THE THIAMINE CONTENT OF RAW AND COOKED FROZEN PORK LOIN

by

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INTRODUCTION

The United States possesses the most generous food supply in the world, yet dietary and clinical studies show that even in normal times, large sections of the population suffer from a thiamine deficiency. This is ample evidence that more attention should be given to the relation of every day diet to good health. War food restrictions and rising food costs increase the importance of making wise food choices. At the same time they make more difficult the task of improving the dietary of an entire nation. Numerous studies have been made on the thiamine content of various foodstuffs, but only recently has meat, and especially pork, been established as a good source of the B complex vitamins. Stiebling and Phipard (1939) in the most comprehensive dietary study of the United States, have shown that an increase in food expenditures resulted in an increase in meat consumption and a correspondingly higher thiamine intake. When families spent 24.2 per cent of the food budget for meat, 30 per cent of the daily thiamine requirement was met. However, in the nation as a whole, per capita consumption of meat has steadily declined in the last century. The Armour Livestock Bureau, and the United States Department of Agriculture furnish figures showing a gradual reduction in consumption from 178 to 131 pounds per person between the years 1839 and 1939.

Present data indicate that the thiamine content of pork may vary widely due to such factors as the diet of the hog, conditions of storage, and methods of cooking. The object of this investigation was to study the thiamine content of certain cuts of pork loin, and to determine the thiamine losses when these cuts were cooked by roasting and braising.

REVIEW OF LITERATURE

The early history of the discovery of thiamine was largely concerned with the recognition of the existence of dietary factors other than fats, carbohydrates, proteins, minerals and water. By the close of the 19th century these were the only nutrients which were known to be necessary to animal life. Furthermore, men of science were absorbed in the study of bacteriology, and were of the opinion that most diseases could be controlled by sanitation. Therefore, it was difficult for them to connect disease with food deficiency.

Takaki, Surgeon General of the Japanese Navy, was one of the earliest to believe that beriberi might be caused by a faulty diet. In 1884, he observed that while the Japanese navy was largely affected by beriberi, the crews of the English vessels, sailing in the same waters, were free from the disease. He concluded that the cause might be dietary, since the two navies maintained the same sanitary standards. He dispatched two vessels over the same route on a nine months cruise. On one vessel the crew ate the usual rations of polished rice, and on the other, the diet included

barley, vegetables, meat, and condensed milk. While beriberi occurred as usual among the crew on the old diet, it was almost nonexistent among the men on the new diet. Although Takaki received recognition from his government for eradicating beriberi from the Japanese navy, the full importance of his work remained unknown to the world. He himself, erroneously believed the cure was affected by increased protein in the ration.

A few years later, Eijkman (1898-99) a Dutch physician, was sent by his government to Java to study beriberi. Quite accidentally, domestic fowls were fed an exclusive diet of polished rice, and developed an illness similar to human beriberi. As a result of this coincidence, he started using chickens as experimental animals, and showed that he could induce polyneuritic symptoms by a strictly polished rice diet, and cure them by feeding rice polishings or by giving an aqueous or alcoholic extract of the same. Although he is credited with the first experimentally induced deficiency disease, he did not at the time recognize that it was of nutritional origin. However, he later agreed with his pupil Grijns who continued this work in Java from 1900-1911 and published the historical statement that the substance in rice polishings capable of curing beriberi was effective because it furnished a necessary dietary factor for the nerves (Schuffner, et al., 1935). British investigators in the Malay States were also working to eliminate beriberi. Fraser and Stanton in 1905, using human subjects, confirmed the work of Eijkman and Grijns. They fed polished rice to one camp of laborers, and unpolished

rice to another camp at a distant location. Regardless of location, or of sanitary conditions, there was always an epidemic of beriberi wherever polished rice was used as the chief article of the diet. Within the same year, Fletcher produced similar results with the inmates of a Malay insane asylum. As a result of this work in the Malay States, the United States Medical Commission in the Philippines, represented chiefly by Chamberlain and Vedder, became convinced that beriberi among the Philippine Scouts could be eradicated by a change in diet. Beans were substituted for a part of the beef ration, and unpolished rice used instead of polished rice, with excellent results (Schuffner, et al., 1935). Further proof of the need of unknown dietary factors was furnished by Hopkins (1912) in England. Experimenting with rats on a purified diet, he found that milk contained substances required for normal growth and life in the rat, and named these the "Accessory Factors". He made the prophetic statement that they were possibly organic complexes that the body could not synthesize, and since they were needed in so small amounts they were likely catalytic in effect. He reminded his fellow workers that although this was a new idea, it was no newer than the one that young animals could not grow on a diet supplying formative and energy making foods alone.

