THEESIS

on

POSSIBLE SOURCES OF CALCIUM AND PHOSPHORUS

IN THE CHINESE DIET

THE DETERMINATION OF CALCIUM AND PHOSPHORUS IN A

TYPICAL CHINESE DISH, CONTAINING MEAT AND BONE

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POSSIBLE SOURCES OF CALCIUM AND PHOSPHORUS IN THE CHINESE DIET

THE DETERMINATION OF CALCIUM AND PHOSPHORUS IN A

TYPICAL CHINESE DISH CONTAINING MEAT AND BONE

INTRODUCTION

That milk is used only for infants and invalids is not merely a prejudice but a dietary practice quite universal among the Chinese. The absence of milk and dairy products in the native Chinese diet has drawn continuous interest because of the sharp contrast in the diet of the occidental world where milk is used freely. The unique place which milk holds in furnishing an abundant supply of calcium, a good source of phosphorus and these elements in favorable ratio for utilization, has led to an inquiry regarding the source of calcium, particularly in the Chinese diet.

There are several food articles in the Chinese diet which might be assumed to be an answer to such a question.

The liberal supply of leafy green vegetables throughout the year in South China may meet the demand to a certain extent. Unfortunately data regarding the availability of minerals in vegetables are contradictory, thus leaving the subject in uncertainty. Blathwick and Long (1) found that calcium and phosphorus derived from vegetables were capable of meeting maintenance needs of man, whereas Sherman and Hawley (2) had evidence to show that children do not seem to utilize the calcium of vegetables as efficiently as they do that of milk. Their experiments have shown that more variable and always less favorable results were obtained when vegetables
replaced about half of the milk as a source of calcium.

Soybean and its various products have been found high in calcium and phosphorus content. At present no definite information can be found regarding their utilization as a main source of supply of these elements. However, Pittman (3) has found that even an intake of four ounces dry weight of navy beans daily was unable to maintain calcium balance. This may also be the case with soybeans.

In addition to vegetables, the common Chinese diet includes fowl, pork and fish as frequently as the American diet includes cheese and milk. Recognizing the low content of calcium in meat in contrast to milk, the question arises as to the possible availability of calcium from the bone of such meat dishes. The method of preparation and of eating may prove to be significant factors in the amount of calcium obtained from such foods.

A commonly used cheap dish of sweet and sour pork spareribs may serve as an illustration. Spareribs are cut up in 1 1/2 inch pieces, slightly browned and cooked slowly until very tender with a large amount of diluted Chinese vinegar, soybean sauce and sugar to taste. The excess liquid is thickened with starch before serving. Due to the long period of cooking, the solution penetrates the soft bone marrow. It is a satisfaction for those who dine with the family to suck and chew the small pieces of bone while eating them.

A similar dish but with a large proportion of bones, usually pig's feet, ginger and higher concentration of vinegar is prepared for the lactating mother as a main dish, in three or four meals a
a day, together with eggs, a small amount of vegetables and occasionally other meats as supplements. This diet is started right after childbirth and lasts 30 to 40 days. It is generally believed that this is good for milk production, blood regeneration, and general vitality.

Such a time-honored custom, while it appears to lack any scientific support, seems worth investigation to determine the actual amount of calcium and phosphorus obtained from the consumption of such a combination of meat-bone and sauce.

The chemical analysis of such a dish holds a two-fold purpose. First, to determine quantitatively the calcium and phosphorus and the distribution in the various ingredients. Second, to acquire the technique in the chemical analysis of these elements in order to make similar studies of other Chinese food products together with their different methods of preparation in which these inorganic substances of the total ash content have not been considered.

