, mixed by rotating the tube, and read. Then 0.1 ml sodium hydrosulfite solution was added, mixed by rotating the tube, and read in 1 minute after addition of the hydrosulfite. Riboflavin fluoresces in light of a wave length of 440 to 500 millimicrons. The intensity of fluorescence is proportional to the concentration of riboflavin in dilute solution. Riboflavin is measured by difference in fluorescence before and after chemical reduction.

This procedure is modified from Methods of Vitamin Assay, 1951. (2)

VITAMIN B_6 ASSAY PROCEDURE

Preparation of Inoculum

1. A stock culture of the yeast strain, Saccharomyces carlsbergensis (ATCC 4228) was used. The organism was carried on malt extract agar slant cultures under refrigeration.

2. Culture medium was prepared as follows:

Yeast extract	0.3 gm
Malt extract	0.3 gm
Peptone	0.5 gm
Dextrose, anhydrous	1.0 gm
Agar	1.5 gm

The medium was made to 100 ml with distilled water, then heated with stirring until the agar dissolved. About 10 ml was put into each tube and the tubes plugged with cotton, then autoclaved at 121° C for 15 minutes. The tubes were cooled in a slanting position and then refrigerated.

Slants were incubated for 16 to 24 hours after transfer and then stored in the refrigerator for a period not longer than 2 weeks. They were then transferred to fresh agar slants.

3. Inoculum: On the day prior to use, the organisms were transferred from a stock culture tube to a fresh agar slant, then incubated for 16 to 24 hours at 30° C. Some of the yeast was then transferred aseptically to tubes of sterile isotonic saline until the proper concentration of yeast was obtained. This should be at 0.1 mg per ml of yeast for use.

Solutions Used

1. Standard Pyridoxine Solution:

Pyridoxine. HCl	100.0 mg

The previously dried and stored U.S.P. Reference Standard anhydrous crystalline pyridoxine. HCl (dried over conc H_2SO_4 in a vacuum desiccator for 24 hours prior to use) was diluted with distilled water to 1 liter in a volumetric flask, using 1 N HCl as needed to dissolve. This solution was stored in a dark bottle in the refrigerator. To prepare the working standard containing 0.01 mcg per ml, 1.0 ml of the standard stock solution was diluted to 100 ml with distilled water in a volumetric flask. One ml of this solution was then diluted to 100 ml, giving 0.01 mcg per ml as the standard for assays. This was prepared fresh for each assay.

Since pyridoxine is light sensitive, care was taken to avoid exposure of the standard and test solutions.

2. Acid hydrolyzed Casein:

One hundred gm of vitamin-free casein was stirred with 250 ml of 95% ethyl alcohol for 15 minutes in an 800 ml beaker and filtered with suction. This was repeated, using another 250 ml portion of alcohol. This was transferred into a round-bottom flask of at least 1 liter capacity, and mixed well with 500 ml of constant boiling HCl. This was then refluxed over a low flame for 8 to 12 hours. A mixture of 1 volume of conc HCl with 1 volume of water gave a 20.1% HCl solution satisfactory for the hydrolysis.

After refluxing, the hydrolysate was concentrated to a thick paste under reduced pressure. The paste was redissolved in approximately 200 ml of water and the concentration repeated to remove additional HCl. (This second concentration is optional).

The hydrolyzed paste was dissolved in about 700 ml of water, and adjusted to pH 3.5 with 40% Na OH. It was then decolorized by stirring with 20 gm of activated charcoal at room temperature. This was stirred until the test filtrate was straw colored. The pH was adjusted to 6.8, the solution diluted to 1 liter, and stored under toluene and over chloroform in the refrigerator.

3. Isotonic Salt Solution:

Na Cl	0.9 gm

The Na Cl was dissolved with shaking in 100 ml of distilled water, and 10 ml quantities were transferred to culture tubes, plugged with cotton, and autoclaved 20 minutes at 121° C.

4. Salt Solution:

KH_2PO_4	2.20 gm
K Cl	1.70 gm
$CaCl_2$	0.50 gm
$MgSO_4$	0.50 gm
$FeCl_3$	0.01 gm
$MnSO_4$	0.01 gm

These were dissolved in distilled water and made to 1 liter.

5. Potassium Citrate Buffer:

Potassium citrate	100.0 gm
Citric acid	20.0 gm

These were dissolved in distilled water and made to 1 liter.

6. Thiamine Solution:

Thiamine. HCl	10.0 mg

This was dissolved in 1 liter of distilled water and gave 10 mcg of thiamine per ml.