When it was established that accessory factors were present in foods, chemists began the search for the pure compounds. The task of isolation and synthesis of thiamine was made more difficult, because of the unknown multiple nature of what was believed to be a single substance.

One of the first important attempts at isolation was performed by Funk (1911) at the Pasteur Institute. He used alkaloidal reagents, and also introduced phosphotungstic acid, and silver nitrate to precipitate the active substance. The resulting crude concentrate was very effective in curing polyneuritis in pigeons. He named this substance the "antiberiberi vitamine", from the words vita, and amine, believing that it was a nitrogenous base, necessary to life. Forecasting that scurvy, rickets, and pellagra, were also deficiency diseases, his book "Die Vitamine" attracted world wide attention.

Within the same year Chamberlain and Vedder (1911) prepared an active concentrate using charcoal as an adsorbent. Edie and associates (1912) suggested the use of baryta for elution and silver nitrate for precipitation of the vitamine from yeast. They proposed the name "torulin". During this period McCollum and Davis (1915) became convinced that a water soluble factor was necessary for normal growth in rats, and named it "water soluble B". The name "vitamin B", was suggested by Drummond (1920).

Interest of various workers turned to the question of the possible multiple nature of vitamin B. Seidell (1916) introduced the use of fuller's earth, as an adsorbent for the active substance. Evidence proving the identity of the water soluble growth promoting substance and the antineuritic factor was first reviewed by Mitchell (1919). Emmett and Luros (1920) suggested that the vitamin consisted of both a heat labile, and a heat stable fraction. However, it was not until six years later that Smith and Hendrick (1926) gave definite proof of its multiple nature

by showing that autoclaved yeast could promote growth, but could not prevent polyneuritis.

During this same period, Goldberger and associates (1925) were seeking a cure for human pellagra. They had observed that the same foodstuffs that were effective for curing this disease, would also prevent polyneuritis, and promote growth in rats. Their work on the preventive foodstuffs for pellagrins helped to arouse interest in the relation of human diets to deficiency diseases.

The first successful isolation of the vitamin was accomplished by Jansen and Donath (1927) in the old laboratory of Eijkman and Grijns. The procedure introduced acid clay for adsorption, but continued to use baryta for elution, and a silver salt for fractional precipitation from a water extract of rice polishings. They adopted the name "aneurin" for the active substance.

Many attempts were made to reproduce the work of Jansen and Donath. In England, Kinnersley and Peters (1928) were unable to secure the same results, but did prepare a very active concentrate somewhat similar in composition. Williams, Waterman, and Gurin (1930) were successful in preparing a concentrate, but its activity was much less than the one of Jansen and Donath. The presence of sulfur in the molecule was established by Windaus and associates (1932), who obtained crystals of the antineuritic vitamin by benzoylation as developed by Seidell (1929), and proposed the correct empirical formala, $C_{12}H_{18}N_4OSCL_2$. Williams, Waterman, and Keresztesy (1934) secured increased yields of the substance from rice polishings. This was accomplished chiefly by

the use of quinine acid sulfate instead of baryta for elution from activated fuller's earth, and resulted in sufficient material for an intense study of its chemical structure. The use of synthetic zeolites as a substitute for fuller's earth was introduced by Cerecedo and Hennessy (1937). This type of adsorbent has the advantage of permitting elution with a strong, cold, neutral salt solution.

Vitamin B_1 was finally synthesized in the laboratory of R. R. Williams. The procedure used is described by Williams and Cline (1936) and by Cline, Williams, and Finkelstein (1937). Williams (1936) showed that the molecule included a substituted pyrimidine and thiazole nucleus.

The Council of Pharmacy and Chemistry of the American Medical Association (1937) selected the official name of thiamin chloride for the vitamin. This name was accepted by the American Society of Biological Chemists, the American Institute of Nutrition, and the Committee on Nomenclature of the American Chemical Society.

The synthesis of pure thiamine made possible further research concerning its role in the physiology of the body, and its relation to enzymes, hormones, and other vitamins. Before the work of synthesis was completed, data had accumulated in the laboratory of Peters (1936) at Oxford to show that vitamin B_1 was concerned with the oxidative breakdown of pyruvic acid in animal tissue. This work was confirmed by Sherman and Elvehjem (1936). Further advancement was made by the isolation of crystalline co-carboxylase from yeast by Lohman and Schuster (1937). They demonstrated that it is the pyrophosphoric ester of thiamine, and that it functions

as a coenzyme in carbohydrate metabolism.