Due to time limit, the author was unable to determine the forms of calcium and phosphorus obtained from such a dish, nor was she able to study the physiological significance involving absorption and utilization in the human and animal species. However, a brief review of the literature concerning the latter is made as an aid in the interpretation and discussion of the result obtained in this study.
Both calcium and phosphorus exist in the human body, constituting a larger proportion of the body weight than any other inorganic elements. The percentage in average human body is 1.5 per cent for calcium and 1 per cent for phosphorus. It is estimated that about 85 per cent of the mineral matter of bone, or at least three-fourths of the entire ash of the body, consists of calcium phosphates. Probably over 99 per cent of the calcium and 70 per cent of the phosphorus in the body belong to the bones and teeth. The remainder of the calcium occurs as an essential constituent of the soft tissues and body fluids (4).

Calcium and phosphorus are also found in teeth in form similar to that of the bone. It differs from bone in having a higher phosphorus content and different organic matrix (5).

Aside from being an essential constituent in the body, calcium and phosphorus serve as physiological regulators in the system of living organisms.

The calcium ion is necessary in the clotting of blood. Clotting is due essentially to the conversion of the soluble protein fibrinogen into the insoluble protein fibrin. The theories concerning the mechanisms controlling such a process are various and conflicting. However, they all agree that the presence of calcium is necessary.

Equally important is the function of calcium salts in the regulation of the muscular action of the heart. Leob (6), the world
known physiologist, performed the classical experiment showing the effect of various salts, including calcium, on heart action. Contractility and irritability disappeared in the absence of these salts but these functions reappeared when the salts were added to the solution in which the heart was placed. With the proper balance of sodium and potassium, calcium salts play an important role in maintaining normal heart activity.

Elements as closely related chemically as sodium and potassium or calcium and magnesium are in some of their functions directly antagonistic in their actions and influence the acid-base balance in the body (4). Meltzer and his associates consider calcium to be the most important regulative element among all inorganic constituents. He states, "Calcium is capable of correcting the disturbances of the inorganic equilibrium in the animal body, whatever the directions of the deviation from the normal may be. Any abnormal effect which sodium, potassium or magnesium may produce, whether the abnormality be in the direction of increased irritability or of decreased irritability, calcium is capable of reestablishing the normal equilibrium."

In metabolism, calcium is a sparer of iron. The organism has a more favorable utilization of even a small quantity of iron if calcium is abundant.

Sjollema (?) found that proper amounts of calcium and phosphorus in the diet increase the reproductive power. Sherman and Booher (8) believe that a higher calcium content of the diet
permits not only earlier maturity but also extends the period between maturity and senescence.

Parallel to calcium, phosphorus has its role in physiological significance. In the living organism phosphorus is used in maintaining the acid-base balance of the blood and in the synthesis of such important cell constituents as phospholipids and nucleoproteins. Phospholipids are essential components of every cell. Bloom (9) found that the more active the tissue the higher its percentage of phospholipids. Brain tissue leads in rank, then the heart, liver, pancreas and kidneys follow, while muscle tissue has the least. Nucleoproteins, as constituents of cell nuclei, are fundamental in the control of the life of the cell.

However, in this study, special emphasis is made on phosphorus in relation to calcium and its functions in the body as such a compound.

**Calcium and Phosphorus Metabolism**

As an important factor in the physiological significance of calcium and phosphorus, the metabolic processes involved in absorption and the utilization of these elements deserve detailed consideration.

**Intake**

Both calcium and phosphorus are taken in orally from natural foods regardless of the forms in which they occur. All kinds of foods contain a certain amount of these elements. Meyer (10) had found even water has some physiological available calcium.
He states that "The percentage of young men fitted for military service is greater in a calcareous region than in other places. A smaller incidence of diseased teeth and a greater proportion of persons with healthier teeth are found in areas where hard, palatable water is used."

Steenbock (11) and his associates have found that calcium in all forms of salt are available to a considerable degree for growth when larger quantities are fed. However, Howe (12) believes that all minerals from inorganic sources can be assimilated only when such minerals are deficient in the body.

**Absorption**

Calcium absorption takes place in the small intestines. The actual amount absorbed is measured by the difference of the daily total intake and the total excreted. The degree of absorption is governed by two main factors, the hydrogen ion concentration within the digestive tract and the relative proportion of other substances in the diet (13). While many authorities believe that vitamin D exerts some effect on calcium absorption, the specific action is still under discussion. If a liberal amount of both calcium and phosphorus are fed, the significance of the ratio of these elements declines in importance.

