July 26, 1965

To the Graduate Council:

I am submitting herewith a thesis written by Jeanne Rebecca Vannoy entitled "The Retention of Vitamin B_6 in Roasts from the Longissimus Dorsi Muscle of Beef When Cooked by Dry Heat." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science with a major in Food Science.

Bernadine Meyer
Major Professor

We have read this thesis
and recommend its acceptance:

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Dean of the Graduate School

THE RETENTION OF VITAMIN B_6 IN ROASTS FROM THE LONGISSIMUS DORSI MUSCLE OF BEEF WHEN COOKED BY DRY HEAT

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Jeanne Rebecca Vannoy

August 1965

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J. R. V.

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CHAPTER I

INTRODUCTION

In the period beginning around 1935, chemical and microbiological methods were developed for determining the amounts of the various B-complex vitamins in foodstuffs and other substances. The work previously had been done by animal assay. With the development of these more rapid and less costly methods, there was considerable research on the effect of cooking on the vitamins of the B-complex in food. The early work was concerned mostly with the effect of cooking on the thiamine, riboflavin, and niacin concentration. Very little data has been published on the effects of cooking on the vitamin-B_6 content of beef.

In one recent study, Lushbaugh, Weichman, and Schweigert (1959) compared microbiological methods with rat bioassay for the determination of the effects of roasting on the vitamin-B_6 content of beef. The results obtained with the two methods were not in good agreement. They reported that 44 per cent of the vitamin was retained after roasting standing-rib of beef when using rat bioassay, and 56 per

cent when measured microbiologically. With Boston cuts, they reported retentions of 56 per cent by rat bioassay and 42 per cent by microbiological methods. In view of the fact that vitamin B_6 is comparatively heat stable, these retentions, based on either method of assay, seem rather low. Additional tests of the effect of oven roasting on the vitamin-B_6 content in beef seem warranted.

For the present study, five tests were conducted of the effect of oven roasting on the vitamin-B_6 content of roasts from the longissimus dorsi muscle of five beef animals. In four of the tests, paired roasts from the right and left loins of the animals were used. The left-side loin was cooked to an internal temperature of 70°C. (medium-done) before analysis of the vitamin-B_6 content. The right-side loins were analyzed raw. For the fifth test, slices were removed from each side of one large ribeye roast for the raw analysis and the remainder was cooked before analyzing. The vitamin content of the meat before and after roasting, and the amount of the vitamin lost in the drippings from the cooked roasts was determined by a yeast-growth microbiological method. When the raw meat, cooked meat, and drippings were sampled for vitamin assays, samples were also taken to

determine the moisture and ether extract of the test material so that cooking retentions could be calculated on a dry, fat-free basis.

It was hoped that this study would serve to increase the amount of information available concerning the effects of roasting on the vitamin-B_6 content of beef.

CHAPTER II

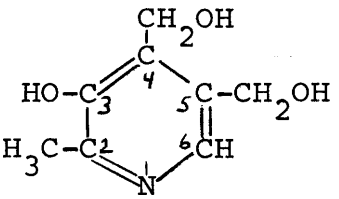
REVIEW OF LITERATURE

I. NATURE OF VITAMIN B_6

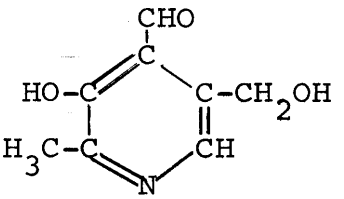
With the identification of thiamine and riboflavin in the early 1930's, the multiple nature of what had been designated "vitamin B" began to be apparent. In tracing the history of vitamin B_6, Cantarow and Schepartz (1962) state that the rather characteristic dermatitis in rats, called "acrodynia," had previously been attributed to a lack of the pellagra-preventive (P-P) factor. It was soon found, however, that niacin had no preventative or curative effect on this lesion. The missing responsible substance (rat-antidermatitis factor) was designated "vitamin B_6" by György (1935). Within a few years, it was isolated from yeast and liver, its chemical structure established, and its synthesis accomplished. This factor was then named "pyridoxine."