Workers began to investigate the function of thiamine in body reactions. Studies were made in the Toronto laboratory of McHenry on the relationship of thiamine to choline and fat. McHenry (1935) established that both choline and thiamine must be present for normal growth in young rats. He also (1937) showed that the thiamine sparing action of fats is affected by the amount of choline in the diet. McHenry and Gavin (1939) demonstrated that thiamine is necessary for the synthesis of fatty acids from carbohydrate in rats and pigeons. The role of thiamine as an antithyrogenic agent was shown by Sure and Buchanan (1937). Their work was confirmed by Peters and Rossiter (1939). Ellis and Zmachinsky (1937) at Columbia University established that riboflavin in some unknown way spares thiamine. Himwich, Goldfarb, and Cowgill (1938) at Yale reported that the amount of thiamine required by dogs appeared to be proportional to the mass of their tissue and the calories in the diet. A positive correlation between the amount of zinc and thiamine in foodstuffs was shown by Eggleston (1939). Working in Shanghai, he found that in beriberi, the zinc content of toenails, fingernails, and skin is reduced. McElroy and Goss (1941) reported that thiamine could be synthesized in the rumen of the sheep and cow. The influence of thiamine on the mobilization of riboflavin in the liver was established by Supplee and associates (1942). The use of pyrithiamine as a thiamine inhibitor was investigated by Woolley and White (1943). Its administration to mice resulted in a deficiency which could be cured by thiamine.

Throughout the period of isolation and synthesis, biological assays were used to measure the vitamin activity of concentrates and foodstuffs. Chemical and microbiological procedures were developed when pure thiamine became available. All three types of assays remain in use and the choice of method depends upon such considerations as, time available, the concentration of vitamin in assay material and the specificity of the method for its proposed use.

The important biological tests were based either on the cure or prevention of polyneuritis, the cure of special symptoms, and the rate of growth of young animals. Pigeons, domestic fowls, and rice birds were used in all early work. Although pigeons are still occasionally used, rats and chicks are more acceptable to most workers at present. McCollum and Simmonds (1918) were among the first to adopt the white rat as an assay animal, making use of the growth curve as an index to the amount of vitamin B present in the assay material. The observation that rats could secure a large percentage of the vitamin B necessary for normal growth by eating their own feces, was made by Steenbock, Sell and Nelson (1923). Salmon (1925) confirmed this work and recommended that test rats be kept on wide mesh wire screens to prevent access to their feces. A basal diet for use in vitamin B assays was introduced by Sherman and Spohn (1923). The phenomenon of refection was reported simultaneously by Fredericia and associates (1927) in Copenhagen and by Roscoe (1927) at the Lister Institute. They recommended the use of sugar or cooked starch instead of raw starch in the diet to prevent this condition. Further studies on

the synthesis of the B vitamins in the digestive tract of the rat were made by Guerrant, Dutcher, and Brown (1937). The type of nerve injury in rat polyneuritis was studied by Church (1935). Coward (1938) outlined procedures for the care and handling of animals during biological assays. Normal growth in the rat was defined as that of four grams per day by Arnold and Elvehjem (1938c). They also emphasized the importance of using a basal ration containing adequate supplies of all essential factors other than thiamine. The chick was introduced for both polyneuritic prevention tests and for growth assays by Kline, Keenan, Elvehjem and Hart (1932).

The Pharmacopeia of the United States (1939) adopted the rat curative procedure of Kline, Tolle and Nelson (1938) as the official method for thiamine assay. This procedure is a modification of the one originated by Smith (1930) and compares the duration of cure of polyneuritis affected by a standard dose of pure thiamine with that resulting from a known amount of assay material. It is specific and can be used for a succession of tests on the same animal. The curative pigeon test of Kinnersley, Peters and Reader (1928) is considered to be less reliable than the rat curative procedure due to the susceptibility of the pigeon to spontaneous cures of polyneuritis. Arnold and Elvehjem (1938b) adopted the chick preventive method which determines the minimum dose of assay material that will protect all chicks on the test from polyneuritis for a period of five weeks. This procedure was modified by Jukes and Heitman (1940).

Rat growth assays have been used with variations by a number of workers. The specificity of this method has been questioned because thiamine is not the only factor which influences growth. Before crystalline thiamine was available as a standard, two units for measuring growth developed, the Chick-Roscoe and the Chase-Sherman units. The Chick-Roscoe unit (1929) measures the amount of vitamin B_1 per day which produces growth at the rate of 10 to 14 grams per week over a five week period. The Chase-Sherman unit (1931) is defined as the minimum amount of vitamin B_1 required to produce a gain in weight of three grams per week during a period of four to eight weeks. The Association of Official Agricultural Chemists adopted the rat growth test of Kline, Hall and Morgan (1941) as official. Young rats are fed a thiamine deficient diet until they cease to grow. A comparison is then made between the growth rate of a control group fed the basal diet with known amounts of standard thiamine added and other groups fed the basal diet with supplements of assay material at various levels.