**Hydrogen Ion Concentration** - Solubility is essential for absorption. Within the digestive tract salts are absorbed in proportion to their relative solubilities in the surrounding media. Calcium salts are found insoluble in alkaline but soluble in acid
solutions at the pH range of 3 to 6.9.

Orr (14) and his coworkers (1924) found that only calcium ions and soluble primary phosphates in solution can pass through the intestinal mucosa. The insoluble forms of calcium phosphates cannot be absorbed. They believed the increase of acidity of the intestinal contents is a favorable factor for the transformation of insoluble secondary calcium phosphates to the soluble primary form, thus promoting the absorption. Kahn (15) and Roe (1926) concluded that calcium can be adequately absorbed even in forms of inorganic salts if they are administered in the interdigestive period when the intestinal reaction is the least alkaline. The failure of absorption, they believed, is due to the improper hydrogen ion concentration for the solubility of calcium. The forms of calcium ingested are less important.

However, Hart, Steenbock and others (16) in their later (1931) experiments on lactating cows, failed to obtain any increase of calcium absorption when 46 to 92 grams of hydrochloric acid were administered with moistened hay for periods of two weeks. Shohl, Bennet and Weed (17) had found, in their rat experiment, that the hydrogen ion concentration had no relationship to the absorption of calcium in food.

Evidently other factors than hydrogen ion concentration must play a part in the absorption of calcium and phosphorus.

**Dietary Factors Affecting Absorption** - All food substances exert some influence on calcium and phosphorus metabolism. However,
they do not seem to favor the absorption of these elements in
the body to the same degree.

Carbohydrates - "Different carbohydrates, sucrose, glucose, starch, dextrin and lactose, added to the diet in large quantities
have decidedly different effects on the absorption of calcium and phosphorus." (18)

Bergeim (19) reported in 1926 in experiments on rats
that 25 to 50 per cent of sucrose or glucose or even maltose and
starch decreased absorption of these minerals. Addition of lactose, on the other hand, showed marked increase of both calcium and phosphorus absorption. According to Bergeim, the favorable result of lactose is due to the formation of lactic acid in the intestinal tract, thus increasing the solubility of calcium phosphates in the acid medium.

Robinson and his associates (20) had a similar result on their experiments of cows. They concluded that lactose or lactate ions themselves exert specific action in facilitating passage of calcium into the blood stream independently of hydrogen ion concentration. Robinson and Duncan (21) recently have been able to show from the measurement of the pH of the intestinal content of rats, that the change to an acid reaction due to lactose feeding occurs only in the lower parts of the alimentary tract where very little or no absorption takes place.

A still more recent (1932) study, that of Kline, Keenan, Elvehjem and Hart (22) has demonstrated that a level of 40 per cent lactose in a rachitic ration had a very favorable effect upon
calcium absorption and skeleton building, also lactose exerted a positive influence in maintaining the acid reaction along the entire length of the intestinal tract. The addition of 40% maltose or 5% citric acid had no effect whatever on calcium absorption or intestinal reaction.

Proteins - Very little can be stated in regard to the relationship of dietary proteins and calcium and phosphorus absorption. Hess (23) agrees with Meyer, who reported some years ago that calcium retention was retarded by adding casein to the diet. He points out that "such a conclusion would fit in well both with biological tests carried out on rats fed a low phosphorus ration and with clinical experience with a diet rich in casein." On the contrary, Sjollema (7) in an experiment on rabbits, found that a high protein diet decreases the fecal calcium output. In serum, Peters and Eiserson (24) have found that the concentration of calcium varies directly with the concentration of protein and inversely with phosphorus.