This term is now used as a group designation for any naturally occurring pyridine derivatives possessing vitamin-B_6 activity. Many authors, however, refer to the alcohol form as pyridoxine. Derivatives containing

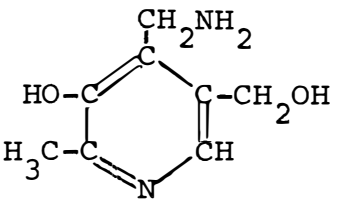
substituents in position four are designated as follows (according to Cantarow and Schepartz, 1962):



Pyridoxol



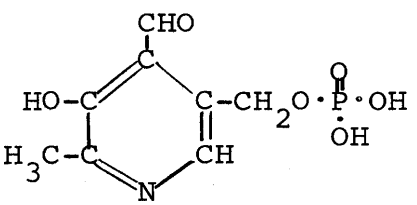
Pyridoxal



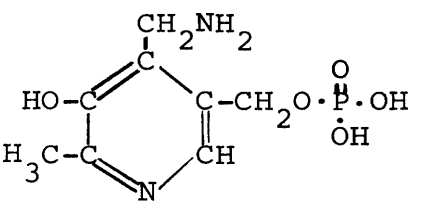
Pyridoxamine

They state also:

> All three forms exhibit "vitamin-B_6" activity, which, however, actually resides apparently in phosphorylated derivatives, pyridoxal phosphate and pyridoxamine phosphate.



Pyridoxal Phosphate



Pyridoxamine Phosphate

These forms occur in nature largely in combination with protein (apoenzymes). The phosphorylation involves the hydroxymethyl group at position five in the pyridine ring.

Sources. Meat, liver, vegetables, and the outer coats (bran) of cereal grains are good sources (Davidson and Passmore, 1963). McIntire, Schweigert, and Elvehjem (1944) reported the following average total vitamin-B_6 ranges (on a moist basis) for muscle meats:

lamb - 2.2-3.3 μg./g.
ham - 2.5 μg./g.
veal - 2.5-4.5 μg./g.
beef - 3.1-4.3 μg./g.

They also reported the following average values for beef organ meats:

tongue - 1.3 μg./g.
heart - 2.9 μg./g.
kidney - 3.9 μg./g.
brain - 1.6 μg./g.
liver - 5.0-9.9 μg./g.

Toepfer et al. (1963), using chromatographic separation of the three forms, have shown that over 90 per cent of the vitamin-B_6 value of lean, dried beef was accounted for as pyridoxal and pyridoxamine, approximately 40 per cent being pyridoxal, 55 per cent pyridoxamine and 5 per cent pyridoxine. The total vitamin-B_6 value for lean, dried beef (not

fractionated) was 14.33 μg./g. The sum of the three fractionated components was 15.93 μg./g.

II. MICROBIOLOGICAL ASSAY OF VITAMIN B_6

The microbiological method most used for vitamin-B_6 determinations was developed by Atkin et al. (1943) using the yeast Saccharomyces carlsbergensis. Cantarow and Schepartz (1962) state that this organism is satisfactory for determinations of total vitamin-B_6 activity, inasmuch as all three forms of the vitamins exert approximately the same growth-stimulating effect on the yeast. Toepfer et al. (1963) reported that the organism responds to a lesser degree to pyridoxamine than to pyridoxal or pyridoxine and that it responds almost equally to pyridoxal and pyridoxine. However, this finding does not agree with findings published by Lushbaugh, Weichman, and Schweigert (1959), who reported almost equal responses to pyridoxamine and pyridoxine and a lesser response to pyridoxal.

The yeast is unable to utilize the bound forms of the vitamin for growth. Therefore, food samples are heated for several hours with acid to hydrolyze the bound forms before the assays are begun.

III. FACTORS AFFECTING THE VITAMIN-B_6 CONTENT OF ANIMAL TISSUES

Some factors that have been investigated for possible effect on the vitamin-B_6 content of animal tissues are differences in diet, location sampled, aging, cooking, and processing.

Variation in Diet

Although very little information is available on the effect of diet upon the vitamin-B_6 content of beef muscle, several studies have been reported in the literature concerning the effect of diet upon the vitamin-B_6 content of rat and mouse tissue.

Vitamin B_6 is very closely related to protein metabolism. The utilization of tryptophan and methionine specifically requires pyridoxine (Tulpule and Williams, 1955). Certain derivatives of vitamin B_6 function as a co-enzyme in the decarboxylation of tyrosine, lysine, arginine, glutamic acid, and in transamination reactions (Schweigert et al., 1946). Therefore, the vitamin-B_6 content of the liver and muscle tissues is most markedly affected by protein intake.

Sheppard and McHenry (1946) reported that an increase in dietary protein caused a greater storage of pyridoxine in the liver. In 1955, Tulpule and Williams demonstrated that the build-up of vitamin B_6 in liver caused a decrease in the concentration of vitamin B_6 in muscle tissues. Sheppard and McHenry (1946) have also reported that there was no measurable effect upon liver or tissue concentration of the vitamin when the carbohydrate or fat intake was increased.