7. Inositol Solution:

Inositol	1.0 gm

This was dissolved in 1 liter of distilled water, giving 1 mg of inositol per ml.

8. Biotin Solution:

Biotin	25.0 mg

The biotin was dissolved in 1 liter of distilled water. Then 40 ml of this stock solution was diluted to 1 liter to give a working solution of 1 mcg per ml.

9. Calcium Pantothenate Solution:

Ca pantothenate	200.0 mg

This was dissolved in 1 liter of distilled water, and yielded 200 mcg per ml.

10. Niacin Solution:

Niacin	1.5 gm

The niacin was dissolved in 1 liter of distilled water, giving 1.5 mg per ml of solution.

Note: All solutions were stored in the refrigerator.

Basal Medium

Dextrose, anhydrous	50.0 gm
Salt soln	250.0 ml
Potassium citrate buffer soln	50.0 ml
Casein hydrolysate soln	50.0 ml
Thiamine soln	2.5 ml
Inositol soln	25.0 ml
Biotin soln	10.0 ml
Ca pantothenate soln	12.5 ml
Niacin soln	1.0 ml

The dextrose was dissolved in the other solutions, which was then adjusted to pH 5.0 to 5.2 with 15% Na OH. Final volume was made to 500 ml with distilled water and this was made fresh for each assay.

Preparation of Sample Solution

The meat sample was weighed into a 400 ml beaker. A two gm sample was covered by 1 ml of 10 N HCl and 179 ml distilled water, giving an acid concentration of 0.055 N. The beaker was covered with a watch glass and autoclaved at 121° C for 4 hours. The sample was then cooled and adjusted to pH 4.5 with 15% Na OH, then transferred to a 200 ml volumetric flask and made to volume with distilled water.

(This solution should contain approximately 0.01 mcg of pyridoxine per ml). The solution was then filtered.

Assay Procedure

Standard pyridoxine flasks (50 ml Erlenmeyer) were prepared by using 0.0 to 4.0 ml of the standard working pyridoxine solution in 0.5 ml increments, or 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0. Distilled water was added to bring the volume in each to 4.0 ml. The prepared meat samples were dispensed in duplicate in the same amounts and the volume brought to 4.0 with distilled water. Finally, 5 ml of the basal medium were added to each flask.

The flasks were plugged with cotton and sterilized at 100° C for 10 minutes. They were then cooled and 1 ml of the inoculum was added to each. They were incubated in the dark, without shaking, for 24 hours. The growth response was then measured turbidimetrically at 660 millimicrons.

This assay procedure is a modification of the method outlined in Methods of Vitamin Assay, 1951. (2) The assay procedure was taken originally from the procedure of Atkin et al. (3)

PANTOTHENIC ACID ASSAY PROCEDURE

Preparation of Inoculum

1. A stock culture of Lactobacillus arabinosus 17-5 (ATCC 8014) was used. The organism was carried on agar stab cultures under refrigeration.

2. Culture medium was prepared as follows:

Yeast extract	2.0 gm
Dextrose, anhydrous	0.5 gm
Na acetate, anhydrous	0.5 gm
Agar	1.5 gm

The medium was made to 100 ml with distilled water, then heated with stirring until the agar dissolved. About 10 ml was put into each test tube and the tubes plugged with cotton, then autoclaved at 121° C for 15 minutes. The tubes were then cooled and refrigerated.

Stabs were maintained by transferring weekly, incubating at 37° C for 48 hours after transfer, then held in the refrigerator.

3. Inoculum: The organisms were transferred from the stock culture to tubes containing approximately 5 ml of the liquid culture medium, prepared without agar. This was incubated for 24 hours at 37° C and the growth suspension obtained was the inoculum. Before inoculation, the organisms were centrifuged and the liquid decanted. After this, the organisms were resuspended in 0.9% KCl solution. This was repeated and the final suspension was used for inoculation.

Solutions Used

1. Pantothenic Acid Stock Solution I:

Ca pantothenate	45.0 mg
0.2 N acetic acid	10.0 ml
0.2 N sodium acetate	100.0 ml

U.S.P. calcium pantothenate was dried at 90° C overnight, then stored in a desiccator over $CaCl_2$. The weighed amount was dissolved in 500 ml distilled water. Acetic acid and Na acetate were added and the volume made to 978 ml with distilled water. This yielded 40 mcg of pantothenic acid per ml. It was stored under toluene in the refrigerator.