Fat - The influence of dietary fat on calcium soaps formation and calcium absorption in the intestine has received considerable study with somewhat conflicting results. Earlier (25) authorities all agreed that an excess of fat in the diet interfered with the calcium absorption through the formation of calcium soap which were excreted in the stools. More recently careful studies have been made to indicate that a liberal amount of fat in diet does favor a better assimilation of calcium.
Telfer (26) in 1921 performed an experiment on the influence of free fatty acids on calcium and phosphorus retention. A diet of milk containing 3 per cent fat was given to a normal infant and three other subjects whose bile had been partially or completely excluded from the gut. He found that from the same amount of fat intake, after complete removal of the bile, these subjects excreted far more calcium soaps and acid phosphate than the normal infant. In the normal subject the higher the percentage of fat fed, the lower the percentage of calcium and phosphorus excreted. This study indicates that the formation of calcium soaps are not due to the amount of fat ingested but more exactly to the secretion of bile by which fat is digested. He points out that "In the presence of gastric juice the greater part of calcium and phosphorus pass into solution. No precipitation of these elements in insoluble form is possible in the intestine owing to the presence of the free acid."

Boyd, Crum and Lyman (27) in 1930 studied the utilization of calcium soaps in normal rats which were fed with a synthetic calcium free diet plus pure calcium soaps made from various fats. He found that the percentage of absorption depends less on the amount but more on the nature of the calcium soaps ingested. Calcium oleate has a greater solubility in bile secretion, thus enabling a greater absorption than the calcium palmitate or calcium stearate. However, all these calcium compounds found in the intestine are well utilized.

In 1932, these same investigators (28) again found that dietary fats favor calcium absorption even taken in the forms
of salts. The retention of calcium chloride was 20.8 milligrams in a fat free diet as compared to 29.3 milligrams in the diet containing 10 per cent of fat in the ration.

According to the later results, it seems safe then to conclude that dietary fats, when properly oxidized, not only exert no harmful effects but even favor calcium retention.

**Acid and Base Forming Foods** - While higher acidity in the intestine is believed to be more favorable for the solubility of calcium phosphates and calcium soaps, the question arises concerning the influence of foods which, being metabolized, yield potential acidity or alkalinity. Bogert and Kilpatrick (29) in 1932 believe base forming foods favor better calcium absorption than the acid forming foods.

Greater calcium absorption was obtained by the addition of orange juice to an orphanage diet, which previously gave an acid residue and was low in vitamins. The amount absorbed was greater than the amount added from the orange. Channey and Blunt (30) believe that this result is due to the basic residue of the orange juice resulting from oxidation. Such potential alkalinity may change the acid residue of the high cereal diet to a nearly neutral one. The initial acidity of the orange juice, before oxidation, may also help to keep an acid condition in the upper intestine and hence to increase the solubility of calcium phosphates.

In a more recent (1932) experiment by Shohl et al (31) they find that when the calcium and phosphorus ratio is high, the
acid producing diet tends to produce a "light healing type" of ricket while neutral and basic diets gave no signs of abnormality. The closer the calcium and phosphorus ratio becomes to 1 : 1 or 2 : 1, the less the importance of the dietary reaction.

As to the opinion concerning oxalic acid in many fruits and vegetables, particularly pineapple and spinach, some authorities have reported that this acid combines with calcium to form calcium oxalate, which is not completely oxidized. According to McLaughlin's (32) finding in the utilization of calcium from spinach, her subjects (adults) obtained about 2 grams of oxalic acid daily, yet gave no evidence of digestive disturbances or ill health, and the calcium retention was noticeable.

Cellulose - Sjollema (7) from his experiments on the adult rabbit was able to find a corresponding increase of fecal calcium with a gradual increase from 3 to 15 per cent of roughage in the diet. In a prolonged period of high roughage, even when the calcium intake was low, there was a large percent of calcium in the feces. In some cases the calcium output was three times greater than the amount present in the food, indicating a waste of considerable amount of calcium.

Hartman and Meigs (33) in their long time experiment on cows, have found that roughage does not interfere with calcium absorption.