In regard to pyridoxine intake, Schweigert et al. (1946) reported that the concentration of vitamin B_6 in mouse tissues increased progressively with increasing consumption of pyridoxine. Their studies also indicated that when vitamin B_6 was omitted from the diet, the amount in the liver decreased whether the percentage of protein in the diet was high or low. Beaton and McHenry (1953) noted that this decrease in the level of the vitamin in the liver of B_6-deprived rats paralleled the levels of the vitamin in the extra-hepatic tissues.

When the total food intake was increased, while holding the vitamin-B_6 intake constant, Cheslock (1958) reported that the vitamin-B_6 content of the liver decreased.

Unpublished data from the University of Tennessee has demonstrated that the type of feed had a slight effect on the vitamin-B_6 content of beef muscle. Samples from the loin and round of grain-finished animals contained an average of 33.2 μg./g. on a dry, fat-free basis as compared to 30.5 μg./g. for the same muscles from grass-finished animals. This represents an approximately 10 per cent greater vitamin-B_6 content in the two muscles of grain-finished animals than in the grass-finished animals. The difference was significant (P=0.01) but not large enough to be of nutritional importance.

Location Sampled

Data obtained at the University of Tennessee (unpublished) indicated a slightly higher vitamin-B_6 content in the semimembranosus than in longissimus dorsi muscle of beef animals on a dry, fat-free basis. The average value found for the longissimus dorsi was 30.6 μg./g. and the average value for the semimembranosus was 33.1 μg./g. This difference was significant (P=0.02) but here, too, would be of no nutritional importance.

Aging

Limited information seems to indicate that aging of beef (ripening) causes practically no change in the vitamin-B_6 content of beef. In unpublished data from the University of Tennessee it was found that loin and top-round muscles from eight steers which had been aged for 42 days contained 32.3 μg./g. of vitamin B_6 as compared to 31.7 μg./g. for unaged samples from the same animals (dry, fat-free basis).

Cooking and Processing

Vitamin B_6 is one of the most heat stable of the B vitamins. Robinson (1951) stated that pyridoxine hydrochloride was not destroyed on heating with 5N acid or alkali at 100°C. or by autoclaving in acid and alkaline solutions. The loss of vitamin B_6 during various cooking processes is probably the result of leaching since the vitamin is very soluble in water in the free form (Henderson, Waisman, and Elvehjem, 1941). It has not been definitely established how much of the vitamin occurs in the free form in beef. Toepfer et al. (1963) found that about 44 per cent of the vitamin was present in the free form in a nonfractionated extract of lean beef and 32 per cent when calculated from

the sum of the fractionated components.

Frying. Early reports indicated that 81-83 per cent of the vitamin B_6 was retained in beef cooked by frying. Henderson, Waisman, and Elvehjem (1941) found 83 per cent of the vitamin retained in an unidentified beef muscle and 70 per cent retention in fried pork ham. Waisman and and Elvehjem (1941) reported an 81 per cent retention of pyridoxine, also after the frying of an unidentified beef muscle.

Roasting. The early work concerning the effect of roasting on the amount of vitamin B_6 in beef indicated that cooking losses were rather high. Henderson, Waisman, and Elvehjem (1941) found the retention of pyridoxine in roasted pork ham to be 55 per cent. They reported also that pyridoxine in pork loin was reduced almost one-half in the roasting process. McIntire, Schweigert, and Elvehjem (1944) reported pyridoxine retentions of 28 per cent in roasted lamb and of 34 per cent in roasted leg and shoulder of veal.

More recently, Lushbaugh, Weichman, and Schweigert (1959) found that standing-rib roast cooked to an internal temperature of 77°C. retained 44 per cent vitamin B_6 as

measured by rat bioassay and 56 per cent as measured microbiologically. With Boston cuts roasted to the same internal temperature, they reported retentions of 56 per cent by rat bioassay and 42 per cent when measured microbiologically. They obtained an average retention of 55.6 per cent by rat bioassay and 53.0 per cent by microbiological assay after roasting the five following cuts: beef standing rib; beef Boston cut; pork ham; lamb leg; and veal leg. Reasons for the lower retentions reported by McIntire, Schweigert, and Elvehjem (1944) are not clear.

Lushbaugh, Weichman, and Schweigert (1959) reported also that the percentage of vitamin B_6 originally present in the fresh meat which was found in the drippings after roasting varied from 1 per cent for fresh ham to 13 per cent for leg of lamb. No other workers have reported the amount of the vitamin lost in the drippings of oven roasts.

Canning. Richardson, Wilkes, and Ritchey (1961) have shown that beef liver which had been heat processed retained 29 per cent of its vitamin-B_6 content immediately after processing, as compared to a frozen control, and 13 per cent after 15 months of storage. Data for boned chicken were

somewhat erratic. Immediately after canning, 57 per cent of the initial amount of vitamin was present. A retention of 26 per cent was obtained after 9 months and 61 per cent after 15 months of storage.