The bradycardia or electrocardiographic method of assay proposed by Drury, Harris and Maudsley (1930) and refined for use by Birch and Harris (1934) measures the lowered heart rate of rats during vitamin B_1 deficiency, and the return to a normal heart rate when the vitamin is restored to the diet. It is not widely used due to the necessity of a skilled operator and to its lack of specificity.

A number of chemical reactions have been proposed for the assay of thiamine, following its isolation and synthesis. Although these methods are rapid and are very promising much work needs to

be done before they can be considered entirely specific. The formaldehyde-azo test of Kinnersley and Peters (1934) is a reaction between diazotized sulfanilic acid and thiamine in formaldehyde solution. A red color is produced which is measured in a colorimeter. The colorimetric method of Prebluda and McCollum (1936) and (1939) utilizes the reaction of diazotized p-amino acetanilide or p-aminoacetophenone to form a red dye which is soluble in selective compounds and can be extracted and determined colorimetrically. Melnick and Field (1939) recommended xylene as a selective solvent for the test. The thiochrome method is widely used for the assay of foodstuffs and of urine. Barger, Bergel, and Todd (1935) oxidized thiamine to thiochrome, a bluish fluorescent compound, by using potassium ferricyanide in alkaline solution. This fluoresence can be measured by a fluorometer as a means of quantitative determination. Pyke (1939a) and (1939b) used the thiochrome method for the assay of foodstuffs and Westenbrink and Goutsmit (1938) adapted it to the determination of thiamine in urine and cereals. The Tauber reaction (1937) uses the color reaction between thiamine and p-dimethyl aminobenzaldehyde in the presence of acetic acid. The production of an orange-red precipitate by thiamine and bismuth potassium iodide was suggested as a color test by Naiman (1937). Raybin (1938) reported that thiamine will react with two, six dibromoquinone chloroimide to give an orange compound which is readily extractable by immiscible solvents.

The growth of microorganism for assay purposes has received attention for many years. A rapid test is made possible by the

short duration of the life cycle of these organisms. Williams and Spies (1938) stated that although such tests are valuable for certain purposes they require rigorous proof of specificity before they can be trusted for general purposes. Williams (1919) noted that the growth of yeast was increased by extracts of antineuritic foods. The catatorulin test of Passmore, Peters and Sinclair (1933) measures in vitro, the oxygen uptake of avitaminous birds' brains. The stimulating effect of thiamine on certain yeasts was used by Williams (1937) to detect as little as one gamma of thiamine. Cachoa and Peters (1938) originated the cocarboxylase test, measuring the amount of CO_2 evolved when pyruvic acid is decarboxylated by alkaline washed yeast in the presence of cocarboxylase. A method for the quantitative estimation of thiamine in the blood by the use of the mould, Phycomyces Blakesleeanus, was presented by Meiklejohn (1937) who found that within limits, the growth of the mould is proportional to the concentration of thiamine.

A large number of assays on a wide variety of animal and vegetable tissues have been made. Daniels and Munsell (1937) have expressed the thiamine content of dietary foods in terms of International Units, while the work of Booher and Hartzler (1939) is expressed in terms of crystalline thiamine. Williams and Spies (1938) have summarized work done on the thiamine content of some animal tissues by the rat growth, bradycardia, pigeon protective and chick growth methods. They state:

> Thiamine is of nearly universal occurrence in quantities usually ranging from 0.1 to 2.0 gamma per gram. These proportions are exceeded in the plant world only in seeds and in yeast grown in rich media. Among the muscular tissues of animals so far as is known, pork is outstanding. In other species the animal organs, liver, heart, and kidney contain somewhat higher concentrations than the body at large.

Hog muscle was investigated as a source of the antineuritic vitamin by Hoagland (1923) who estimated that five per cent in the ration fully protected pigeons from polyneuritis. Ten years later, Plimmer, Raymond and Lowndes (1933) in England, using the pigeon preventive assay, compared the vitamin B_1 content of fresh animal foodstuffs with that of dried yeast as a standard, ranking them lower than cereals but higher than fruits and vegetables. Improvement in assay methods contributed to a more accurate measurement of the vitamin, resulting in even higher thiamine values for pork than was indicated by early work. This fact gained wider recognition through the work of Elvehjem and his group. Using the chick growth method, Elvehjem, Sherman and Arnold (1935) assayed pork muscle, kidney, heart, liver and lung for vitamin B_1. The values ranged from 19.8 mcg per g in dried pork muscle, down to four micrograms per gram of dried lung tissue. Reports from the same laboratory by Mickelson, Waisman and Elvehjem (1939) showed that a diet composed of seven per cent meat can furnish a considerable fraction of the daily thiamine requirement. Additional experiments by Waisman and Elvehjem (1941) using the rat growth method of assay, estimated that although both ham and loin cuts varied in vitamin content, both contained large amount of thiamine. Six different ham samples showed ranges of