2. Pantothenic Acid Stock Solution II:

Stock solution I	100.0 ml
0.2 N acetic acid	9.0 ml
0.2 N sodium acetate	90.0 ml

This solution provided 4 mcg per ml. It was made to 1 liter with distilled water and stored under toluene in the refrigerator.

3. Pantothenic Acid Stock Solution III:

One ml of stock solution II was made up to 100 ml with distilled water. This amount provided 0.04 mcg per ml which was used as the standard for assays.

4. Acid hydrolyzed Casein Solution:

Vitamin-free casein	10.0 gm
3 N HCl	15.0 ml

This was autoclaved at 121°C for 4 hours. After removing from the autoclave, the solution was immediately made to pH 3.5 with 1 N KOH. It was then filtered and diluted to 100 ml with distilled water.

5. Cystine-Tryptophan Solution:

L-Cystine	2.0 gm
DL-Tryptophan	1.0 gm

The cystine was put into solution first, using conc HCl as needed to dissolve the cystine. The total volume was then made to 500 ml with distilled water.

6. Adenine-Guanine-Uracil Solution:

Adenine. SO_4	200.0 mg
Guanine. HCl	200.0 mg
Uracil	200.0 mg

These were dissolved with heat in conc HCl, then made to 200 ml with distilled water. This provided 0.1% of each in acid.

7. Riboflavin-Thiamine. HCl-Biotin Solution:

Riboflavin	10.0 mg
Thiamine. HCl	5.0 mg
Biotin	*0.2 ml (20 mcg)

These were dissolved in 5 N HCl, diluted to 100 ml with distilled water and stored under toluene.

*Prepared by diluting 10 mg biotin to 100 ml.

8. p-Aminobenzoic-Niacin-Pyridoxine. HCl Solution:

PABA	5.0 mg
Niacin	25.0 mg
Pyridoxine. HCl	20.0 mg

The vitamins were dissolved in 25% ethyl alcohol, then brought to 100 ml in the alcohol.

9. Salt Solution A:

KH_2PO_4	25.0 gm
K_2HPO_4	25.0 gm

This solution was made to 500 ml with distilled water.

10. Salt Solution B:

$MgSO_4 \cdot 7H_2O$	10.0 gm
NaCl	0.5 gm
$FeSO_4 \cdot 7H_2O$	0.5 gm
$MnSO_4 \cdot 4H_2O$	0.5 gm

The salt solution was made to 500 ml with distilled water.

Note: All solutions were stored in the refrigerator.

Basal Medium

Acid hydrolyzed casein soln	25.0 ml
Cystine-tryptophan soln	25.0 ml
Adenine-guanine-uracil soln	5.0 ml
Riboflavin-thiamine-biotin soln	1.0 ml
PABA-niacin-pyridoxine soln	1.0 ml
Salt A soln	5.0 ml
Salt B soln	5.0 ml
Dextrose, anhydrous	10.0 gm
Na acetate, anhydrous	5.0 gm

The dextrose and sodium acetate were dissolved in the liquid ingredients. The pH was adjusted to 6.8 with 5 N KOH and the final volume made to 250 ml with distilled water.

Preparation of Sample Solution

Approximately 0.5 gm of the meat sample was weighed on an analytical balance. The sample was then suspended in 25 ml of distilled water and brought to pH 6.8. It was then autoclaved for 15 minutes at 121° C, cooled and 1 ml of 2.5 M Na acetate added. Following this, the solution was brought to pH 4.8 with 0.5 N HCl and 50 mg of the enzyme, mylase, added. (42) The solution was then incubated under toluene for approximately 20 hours at 37° C, after which it was again brought to pH 4.8, then made to 100 ml with distilled water. The sample was then filtered and 20 ml of the filtrate was made to 50 ml for dispensing into racks. This was a final dilution of 1 to 500.

Mylase blanks were run to determine their pantothenic acid content and showed such negligible activity, it was not considered necessary to correct for the amount in calculating.

Assay Procedure

Standard pantothenic acid tubes were prepared by adding 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the standard pantothenic acid

solution III to duplicate test tubes in two rows in each rack. The prepared meat samples were dispensed in duplicate in the same amounts and the volume brought to 1.0 ml with distilled water in all of the tubes. Finally, 1.0 ml of the basal medium was dispensed into each tube.

The racks were autoclaved for 5 minutes at 121° C. At the same time, a syringe and approximately 50 ml of 0.9% KCl solution were sterilized. Racks were then cooled and inoculated aseptically with 1 drop of the inoculum. After shaking, the racks were incubated at 37° C for 72 hours and then titrated with 0.05 N KOH to pH 7.3.