Irradiation. The amount of vitamin B_6 retained in irradiated foods is essentially the same or greater than in heat processed foods according to Richardson, Wilkes, and Ritchey (1961). Their research indicated that beef liver irradiated with 2.79 megarads* of gamma rays retained 100 per cent of its initial content of vitamin B_6 when compared to a frozen control. After 15 months of storage, the retention was reduced to 53 per cent. When beef liver was exposed to 5.58 megarads, the initial retention was 82 per cent and after 15 months of storage, the vitamin-B_6 content decreased to 57 per cent of that of the frozen control. When boned chicken was exposed to 2.79 megarads, the initial retention was 68 per cent, and after 15 months of storage, the retention increased to 85 per cent. This apparent

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*Rad indicates a unit of ionizing radiation corresponding to an absorption of energy in any medium of 100 ergs/g. One rad in tissue = 100/93 rep. (rep = roentgen equivalent physical).

increased retention was not explained. Boned chicken, immediately after exposure to 5.58 megarads of gamma rays, retained 63 per cent of its initial vitamin B_6 content. Retention was reduced to 54 per cent after 15 months of storage.

Day et al. (1957) reported that a gamma irradiation dose of 3.0 megareps destroyed 26 per cent of the pyridoxine content of raw ground beef. The United States Army Quartermaster Corps (1957) reported that 2.8 megareps of gamma irradiation produced 27 per cent destruction of vitamin B_6 in beef.

In a study to compare the destruction of vitamin B_6 when mixed with vitamin-free casein and irradiated dry, to the destruction which occurs when in solution, Richardson, Martin, and Hart (1958) used 2.79 megarads of gamma rays. They reported that no destruction of pyridoxine occurred when the dry mixture was irradiated. However, there was some destruction of vitamin B_6 when in an aqueous solution and, although they could not estimate the accurate percentage, they reported that this loss was slightly higher than that obtained for riboflavin and pantothenic acid.

Other methods of cooking and processing. The reported retentions of vitamin B_6 for some other methods of cooking and processing meats (boiling, stewing, smoking, curing, and tenderizing) are summarized in Table I. It will be noted that there is a wide variation in the reported retentions. Again, reasons for the variations are not clear.

TABLE I

SOME REPORTED RETENTIONS OF VITAMIN B_6 IN COOKED AND PROCESSED MEAT

Method of cooking or processing	Cut and/or kind of meat	Per cent retention	Source
Boiling	Pork ham	Appr. 50	Henderson, Waisman, and Elvehjem (1941)
Stewing	Lamb (stew meat)	16	McIntire, Schweigert, and Elvehjem (1944)
	Veal (stew meat)	18	McIntire, Schweigert, and Elvehjem (1944)
	Beef kidney	Appr. 66	Henderson, Waisman, and Elvehjem (1941)
Smoking	Pork ham	Appr. 50	Henderson, Waisman, and Elvehjem (1941)
Curing	Pork ham	57	McIntire, Schweigert, and Elvehjem (1944)
Tenderizing	Pork ham	40	Henderson, Waisman, and Elvehjem (1941)

CHAPTER III

PROCEDURE

I. SOURCE OF MEAT

In the present study, five tests were conducted of the effect of oven roasting on the vitamin-B_6 content of roasts from the longissimus dorsi muscle of beef. In four of the tests, paired loin roasts from grain- or grass-finished animals were used. The paired roasts were taken from the same location in the left and right loins of each animal. These roasts were aged for twenty-one days at $2^{\circ}C$. and then held in freezer storage until tested. The left-side loins were roasted to an internal temperature of $70^{\circ}C$. (medium-done) before analysis of the vitamin-B_6 content. The right side loins were analyzed raw. For the fifth test, one large ribeye roast was procured from the Animal Husbandry Department of the University of Tennessee. Slices approximately one-inch thick were removed from each side of the roast for the raw analysis and the center four-inch section was roasted. All roasts were four inches thick, boneless, and averaged approximately two pounds in weight.

II. COOKING AND PREPARATION OF SAMPLES

The frozen roasts were thawed for two days at refrigerator temperatures prior to cooking. The roasts were weighed to the nearest gram and placed in tared, shallow, aluminum pans with racks. The roasting was done in an electric, household range at an average temperature of 149°C. At the setting used, the thermostat of the oven cycled in a range of 141-166°C. A meat thermometer was inserted into the center of the roast and the roast was removed from the oven when the internal temperature reached 70°C. After cooling to an internal temperature of 60°C., the weights of the cooked roast and the drippings were taken to permit calculation of percentage cooking losses.