33 to 78 mcg per g of dried material. Eight samples of pork loin were assayed and three samples contained 39 mcg per g. Five others ranged from 48 to 78 mcg per g. The muscles of other species contained approximately one-third to one-fourth the thiamine content of pork. In England, Pyke (1940) studied the thiamine content of the skeletal muscles of 17 species of vertebrates by the thiochrome method and reported values from 170 to 510 I.U. per 100 gram of fresh pork, which is 5.1 to 15.3 mcg per g of fresh tissue.

The effects of cooking, storage and commercial processing were investigated in various laboratories. Christensen, Latzke and Hopper (1936) found that dried lean pork contained 36 mcg per g and that losses in cooking were 12 per cent and those in autoclaving 21 per cent. Meat canned under vacuum and stored for two years was found by Arnold and Elvehjem (1938a) to retain 80 per cent of its thiamine. A destruction of 70 to 80 per cent was found by the same authors (1939) in pork subjected to commercial processing for one hour and 50 minutes at a temperature of 121° C. Retention after cooking was reported by Aughey and Daniels (1940) to be 57 per cent for roast loin and 85 per cent for braised loin. McIntire and associates (1943) found a wide variation in the thiamine content of different pork carcasses. After cooking, considerable thiamine was found in the drippings, particularly from braised loin cuts. The average retention of thiamine in the meat was 70 per cent after roasting and 50 per cent after braising. Schweigert and co-workers (1943) showed that after roasting the average retention in meat and drippings

was 70 per cent. After frying, the average retention in meat and drippings was 92 per cent. The drippings alone contained 10 per cent of the thiamine in each sample. Reedman and Buckley (1943) in Canada, investigated meat processed for the Canadian Army and found losses of 41 per cent of the original vitamin. Synthetic thiamine added to the meat before processing showed no greater destruction in processing than did the natural thiamine. The great variation sometimes found in the thiamine content of different pork carcasses was studied by Miller and associates (1943) who were able to show that the thiamine content of the pork may be influenced by the amount of thiamine in the rations of the animal.

It is now well established that pork furnishes an excellent source of thiamine. In view of the fact that the actual thiamine content of the tissue is variable even for different muscles in the same carcass, additional data will be of value.

PROCEDURE

The rat curative procedure of Kline, Tolle and Nelson (1938) as adopted by the United States Pharmacopeia (1939) was used with only minor variations for this test. Normal white rats, not over 30 days of age and weighing between 40 and 50 g, were placed in individual cages on wire screens of one-half inch mesh to prevent coprophagy. A thiamine free basal diet and water were supplied at all times. The basal diet, which was mixed each week, consisted of the following ingredients:

	per cent
Cane sugar.....	61.25
Vitamin free casein.....	18.00
Autoclaved peanuts.....	10.00
Autoclaved yeast.....	4.00
Osborne and Mendel salt mixture.....	4.00
Codliver oil.....	2.00
Purified liver extract.....	0.75

In order to destroy the thiamine, the Brewer's yeast was spread in shallow pans, in layers not more than one-fourth inch thick, autoclaved for six hours at 15 pounds pressure and dried at a temperature of not more than 65° C. After drying it was ground to almost its original fineness in a burr mill and stored in tight containers away from the light.

No. 1 raw shelled peanuts were ground until fine but not plastic, spread in layers one-fourth inch thick, in shallow pans, and autoclaved for six hours at 15 pounds pressure. They were air dried and stored in the refrigerator.

Commercial vitamin free casein was used and Wesson's modification of the Osborne and Mendel salt mixture (1932) was prepared in the laboratory.

Commercial liver extract was made thiamine free by dissolving in water and precipitating with ethyl alcohol and ether. A solution of the precipitate in water was dried on casein, air dried until free from ether and oven dried at a temperature of 65° C. until suitable for grinding and storing.