While there are contradictory results in these investigations, later (1931) experiments of Ascham (34) using cellulose
flour or agar agar in the diet for dogs, fails to obtain enough evidence to draw definite conclusions. Thus, the question of crude fiber being an influencing factor on calcium retention is still a matter for further investigation.

Inorganic Salts - As Sherman has stated that some inorganic elements, even if they are chemically closely related, are antagonistic in their actions. Chemically, magnesium resembles calcium in many respects. In the earlier studies these two elements were either considered together or the magnesium neglected. More reliable evidences have been found by McCrudden (23), and later by Schloss and Casemann that rachitic bones contained more magnesium and less calcium than the normal, indicating the antagonistic action of the two elements. Hess (23) quotes the result of Schueler that calcium and magnesium have a tendency to go in parallel rather than in an opposite direction. The fact that in four cases of severe rickets, where the calcium balance was negative, the same was true for magnesium. This opposes the theory of a replacement of the losses of calcium in the bone by a deposition of magnesium. Mendel and Benedict (5) noted that an increased excretion of magnesium in rats could be induced by the administration of calcium, and that an increased elimination of calcium could be brought about by the administration of magnesium. Similar results have been shown to hold for men by Bogert and McKittrick (35) (1922)

Again, Hart (36) and Steenbock (1913) and also Haag (37) and Palmer (1928) have found that the addition of magnesium
salts to an otherwise well-balanced ration tends to cause a loss of
calcium from the body.

Evidently there are reasons enough to believe that
magnesium has antagonistic action toward calcium absorption.

**Fluorine** - Among other so-called minor minerals, fluorine
has been found, by several authorities, to produce a detrimental
effect on calcium metabolism. Earlier literature has mentioned that
the feeding of animals with commercial rock phosphates as a source of
calcium and phosphorus, resulted in a disturbance in health with
faulty bones and teeth. McClure and Mitchell (38) have performed
experiments on feeding rats with certain percentage of sodium
fluoride and calcium fluoride in the ration. They found that when
as little as 0.03 per cent of sodium fluoride was added in the ration,
growth was inhibited. When the percentage was increased to 0.06
per cent, the effect was marked. Subnormal growth, lower calcium
absorption and poor bones and teeth were the results. The insoluble
calcium fluoride as well as the soluble sodium salts produced the
same effect.

Smith and Smith (39) later in their clinical
observations have found that mottled enamel, a disease of human
teeth, was due to the high percentage of fluoride in drinking water.

**Calcium and Phosphorus Ratio** - Daniels (40) states that
"optimum development would seem to demand the retention of optimum
amount of calcium and phosphorus in the ratio of approximately two
parts of calcium to one part of phosphorus." Based on the result of
the mineral metabolism of children, Orr, Holt and others (14) conclude: "Excessive amounts of calcium in the diet tend to increase the total absorption and retention of calcium but tend to impair phosphorus retention. Excessive amounts of phosphorus in the diet exercise an unfavorable influence on the calcium metabolism and are accompanied by an increase in the calcium loss in the feces". This antagonistic action, as they believe, can only be explained by the formation of insoluble phosphates of calcium which cannot be absorbed.

Lately (1932) Bethke and associates (41) have performed experiments on the effect of calcium and phosphorus relationship on growth, calcification and blood composition of rats. They find that the calcium and phosphorus ratio has a greater importance than the amount of these elements in the ration. The wider the ratio the greater the demand of vitamin D for the absorption of available amounts of these elements for biological function, calcification and growth. A calcium and phosphorus ratio of two to one is found to require the least amount of vitamin D.

At the same time, Brown, Shoel and coworkers (42) from their chemical and histological studies on rachitic rats, concluded that for the same calcium and phosphorus ratio, the higher the amount of these elements the less rachitic the condition of the bones.