When the cooked roast cooled to an internal temperature of 50°C., it was prepared for vitamin assay. The roast was diced and then ground twice in an electric grinder, first through a course plate (openings 3/8 inch in diameter) and then through a fine plate with 3/16-inch holes. The samples were thoroughly mixed after each grinding and reweighed before sampling for vitamin assay. Samples of the ground meat were placed in freezer storage in the event they

should be needed for repeat assay. The raw roast was diced, ground, reweighed, sampled, and stored under the same conditions.

To prepare the drippings for vitamin assay, the drippings were poured into a counter-balanced Waring-Blendor jar. The pan was rinsed twice with hot distilled water using 50 ml. for each rinsing. A rubber scraper was used to clean out the pan. After blending for one minute, the weight was recorded as weight of drippings for the moisture, ether extract, and vitamin determinations. The drippings remaining after the samples were removed were put in brown bottles and frozen.

III. MOISTURE AND ETHER-EXTRACT DETERMINATIONS

Since the vitamin content was determined on the undried meat and drippings, it was necessary to determine the moisture and ether extract so that the values could be expressed on a dry, fat-free basis.

Raw and cooked meat. Approximately 10-12 g. samples of the ground raw and cooked meat, weighed by difference to 0.001 g., were transferred into dried, tared, asbestos shells

and dryed first in air at about 93°C. and then at 62°C. under a vacuum of twenty-nine inches until a constant weight was obtained. The shells were then placed in a Soxhlet apparatus and extracted with petroleum ether for sixteen hours, dried, and reweighed. The extraction procedure was repeated for two-hour periods, drying after each extraction, until a constant weight was reached.

Drippings. While the drippings were still hot, triplicate samples of approximately 10 ml. were transferred into tared, 30-ml., glass-stoppered bottles. The sample weight was determined to 0.001 g. on a Mettler P-120 balance. After cooling, the samples were shaken for thirty seconds with 10 ml. of ethyl ether and centrifuged for five minutes at 2500 r.p.m. The ether layer was siphoned off and discarded after centrifuging. This extraction procedure was repeated six times. After removal of the ether extract in the above procedure, the drippings were dried first in a boiling water bath and then under vacuum to a constant weight.

IV. DETERMINATION OF VITAMIN B_6

The total vitamin B_6 was determined by microbiological assay, using Saccharomyces carlsbergensis. The procedure followed was that of Atkin et al. ((1943) with the modifications employed by Lushbaugh, Weichman, and Schweigert (1959). Vitamin B_6 is light sensitive and therefore, solutions containing the vitamin had to be protected against exposure to light and most of the work was done in darkened rooms.

Preparation of samples. Approximately 5-g. samples of the ground, raw, and cooked meat were weighed to 0.001 g. on a Mettler P-120 balance. Three to four grams of the hot drippings were weighed to 0.01 g. on a torsion balance. The samples were suspended in 170 ml. of distilled water and 10 ml. of 1 N HCl were added. These samples, having a pH of about 1.6, were autoclaved for four hours at 15 lbs. pressure to extract and hydrolyze the bound vitamin. Only total vitamin B_6 was measured in this study. The amount of free vitamin B_6 was not determined nor was an attempt made to determine the distribution of vitamin B_6 among the three active forms.

The digests were cooled overnight and adjusted to pH 4.5 with 40 per cent NaOH. This pH resulted in a nearly complete precipitation of protein and other materials which could cause turbidity. The digests were transferred quantitatively to 250-ml. volumetric flasks and diluted to volume with distilled water. The solutions were filtered (discarding the first few milliliters of the filtrate) into brown glass bottles to prevent exposure to light. The filtrates were diluted as required for measurement of the vitamin-B_6 content.

A standard recovery was run with each set of samples. To do this, 30 μg. of pyridoxine hydrochloride was added to one of the meat samples before hydrolyzing. The per cent recovery was considered as a measure of the accuracy of the determination. Only assays with a total recovery of ± 10 per cent and added recoveries of ± 20 per cent were accepted.

All assays were run in duplicate. Only vitamin values agreeing within 10 per cent on duplicate assays were accepted.

Procedure for assays. The working standard pyridoxine solution (0.01 μg./ml.) was pipetted in triplicate into

50-ml. Erlenmeyer flasks in quanitites of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 ml. Water was added so that the volume in each flask was 5 ml.

Samples of the diluted extract of the raw meat, cooked meat, and drippings were pipetted in duplicate into 50-ml. flasks in quantities of 1.0, 2.0, 3.0, and 4.0 ml. Water was added to total 5 ml. in each flask.