During the first part of the depletion period the rats were weighed weekly. When growth ceased they were weighed daily. The approach of polyneuritis was detected by such early symptoms as roughening of the hair, continued loss of energy, steadily declin- weight, increased lack of control of the hind quarters and a tendency to cling tightly to the cage and to the worker's hands. At the appearance of these symptoms, between the third and fourth week of the test, the rats were twirled daily by the tail. When the rat developed polyneuritis it responded to this treatment by a characteristic loss of equilibrium and lack of coordination. These symptoms did not always appear at the same time, the average time being from the 28th to the 35th day of the depletion period, neither did they show in all rats in the same manner. Some had tremors, with the rat prostrate on its stomach, eyes closed and legs extended. Others rolled rapidly over and over with a barrel-like motion, while some ran in continuous circles. In rare cases head retractions occurred. Three stages of polyneuritis were noted, the slight, the acute and the severe. The acute stage was used for this test. In the slight stage, recovery from loss of equilibrium was completed immediately. Recovery from the acute stage occurred within a few seconds, while in the severe cases continuous uncontrolled movements resulted and often appeared before spinning.

When the acute stage was reached, the assay period began with the administration of six micrograms of thiamine and the duration of the curative period was recorded. A stock solution of standard thiamine was prepared by weighing 30 milligrams of

crystalline thiamine and dissolving it in 50 ml. of 25 per cent ethyl alcohol made 5/100 N. with HCL. This solution and all dilutions were kept in the refrigerator. A 1-10 dilution was made each week and fed to the rats by mouth with a 0.5 ml. tuberculosis syringe. Each 0.1 ml. dose contained six micrograms of thiamine.

At the reappearance of acute symptoms, those rats, showing a curative period of not less than five days and not more than nine days, were fed a weighed sample of the pork to be assayed, and the duration of that curative period was also recorded. It was then possible to make a comparison of the length of the curative period produced by the six micrograms of thiamine with that produced by the sample of pork.

Twelve to 16 animals were used for each assay level and three or more levels were fed until that amount containing approximately six micrograms of thiamine was found. The various levels used were weighed on the balance into small glass jars, tightly covered and stored in the freezing compartment of the refrigerator. They were fed to the animals as soon as possible after weighing. Great care was taken to avoid loss of meat during feeding.

The meat used was obtained from the Department of Animal Husbandry, Kansas State College. It had been stored in a freezer locker and was thawed before grinding or cooking. Paired cuts from the same animal were used. Meat used for the raw samples was prepared by removing the outside fat and the bones. The cooked samples were braised or roasted before removing the bones and outside fat. The remaining lean meat from both the raw and

the cooked samples was ground three times through a household food chopper and carefully mixed during each grinding period. Cooking methods employed were those recommended by the Committee on Preparation Factors, National Cooperative Meat Investigations. An electric oven, heated to a temperature of 350° F. was used for roasting. The meat was cooked in a tin pan 7½ by 7½ by two inches until an internal temperature of 180° F. was reached. The chops were braised in a preheated aluminum skillet and were turned after 1½ minutes, and again at the end of 5½ minutes. After cooking seven minutes, a thermometer was placed in the chop, 15 ml. of water were added and the skillet closely covered. The chops were simmered until they showed an internal temperature of 180° F.

DISCUSSION OF RESULTS

Both a raw and a cooked portion of four samples of pork chops and three samples of pork loins were assayed. Data used to calculate the micrograms of thiamine per gram of meat were recorded as in Tables 1 to 6. For each assay level, a comparison was made between the average duration of cure resulting from six micrograms of standard thiamine and that from the weighed samples of meat. When these periods were equal, it was an indication that the meat contained six micrograms of thiamine. Therefore the amount of the vitamin in one gram of pork could be calculated from these data by simple proportion.

The most satisfactory comparison resulted when the average curative period from the standard, equaled the average curative period from the test material. Data in Table 2, furnish an ex-

Table 1. Curative response of thiamine deficient rats to 6.0 mcg thiamine and 0.8 g cooked loin No. 103.

Rat no.	Standard 6.0 mcg		Meat 0.8 g	
	Wt. g	Days	Wt. g	Days
2317 f	10	5	7	6
2326 m	6	6	4	3
2327 m	8	5	2	11
2344 f	7	5	9	7
2347 m	4	5	4	8
2348 f	5	7	4	3
2350 m	7	6	11	7
2357 m	5	5	3	3
2358 f	8	7	5	8
2368 f	10	6	5	2
2370 f	11	8	4	2
2380 m	8	6	8	4
2381 m	8	6	7	3
2382 m	4	5	4	3
2383 f	6	5	5	10
2378 m	7	7	9	7
Total	114	94	91	87
Ave.	7.1	5.8	5.6	5.4

Table 2. Curative response of thiamine deficient rats to 6.0 mcg thiamine and 1.0 g cooked loin No. 103.