In this connection one may take Sherman's conclusion "it is well to remember that a moderate fluctuation in the absolute
intake of either (calcium or phosphorus) may be serious when the intakes are low, but cannot seriously disturb the ratio when the intakes of both are liberal". (4)

**Various Food Articles** - Besides the notable dietary factors which affect calcium absorption, there are some special food articles possessing such property, but their specific action has not been found.

The prolonged meat diet (43) (high in fat and protein and low in minerals) of two Arctic explorers showed a slight negative calcium balance, while the general physical welfare of these subjects remained normal. Whether this loss of calcium was due to the influence of high fat or high protein or acid residue is not determined. Tao and others (44) have found that a diet containing 70 per cent cereal and 30 per cent egg yolk for rats is adequate for normal growth and reproduction throughout four generations, whereas the same cereal ration supplemented by 2 per cent butter for vitamin D and fats was unsatisfactory. Tao concludes that egg yolk furnishes a specific substance for the mobilization and utilization of an apparently limited amount of calcium in the diet. Another recent study also confirms this result. Komm (45) has prepared an egg yolk extract which he calls "Hellicitin". He finds that 100 milligrams of this substance fed with a rachitic diet prevents the appearance of rickets and maintains a normal blood calcium and phosphorus.

**Cereals** - Mellanby (46) in 1921 startled the world by stating that among all the cereals those containing the higher
amounts of calcium and phosphorus (oatmeal and wheat germ) were the most anticalcifying, while those containing the lesser amounts of these minerals (white flour and rice) were the least detrimental. This showed that the anticalcifying action is not due to the amount of calcium and phosphorus present, nor the acid forming effect of the food, since both rice and white flour are lower in these elements and the amount of potential acidity is similar. Later, he has been able to extract a substance from oatmeal, which he calls "toxamin". Recently Mirvish (47), using a similar method, has obtained from oatmeal an extract which will lower the blood calcium of rabbits 30 per cent in from twenty-four to forty-eight hours after intraperitoneal injection with a return to normal in about seventy-two hours.

However, Mellanby can demonstrate physiologically the presence and effect of this anticalcifying substance only in the diet which is deficient in vitamin D. He, himself, has shown that the antagonistic effect of cereal may be entirely nullified by supplementing the diet with adequate amounts of vitamin D with calcium and phosphorus.

While considerable amount of work has been done on the various dietary constituents influencing the absorption of calcium and phosphorus, yet no general laws have been formulated as to the primary factors and conditions favoring the optimal absorption of these minerals.
Assimilation

According to Hawk and Bergeim (48) in the normal blood the serum calcium has a range from 9 to 11 milligrams and 3 to 4 milligrams inorganic phosphorus per 100 cubic centimeters. Concentrated forms of calcium phosphates are broken down into more simple compounds before absorption. Referring to the recent study of Benjamin and Hess (49) they state "Calcium is present in normal serum in at least four forms, two of which are diffusible and two non-diffusible. Of the diffusible calcium, about two-thirds is in the form of adsorbable calcium-phosphorus complex; the remainder contains the calcium ion. Of the non-diffusible calcium, about one-fourth is adsorbable by barium sulfate and is regarded as part of a second, non-filtrable complex. The remainder, which is not adsorbable, contains the calcium usually described as 'protein bound' calcium". (1933)

The inorganic phosphorus in normal serum is found to be present in at least two forms, one of which is the filtrable, adsorbable calcium-phosphorus complex. The remainder may be ionic.

There are several regulatory mechanisms of calcium and phosphorus content in blood. The concentration of serum calcium is directly affected by the functioning of the parathyroid glands, which are called by Cannon (50) regulators of the homeostasis of blood calcium. Parathormone is known as a mobilizer in calcium metabolism (18). Removal of the parathyroids lowers the serum calcium. Injection of parathormone, as well as in certain
abnormality of the parathyroid glands, such as parathyroid tumor, tends to increase blood calcium and calcium excretion. If the injection is prolonged or the disease is not checked, the calcium excretion continues at the expense of the bone where this element is less carefully conserved than other substances.