This assay procedure is a modification of the method outlined in Official Methods of Analysis of the Association of Official Agricultural Chemists, 8th Edition. 1955. (1)

VITAMIN B_{12} ASSAY PROCEDURE

Preparation of Inoculum

1. A stock culture of Lactobacillus leichmannii ATCC 7830 (313) was used.

2. The microorganisms were carried in agar stab cultures. Culture medium was prepared as follows:

Yeast extract	0.75 gm
Peptone	0.75 gm
Dextrose, anhydrous	1.00 gm
KH_2PO_4	0.20 gm
Tomato juice filtrate[1]	10.00 ml
Tween 80 soln	1.00 ml
Agar	1.50 gm

The medium was brought to pH 6.8 with 3 N KOH, then made to 100 ml with distilled water and heated with stirring until the agar dissolved. About 10 ml was put into each tube and the tubes plugged with cotton, then autoclaved at 121° C for 15 minutes. The tubes were then cooled and refrigerated.

Stabs were maintained by transferring daily, incubating for 24 hours at 37° C after transfer, then held in the refrigerator. New stab medium was made at least once a week.

3. Inoculum: The organisms were transferred from the stab culture to the liquid culture medium 6 to 8 hours before time of inoculation of the assay. The liquid culture medium was the same as for the stabs but with the agar omitted. New liquid culture medium was made at least once a week. It was necessary to have the organism quite active (10 successive transfers) before using for an assay.

The culture medium was incubated for 6 to 8 hours at 37° C and the growth suspension obtained was the inoculum. The 8 hour culture was washed 3 times by centrifuging, decanting the supernatant, and resuspending in 0.9% KCl solution, and 1 drop of the suspension added to each tube from an inoculating syringe.

Solutions Used

1. One hour Casein Hydrolysate:

One gm of vitamin-free casein was covered by 3 ml of 3 N HCl and autoclaved at 121° C for 1 hour. It was made to pH 7.0 immediately after removal from the autoclave; then made to 100 ml volume and filtered. The solution contained 10 mg of casein per ml. This casein hydrolysate was added as a source of stimulatory peptides.

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1. The tomato juice was centrifuged and filtered through Filter-cel until the filtrate was pale yellow.

2. Amino Acid Solution: (51)

DL-Alanine	0.8 gm
L-Arginine. HCl	0.4 gm
L-Asparagine	1.6 gm
L-Cysteine. HCl	1.6 gm
L-Glutamic acid	1.6 gm
Glycine	0.4 gm
L-Histidine. HCl	0.4 gm
DL-Isoleucine	0.8 gm
L-Leucine	0.4 gm
L-Lysine	0.8 gm
DL-Methionine	0.8 gm
DL-Phenylalanine	0.4 gm
L-Proline	0.2 gm
DL-Serine	0.4 gm
DL-Threonine	0.8 gm
DL-Tryptophan	0.8 gm
L-Tyrosine	0.4 gm
DL-Valine	0.8 gm

The amino acids were made to 250 volume with conc HCl and hot distilled water.

3. Adenine-Guanine-Uracil Solution:

Adenine. SO_4	0.1 gm
Guanine. HCl	0.1 gm
Uracil	0.1 gm

These were dissolved with heat in 1 N HCl and made to 100 ml in distilled water.

4. Xanthine Solution:

Xanthine	0.1 gm

This was dissolved in 5 N KOH and made to 100 ml with distilled water.

5. Tween 80:

Polyoxyethylene sorbitan monooleate	10.0 gm

The Tween solution was dissolved and made to 100 ml volume in distilled water.

6. Salt Solution A:

$K_2H\ PO_4$	10.0 gm
$KH_2\ PO_4$	10.0 gm

The salts were made to 200 ml with distilled water.

7. Salt Solution B:

$MgSO_4 \cdot 7H_2O$	4.0 gm
NaCl	0.2 gm
$FeSO_4$	0.2 gm
$MnSO_4 \cdot 4H_2O$	0.2 gm

These were combined and made to 200 ml with distilled water.

8. Vitamin Solution I:

Riboflavin	5.0 mg
Thiamine. HCl	5.0 mg
Niacin	10.0 mg
Biotin	*50.0 mcg

The vitamins were made to 100 ml with distilled water, using 5 N HCl to effect solution.

*One ml of a solution containing 50 mcg per ml.