Rat no.	Standard 6.0 mcg		Meat 1.0 g	
	Wt. g	Days	Wt. g	Days
2345 m	5	5	8	7
2348 f	6	5	7	6
2487 m	0	5	3	3
2549 m	7	5	9	7
2556 m	8	7	4	3
2561 f	4	5	6	6
2566 f	1	5	5	10
2567 m	5	5	3	4
2568 f	3	5	6	7
2570 f	4	5	3	4
2583 f	7	5	6	3
2593 m	6	5	7	5
2596 f	6	5	4	3
2604 m	4	6	3	3
2607 f	5	5	9	7
Total	71	78	83	78
Ave.	4.7	5.2	5.5	5.2

Table 3. Curative response of thiamine deficient rats to 6.0 mcg thiamine and 1.5 g of cooked loin No. 103.

Rat no.	Standard 6.0 mcg		Meat 1.5 g	
	Wt. g	Days	Wt. g	Days
2326 f	4	5	10	8
2330 m	4	6	8	12
2338 f	5	5	6	11
2341 f	3	9	6	12
2347 m	2	6	7	9
2348 f	5	7	10	10
2352 f	5	5	8	15
2357 m	10	6	10	11
2361 f	6	5	9	9
2365 f	6	6	10	10
2369 m	5	5	0	5
2370 f	6	5	10	11
2372 f	4	5	8	10
2377 m	9	5	6	10
2373 f	5	9	8	9
Total	79	89	116	152
Ave.	5.2	5.9	7.7	10.1

Table 4. Curative response of thiamine deficient rats to 6.0 mcg thiamine and 0.8 g of raw loin No. 101.

Rat no.	Standard 6.0 mcg		Meat 0.8 g	
	Wt. g	Days	Wt. g	Days
2330 m	7	7	6	6
2333 f	7	8	6	8
2336 m	9	9	5	9
2338 f	6	9	6	6
2339 f	4	9	8	9
2340 f	6	6	8	8
2341 f	6	8	5	7
2363 f	7	8	5	5
2374 f	7	8	5	5
2377 m	6	6	2	5
2380 m	8	7	0	2
2381 m	8	7	7	3
2373 f	5	5	5	3
2376 m	6	5	8	7
2379 m	8	9	6	8
Total	98	112	81	94
Ave.	6.5	7.4	5.4	6.2

Table 5. Curative response of thiamine deficient rats to 6.0 mcg thiamine and 1.0 g of raw loin No. 101.

Rat no.	Standard 6.0 mcg		Meat 1.0 g	
	Wt. g	Days	Wt. g	Days
2319 f	4	5	7	6
2338 f	6	6	9	13
2341 f	5	6	6	9
2345 f	6	5	7	7
2348 f	7	7	7	5
2349 m	4	5	3	6
2352 f	5	6	6	10
2357 m	8	7	5	5
2358 f	7	6	6	6
2379 m	5	5	7	6
2382 f	6	6	8	6
2383 f	2	5	3	6
2462 f	3	6	5	9
2459 f	5	7	2	3
2461 f	5	5	5	7
2373 f	6	8	0	2
Total	84	95	86	106
Ave.	5.2	5.9	5.3	6.6

Table 6. Curative response of thiamine deficient rats to 6.0 mcg thiamine and 1.2 g of raw loin No. 101.

Rat no.	Standard 6.0 mcg		Meat 1.2 g	
	Wt. g	Days	Wt. g	Days
2318 f	7	6	7	6
2333 f	5	5	11	13
2340 f	5	6	8	9
2343 f	6	6	6	7
2344 f	7	6	7	7
2345 f	6	5	8	11
2349 m	5	5	5	13
2358 f	6	5	2	11
2360 m	11	5	8	6
2361 f	9	6	7	6
2362 f	7	5	8	7
2368 f	7	7	6	6
2373 f	5	5	6	7
2375 m	2	5	7	9
2379 m	6	5	7	14
2381 m	8	5	8	5
Total	102	87	111	137
Ave.	6.3	5.4	6.9	8.9

ample of such a comparison. One gram of roast loin No. 103, and six micrograms of thiamine, both resulted in an average curative period of 5.2 days. In an effort to find the correct level of pork, two other weighed portions had been fed. It will be noted in Table 1 that 0.8 grams of meat resulted in an average curative period less than that of the standard dose, while 1.5 grams of the pork, as shown in Table 3, produced a curative period greater than that of six micrograms of thiamine.