Five milliliters of the basal medium containing all of the nutrients required for growth of S. carlsbergensis except pyridoxine were added to both the standard and the assay flasks. The basal medium contained per 500 ml.:

50.0 g.	glucose
50.0 ml.	potassium citrate buffer solution
50.0 ml.	casein hydrolyzate (acid hydrolyzed)
2.5 ml.	thiamine solution (10 μg./ml.)
25.0 ml.	inositol solution (1 mg./ml.)
10.0 ml.	biotin solution (1 μg./ml.)
12.5 ml.	calcium pantothenate solution (200 μg./ml.)
1.0 ml.	niacin solution (1.5 mg./ml.)
250.0 ml.	salt solution*

The pH was adjusted to 5.0-5.2 with 15 per cent NaOH.

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*The salt solution contained per liter:

2.2 g.	potassium phosphate (monobasic)
1.7 g.	potassium chloride
0.5 g.	calcium chloride
0.5 g.	magnesium sulfate
0.01 g.	ferric chloride
0.01 g.	manganese sulfate

All flasks were stoppered with cotton plugs and steamed at 100°C. for ten minutes. The next day each flask was inoculated with one drop of inoculum from a sterile 10-ml. pipette. The inoculum was prepared by dispersing 3 to 4 loopsful of the organism, which had been serially transferred twenty-four hours earlier, into 10 ml. of physiological saline solution. The inoculum gave absorbance values from 113 to 160 (with an average of 139) on a Klett-Summerson colorimeter.

The flasks were incubated at 30°C. After twenty-four hours, growth was stopped by steaming at 100°C. for ten minutes. Absorbance was read in a Klett-Summerson photoelectric colorimeter with a blue filter as a measure of the density of cell growth.

Calculation of vitamin-B_6 content. A standard curve was drawn by plotting the absorbance values against the micrograms of pyridoxine per tube in the standard series. The vitamin-B_6 content of the tubes in the unknown samples was determined by interpolation of the turbidometric readings on the standard curve. The vitamin-B_6 content was calculated from the average value per milliliter of test

solution obtained at not less than three levels according to the following formula:

$$\mu g./g. = \frac{\mu g./ml. \times volume \times dilution\ factor}{wt.\ of\ sample\ in\ g.}$$

The per cent recoveries were calculated according to formulae below:

$$\text{Added recovery} = \frac{\mu g./ml._{(R)} - \mu g./ml._{(T)}}{\mu g./ml._{(S)}} \times 100$$

$$\text{Total recovery} = \frac{\mu g./ml._{(R)}}{\mu g./ml._{(S)} + \mu g./ml._{(T)}} \times 100$$

where, R = Recovery sample
T = Test material
S = Standard added to recovery sample

V. CALCULATION OF VITAMIN-B_6 RETENTION

Every attempt was made in pairing the roasts to make them exact duplicates. However, it could not be assumed that the moisture and fat contents were identical and, therefore, the retentions could not be calculated on the moist, fat basis. The per cent of the vitamin retained in the cooked roast and the amount transferred to the drippings were calculated according to the following formulae:

$$\text{Per cent retained in meat} = \frac{\text{Total wt. of C (g.)} \times \mu\text{g./g. of } B_6 \text{ in C}}{\text{Total wt. of R (g.)} \times \mu\text{g./g. of } B_6 \text{ in R}} \times 100$$

$$\text{Per cent transferred to drippings} = \frac{\text{Total wt. of D(g.)} \times \mu\text{g./g. of } B_6 \text{ in D}}{\text{Total wt. of R(g.)} \times \mu\text{g./g. of } B_6 \text{ in R}} \times 100$$

where, C = Cooked roast
R = Raw roast
D = Drippings

The retentions were not calculated on a gram-per-gram basis but involved total dry, fat-free weights of the raw meat, cooked meat, and drippings, and the calculated vitamin-B_6 content per gram of dry, fat-free material. The total retention was recorded as the sum of the vitamin B_6 retained in the cooked meat and transferred to the drippings.

CHAPTER IV

RESULTS AND DISCUSSION

I. COOKING LOSSES

Data on the per cent cooking losses of roasts containing the longissimus dorsi muscle of beef after roasting to an internal temperature of 70°C. are presented in Table II. Percentage cooking losses were obtained for only four of the five tests. Loss due to evaporation ranged from 12.5 per cent to 19.5 per cent with an average of 15.7 per cent. Loss due to drippings ranged from 3.7 per cent to 14.2 per cent, averaging 8.6 per cent. Total losses (evaporation plus drippings) ranged from 23.1 per cent to 26.7 per cent with an average of 24.2 per cent.