9. Vitamin Solution II:

PABA	10.0 mg
Ca pantothenate	5.0 mg
Pyridoxine	20.00 mg
Pyridoxal	20.00 mg
Pyridoxamine	4.0 mg
Folic acid	*1.0 mg

These were mixed in a beaker and made to 100 ml with distilled water.

*Five ml of a 200 mcg per ml folic acid solution made up in 0.01 N KOH in 50% ethyl alcohol.

10. Buffer Solution: (68)

The buffer was made by combining 8.2 gm sodium acetate and 10.0 mg KCN. These were made to 1 liter (0.1 N sodium acetate). The solution was then made to pH 4.5 with 1 N acetic acid. After preparation, this solution could be kept for several weeks. Just before using, 1.0 mg of Na meta bisulfite was added per ml of buffer soln.

11. Standard B_{12} Solution:

Standard B_{12} (cyanacobalamin) was obtained from Merck and Co., Inc., and contained 20 mcg per ml. A stock solution of 500 mmcg per ml was prepared, and from this, a 5 mmcg per ml standard. For each assay, the latter was diluted 1 to 100, or 0.05 mmcg of B_{12} per ml for dispensing.

Note: All solutions were kept in the refrigerator.

Basal Medium

Amino acid soln	10.0 ml
Casein hydrolysate soln	10.0 ml
Adenine-guanine-uracil soln	2.0 ml
Xanthine soln	2.0 ml
Vitamin I soln	2.0 ml
Vitamin II soln	2.0 ml
Salt A soln	2.0 ml
Salt B soln	2.0 ml
Tween 80 soln	2.0 ml
Dextrose, anhydrous	4.0 gm
K acetate, anhydrous	2.0 gm
Ascorbic acid	0.4 gm

The dry ingredients were dissolved in the liquid solutions. The medium was made to pH 6.0 with 5 N KOH and then to 100 ml volume with distilled water.

Preparation of Sample Solution

One-half gram of meat sample was weighed on an analytical balance. 25.0 ml of the buffer solution was added and the sample autoclaved for 20 minutes at 121° C. After cooling, the sample was brought to pH 6.0 with 1 N KOH; then made to 100 ml volume with distilled water and filtered. For dispensing into the tubes, 10 ml of the filtrate was made to 50 ml or a dilution of 1 to 1000.

Assay Procedure

Standard B_{12} tubes were prepared by adding 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the standard B_{12} solution to duplicate test tubes in two rows in each rack. The prepared meat samples were dispensed in duplicate in the same amounts and the volume brought to 1 ml with distilled water in all the tubes. Finally, 1 ml of the basal medium was dispensed into each tube. The racks were autoclaved for 5 minutes at 121° C. At the same time, a syringe and approximately 50 ml of 0.9% KCl solution were sterilized. The racks were then cooled and inoculated aseptically with 1 drop of the inoculum. After shaking, the racks were incubated at 37° C for 40 hours and then titrated with 0.05 N KOH to pH 7.3.

This procedure is a modification of the method from the Pharmacopoeia of the United States. 1955. (39)

APPENDIX B

Meat Cutting and Cooking Procedures Followed in This Study

Meat Cut	Cutting Procedure	Method of Cooking
Beef		
Round Steak (Top and Bottom)	Cut 3/4 inch thick and from a midway point between superior and inferior extremity of the femur. Excess fat was removed from top round to a half inch. Bottom should contain eye of the round.	Braise at low tepera-ture (below boiling point), allowing 45 minutes to 1 hour total cooking time.
Sirloin Steak	Cut 1 inch thick, the tip removed and cut from midway point on wholesale break at about second sacral vertebra.	Broil at 350° F at top surface of meat. Allow 10 to 12 minutes per side.
T-Bone Steak	Cut 1 inch thick, close trimmed flank and cut from over 4th lumbar vertebra.	Broil at 350° F at top surface of meat. Allow 10 to 12 minutes per side.
Standing Rib Roast	Cut 7 inches of rib length, using a 5 to 6 pound roast, of the 9th to 11th ribs.	Roast at 300° F until meat thermometer registers 160° F, allowing 2 to 2½ hours total cooking time.
Standing Rump Roast	A 5 to 6 pound cut. Remove the excess fat to a half inch.	Braise at low temperature, allowing 3½ to 4 hours total cooking time. (175° to 180° F internal temperature)
Corned Beef	Whole boneless brisket, deckle cut and split into two equal portions by weight, about 3½ to 4 pounds each.	Cook slowly at a simmering temperature (185° F at sea level) in enough water to cover, allowing 3½ to 4 hours total cooking time.
Ground Beef	75% lean/25% fat mixture from beef trimmings plus enough fat to make the proper ratio.	Shape into 4-ounce patties (1 inch thick, and 3 inches in diameter) and broil 10 to 12 minutes per side at 350° F.