However, it was not always possible to achieve perfect results. If all the weighed samples had been fed and the results showed the average curative periods to be unequal, the correct amount of pork containing six micrograms of thiamine was determined from the data recorded by calculation as proposed by Birch and Harris (1934). Coward (1938) agreed with their suggestion, that since a straight line relationship exists between dose and duration of cure of polyneuritis, simple proportion may be used to calculate the correct amount of the assay material which would contain six micrograms of thiamine. Tables 4, 5 and 6 compare the curative periods from pork and thiamine for three different levels of raw loin No. 101. No two curative periods were equal. In Table 5, one gram of the pork resulted in an average curative period of 6.6 days, while that of six micrograms of thiamine was 5.9 days. Table 6 shows the average duration of cure resulting from 1.2 grams of the sample to be 8.9 days, while that of the standard was 5.4 days. Table 4 shows a curative response to the pork of 6.2 and that of the standard dose 7.4 days. Since no two curative periods were equal the correct level con-

taining six micrograms of thiamine was calculated by simple proportion from the data recorded to be 0.9 g.

Results of all assays made, expressed in micrograms per gram of thiamine were recorded in Table 7. The thiamine content of raw loins No. 117, 101 and 107 were five, 6.6 and 6.6 micrograms per gram. After roasting the corresponding samples No. 119, 103 and 105 contained four, six and 5.4 micrograms per gram respectively. Thiamine retention after roasting was 80, 90 and 81 per cent. Therefore the average retention of thiamine in the roasted pork loins was 83 per cent.

Four samples of raw and braised pork chops were assayed. The thiamine content of raw chop No. 85 was four micrograms per gram. The corresponding braised chop No. 86 contained 3.3 micrograms per gram, showing a retention of 82 per cent of the original vitamin. Chop No. 125 contained 6.6 micrograms per gram while braised chop No. 126 showed five micrograms per gram of thiamine and a retention of 75 per cent. In the other two chops, the thiamine content was reduced from 3.7 to 3.3 in samples No. 155 and 154 and from five to 4.6 micrograms per gram in samples No. 156 and 157, showing a retention of 89 and 92 per cent respectively. The average retention was 84 per cent for the four pork chops.

The thiamine content of the three loin cuts and the four pork chops assayed, ranged from 3.7 to 6.6 micrograms per gram of the raw meat. These figures correspond to the lower range of thiamine values reported in the literature. Pyke (1940) found values from 5.1 to 15.3 micrograms per gram of fresh muscle. Similar

Table 7. Thiamine content of paired pork loins and chops, showing thiamine retention after braising and roasting.

Pork	Samples no.	Cooking process	Mcg/g thiamine	Per cent thiamine retention
Loin	117	None	5.0	
"	119	Roasted	4.0	80
Loin	101	None	6.6	
"	103	Roasted	6.0	90
Loin	107	None	6.6	
"	105	Roasted	5.4	81
Average				83
Chop	85	None	4.0	
"	86	Braised	3.3	82
Chop	125	None	6.6	
"	126	Braised	5.0	75
Chop	155	None	3.7	
"	154	Braised	3.3	89
Chop	156	None	5.0	
"	157	Braised	4.6	92
Average				84

results were obtained by Waisman and Elvehjem (1941) who assayed nine fresh pork loins and reported a range from 11.1 to 18.7. McIntire and associated (1943) showed that fresh pork loins contained from 7.4 to 15.2 mcg per gram.

The braised pork chops showed an average retention of 84 per cent, which agrees with similar work by Aughey and Daniels (1940) who reported a retention of 85 per cent. However, it is much higher than a retention of 50 per cent in the braised loin assayed by McIntire and associates (1943). The average retention of

thiamine was 83 per cent for loin roasts and 84 per cent for the braised pork chops. Therefore the results of this study indicate little difference between braising and roasting in thiamine retention. McIntire and associates (1943) reported that although there was only slight variation in the total vitamin destruction in braising, roasting or broiling, more thiamine was found in the drippings after braising.

A rather wide variation in the per cent of thiamine retained, was shown in this investigation. Pork chop No. 126 retained 75 per cent of its thiamine when braised, while No. 157 retained 92 per cent. Waisman and Elvehjem (1941) reported a similar variation in fried pork chops, and stated that there was as yet no explanation why one chop showed a thiamine retention of 47 per cent and another had retained 65 per cent of its original vitamin.

SUMMARY

1. Thiamine assays were made by the rat curative procedure on seven cuts of pork loin, to determine the thiamine content of the raw meat and losses in cooking resulting from braising and roasting. Paired cuts from the same animal were used for the raw and cooked samples.

2. The thiamine content of the raw pork loin ranged from 3.7 to 6.6 micrograms per gram. The braised chops ranged from 3.3 to 5.0 micrograms per gram while the loin roasts contained from four to six micrograms of thiamine per gram of meat.

3. The average retention of thiamine after roasting was 83 per cent and after braising it was 84 per cent.

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