Published data by Meyer et al. (1960) indicated similar losses in longissimus dorsi roasts from grain- and grass-finished animals which had been aged for twenty-one days at 2°C. and roasted to an internal temperature of 70°C. at approximately the same oven temperature. A revolving hearth oven was used in their study rather than a household range. They reported evaporation losses of 11.7-12.2 per

TABLE II

PER CENT COOKING LOSSES IN ROASTED LONGISSIMUS DORSI MUSCLE OF BEEF

Test number	Type of roast	Evaporation	Drippings	Total
1	Ribeye	19.5	3.7	23.2
2	Loin	_[a]	_[a]	_[a]
3	Loin	12.5	14.2	26.7
4	Loin	14.3	9.6	23.9
5	Loin	16.4	6.7	23.1
Avg.		15.7	8.6	24.2

[a] Data was not obtained.

cent and drippings losses of 7.3-10.1 per cent which are more consistent than the values obtained in the present study. The total loss reported by Meyer et al. (1960) for the roasted longissimus dorsi ranged from 19.5 per cent to 21.8 per cent as compared to the slightly higher total loss of 23.1-26.7 per cent found in the present study. The higher loss in this study may have been due to the fact that the household range, although averaging 149°C., cycled as high as 166°C. during the roasting process, whereas the revolving hearth oven used by Meyer et al. (1960) was maintained at a uniform temperature of 149°C. In addition, the roasts used in the present study were stored at freezer temperatures and then thawed before cooking and analyzing, whereas the roasts used by Meyer et al. (1960) were not frozen prior to roasting. This is another possible reason for the higher loss reported for the present study.

II. VITAMIN-B_6 CONTENT OF RAW LONGISSIMUS DORSI

The vitamin-B_6 content of the raw meat, roasted meat, and drippings is shown in Table III. The results are expressed in μg./g. and are presented on the moist, fat basis as well as the dry, fat-free basis.

TABLE III

AVERAGE VITAMIN-B_6 CONTENT OF RAW MEAT, ROASTED MEAT AND DRIPPINGS OF LONGISSIMUS DORSI MUSCLE OF BEEF

Test number	Type of roast	Moist, fat basis[a]			Dry, fat-free basis[a, b]		
		Raw	Roasted	Drippings	Raw	Roasted	Drippings
1	Ribeye	4.9	6.1	2.9	23.1	23.0	70.1
2	Loin	4.6	5.4	3.8	24.0	19.6	95.1
3	Loin	6.3	6.0	5.6	28.6	20.6	94.4
4	Loin	5.6	4.9	4.0	28.2	18.5	84.3
5	Loin	5.1	5.5	4.4	22.5	19.0	74.0
Avg.		5.3	5.6	4.1	25.3	20.1	83.6

[a] Values expressed as µg./g. Each value is the average of two determinations.

[b] Average per cent dry, fat-free weight of raw meat was 20.9; cooked meat, 27.6; and drippings, 5.0.

On a moist, fat basis, the vitamin-B_6 values for the raw longissimus dorsi ranged from 4.6-6.3 μg./g. with an average of 5.3 μg./g. The roasted meat contained 4.9-6.1 μg./g. with an average of 5.6 μg./g. The range of vitamin-B_6 content in the drippings was 2.9-5.6 μg./g. The average vitamin-B_6 content of the drippings was 4.1 μg./g.

On a dry, fat-free basis the raw longissimus dorsi contained from 22.5-28.6 μg./g. with an average content of 25.3 μg./g. The cooked meat content ranged from 18.5-23.0 μg./g. with an average of 20.1 μg./g. The drippings contained 70.1-95.1 μg./g. with an average of 83.6 μg./g.

The average vitamin-B_6 values for muscle meats of beef reported by McIntire, Schweigert, and Elvehjem (1944) ranged from 3.1-4.3 μg./g. on a moist basis. The raw longissimus dorsi muscles assayed in the present study were slightly higher, ranging from 4.6-6.3 μg./g. The particular muscles assayed by the above experimenters were not identified.

Lee et al. (1950) assayed the longissimus dorsi of beef with the fungus Neurospora sitophila according to the method of Stokes et al. (1943), as modified by Barton-Wright (1945). They reported a pyridoxine content of 10.98 μg./g.

on a dry, fat-free basis. This particular organism has been reported to measure only the alcohol form of the vitamin (Barton-Wright, 1945). Therefore, "pyridoxine" as measured by these experimenters could have involved only one form of vitamin B_6. Consequently, the values published by Lee et al. (1950) cannot be compared to the values obtained in the present study where the organism responded to all three forms of vitamin B_6.

III. RETENTION OF VITAMIN B_6 AFTER ROASTING

Data on vitamin-B_6 retention after the roasting of longissimus dorsi of beef are shown in Table IV. Retention in the cooked meat ranged from 62.7 per cent to 95.9 per cent with an average of 76.8 per cent. The amount of the vitamin transferred to the drippings ranged from 11.0 per cent to 21.4 per cent. The average recovered in the drippings was 16.6 per cent. The total retention ranged from 76.6 per cent to 107.0 per cent with an average retention of 93.4 per cent.