Meat Cut	Cutting Procedure	Method of Cooking
Lean Ground Beef	85% lean/15% fat mixture from lean beef trimmings plus fat to make the desired ratio.	Shape into patties as for ground beef, and broil at 350° for 10 to 12 minutes per side.
Stew Meat	Cut 1½ inch cubes of beef from the chuck and round.	Brown cubes on all sides. Cook slowly at simmering temperature in water to cover, allowing 2½ to 3 hours total cooking time.
Veal		
Standing Rump Roast	A 4 to 6 pound roast cut and trimmed from the rump.	Roast at 300° F until meat thermometer registers 170° F, allowing 2½ to 3 hours total cooking time.
Loin Chop	One half inch thick chops cut from the heavy end of the loin and excess flank removed.	Braise at low temperature, below boiling point, allowing 45 minutes to 1 hour total cooking time.
Cutlet (from Round)	Cut ½ inch thick, from a midway point between the superior and inferior extremity of femur and perpendicular to it.	Braise at low temperature. Allow 45 minutes to 1 hour total cooking time.
Sirloin Roast	Cut and trimmed to a weight of 3 to 5 pounds.	Roast at 300° F until meat thermometer registers 170° F, allowing 2½ to 3 hours total cooking time.
Lamb		
Loin Chop	Cut 1 inch thick. The fell removed and excess flank meat cut off.	Broil at 350° F at top surface of meat, allowing 5 to 7 minutes per side.
Leg Roast	Five to 7 pound frenched leg of lamb with sirloin chops removed. Leave fell on the roast.	Roast at 300° F until meat thermometer registers 175° F, allowing 3 to 3½ hours total cooking time.
Pork		
Cured Ham	Cut from a cured, smoked ham.	Roast in 300° F oven until meat thermometer registers 185° F.

Meat Cut	Cutting Procedure	Method of Cooking
Center Ham Slice, Fresh	Cut 3/4 inch thick, from a 12 to 14 pound fresh ham. Remove slice midway between superior and inferior extremity of the femur bone and perpendicular to it.	Braise at low temperature (below boiling point), allowing 45 minutes to 1 hour total cooking time.
Loin Chop	Cut 3/4 inch thick chops from a 12 to 14 pound pork loin.	Braise at a low temperature, allowing 45 minutes to 1 hour total cooking time.
Sirloin Roast	A roast of 4 to 5 pounds cut from a 12 to 14 pound pork loin. Maximum fat thickness is $\frac{1}{4}$ inch.	Roast in 350° oven until meat thermometer registers 185° F, allowing 3 to $3\frac{1}{2}$ hours total cooking time.
Canadian Style Bacon	Center slices from different packer's brands. Slice $\frac{1}{4}$ inch thick.	Panbroil at moderate temperature until meat is browned on both sides.
Fresh Pork Link Sausage	Three different packer's brands used.	Place in frying pan, add $\frac{1}{4}$ cup water, cover and simmer for 8 to 10 minutes. Do not prick links. Then remove cover and brown the links.

The cutting procedures are from 101 Meat Cuts (34a).
The cooking procedures are taken from Meat Manual (34).

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Biographical and Other Items:

Born: July 27, 1926, at Stigler, Oklahoma

Undergraduate Study: Attended grade school at Stigler, Oklahoma; graduated from Stigler High School in 1944; received the Bachelor of Science degree from Oklahoma Agricultural and Mechanical College, with a major in Household Science, in May, 1948.

Graduate Study: Entered the University of Michigan Hospital, Ann Arbor, Michigan, in September, 1948, for a one-year dietetic internship which was completed in 1949. Entered Oklahoma Agricultural and Mechanical College in September, 1956, pursuing work toward the degree of Master of Science, and completed the requirements in August, 1957.

Experiences: Chief Dietitian, Children's Hospital, Louisville, Kentucky, 1949-1952. Administrative Dietitian, University of Chicago Clinics, Chicago, 1952-1956. Research Assistant, Department of Food, Nutrition, and Institution Administration, Oklahoma Agricultural and Mechanical College, 1956-1957.

Organizations: American Dietetic Association, Mortar Board, Who's Who in American Colleges and Universities, Omicron Nu, Phi Sigma, Sigma Xi, Phi Kappa Phi.

Date of Final Examination: August, 1957.