There is no apparent reason for the high total retention with the ribeye roast but inspection of the data seems

TABLE IV

COOKING RETENTION OF VITAMIN B_6 IN ROASTED LONGISSIMUS DORSI MUSCLE OF BEEF[a]

Test number	Type of roast	Total vitamin-B_6 content (µg.)			Per cent retentions		
		Raw roast	Cooked roast	Drip-pings.	Cooked roast	Drip-pings.	Total
1	Ribeye	3160.6	3032.1	349.4	95.9	11.0	107.0
2	Loin	4631.5	3580.2	961.0	77.3	20.8	98.1
3	Loin	5393.9	3637.7	1152.8	67.4	21.4	88.8
4	Loin	5091.0	3192.0	702.4	62.7	13.9	76.6
5	Loin	3726.6	3001.3	593.5	80.5	15.9	96.5
Avg.		4400.7	3288.7	752.6	76.8	16.6	93.4

[a] Calculated from the total vitamin-B_6 content on a dry, fat-free basis.

to indicate that the amount retained in the cooked meat is abnormally high. This was also the roast which had the highest evaporation loss during cooking. However, it is not apparent whether the inconsistency comes from differences in the cooking process or some errors in the vitamin-B_6 analyses themselves. However, if this 107 per cent retention were excluded from the average, the total retention in the present study would still have been 90.0 per cent.

The one investigation which is very comparable to that in the present study was the test by Lushbaugh, Weichman, and Schweigert (1959) in which a standing rib of beef was roasted to an internal temperature of 77°C. This cut of meat involves the same muscle (longissimus dorsi) as that used in the present study. They reported 56 per cent retention in the meat of the original vitamin B_6 content as measured by microbiological assay. The average retention of 76.8 per cent obtained in the present study is approximately 37 per cent higher than their value.

The reason for the higher retention obtained in the present study is not entirely clear. The higher internal temperature (77°C.) in the study by Lushbaugh, Weichman, and Schweigert (1959) may have caused lower retentions in

the meat, but should not have affected total retention. Perhaps undue exposure to light was partially responsible for increased destruction of vitamin B_6 in their study. Although they stated that the inoculated assay flasks were incubated in the dark, it was not stated that the samples were protected from light destruction throughout the experimental period.

In the present study, only 7 per cent of the vitamin B_6 was not recovered after roasting beef longissimus dorsi cuts. In view of the fact that the pyridoxine should not be destroyed by heating, the 93 per cent retention obtained here is consistent with the retention which could theoretically be expected. In spite of efforts to avoid exposure of the meat and samples to light, some loss through photo destruction could have occurred. It is also possible that this 7 per cent loss obtained in the present study is merely a reflection of the lack of precision in the microbiological method.

CHAPTER V

SUMMARY

I. SCOPE OF STUDY

Vitamin B_6 is one of the most heat stable of the B-vitamins. However, literature available concerning the effect of roasting on the amount of this vitamin in meat has indicated low retentions. Because meat is one of the common sources of vitamin B_6 in the diet, further investigation of the effect of cooking on retentions of this vitamin in meat seemed warranted.

For the present study, five tests were conducted of the effect of oven roasting on the vitamin-B_6 content of roasts from the longissimus dorsi muscle of beef. In four of the tests, paired loin roasts were used. The paired roasts were taken from the same location in the left and right loins of each animal. The left-side loins were roasted at 149°C. to an internal temperature of 70°C. (medium-done) before analysis of the vitamin-B_6 content. The right-side loins were analyzed raw. For the fifth test, one large ribeye roast was procured. Slices

approximately one inch thick were removed from each side of the roast for the raw analysis and the center four-inch section was roasted. All roasts were four inches thick, boneless, and averaged approximately two pounds in weight. The vitamin-B_6 content of the meat before and after roasting and the amount of the vitamin lost in the drippings from the cooked roasts was determined by a yeast-growth microbiological method. The cooking retentions were calculated from the total vitamin-B_6 content of the raw roasts, cooked roasts, and drippings on a dry, fat-free basis.

II. PRINCIPAL FINDINGS

The average amount of vitamin B_6 retained in the longissimus dorsi roasts after cooking to the medium-done stage was 76.8 per cent. On the average, 16.6 per cent of the vitamin B_6 was transferred to the drippings giving a total average retention of vitamin B_6 in roasted beef of 93.4 per cent. This retention is somewhat higher than that which had been reported previously, but is consistent with what is known concerning the stability of vitamin B_6 in the presence of heat.

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