According to Hess (23) the administration of irradiated ergosterol will promptly raise the serum calcium to the normal level. However, after the complete removal of the parathyroid glands, even repeated doses of the irradiated product cannot bring up the calcium to the normal level.

The function of vitamin D has been found to be vital in calcium and phosphorus metabolism, and the prevention of rickets. As to the methods of control, there is still a question. It is believed that vitamin D in various forms improves the permeability of the intestinal wall toward calcium and phosphorus. Bethke, Kick, Willard (41) in their rat experiments indicate that the inclusion of vitamin D not only tends to stabilize the calcium and phosphorus concentration in blood serum, but also to make a greater percent of these elements available for such biological functions as calcification and growth.

In 1929, Kramer, Shear and McKenzie (51) had found that both cod liver oil and irradiated ergosterol tended to increase the serum calcium but had no effect on calcium content of the bone.

In a recent (1933) experiment of Tamplin and Steenbock (52) on the conservation of calcium in adults on a low calcium diet, similar results were obtained. Groups of ten adult female rats were fed on the same low calcium ration. At the end of 8 to 11 weeks the group
which had been irradiated had a serum calcium content of 10.9 milligrams per 100 cubic centimeters, while the non-irradiated group had 7.5 milligrams as compared to 11.9 milligrams of the control group. In both experimental groups, the percentage of ash in the bone was lower than the normal. This confirms Sherman's statement that when the raw materials, calcium and phosphorus are deficient, vitamin D may help to maintain bone growth and formation, but the composition of such bone will be soft and watery (4).

The efficiency of irradiation is further tested by Jones and Rapoport (47) on dogs. When standard doses of calcium salts (calcium gluconate) were fed on the basis of 0.1 grams of calcium per kilogram of body weight, only a slight rise in the blood calcium was observed. After the dogs had been given irradiated ergosterol, however, the same amount of calcium salts produced a much greater rise in blood calcium. When calcium and phosphorus were fed in the form of insoluble dicalcium phosphate, no increase of either calcium or phosphorus could be obtained.

Storage

According to Bauer, Aub and Albright (53) the excess calcium in the blood is temporarily stored in the trabeculae at the epiphyseal ends of the growing bone. The trabeculae are lace-like structures closely related to bone marrow. They are located where the blood supply is the greatest, and where the deposition of calcium occurs largely. In a high calcium diet the trabeculae are
increased, thus allowing a large amount of the calcium to be deposited in the ends of the bones but not in the cortex. The cortex is believed to be uninfluenced by calcium metabolism, unless a great demand requires its output from the stored calcium. When the calcium retention equals the output, or when there is a negative balance, no deposition is found. When parathormone is injected, the number of trabeculae is reduced even in a diet comparatively high in calcium.

The trabeculae serve as a temporary storage for the conveniently available calcium. Whenever an excess amount of calcium is demanded such as for pregnancy and lactation or tooth formation, this stored calcium will be given up promptly to the blood and be carried to the place where calcium is most needed.

From the study of Holmes, Pigott and Campbell (54) on the calcium and phosphorus ratio in tibiae of chicks, there is no correlation between the calcium-phosphorus ratio of tibiae and the ratio in the ration or the ratio in permanent bone ash. These authors believe that the calcium and phosphorus are deposited at a constant ratio regardless of the complete or incomplete bone development.

**Paths of Excretion**

Calcium is excreted partly by the kidneys but mostly by the large intestines. Inorganic constituents which form soluble salts with calcium favor its appearance in the urine, while the insoluble compounds appear in the feces with phosphates or fats. Kugelmass (55) believes that one-fourth of the calcium intake is normally
found in the feces in the form of soaps. When the intake of calcium is low, diarrhea ensues with decrease of calcium soaps and an increase of free fatty acids in the stools. Hence, calcium soaps have a regulative action on the bowel.

He further states, "Phosphorus is excreted as phosphate in the urine and as calcium phosphate in the feces. A diet high in calcium precipitates phosphates and the excretion is through the feces, while a protein diet favors excretion through the urine."

According to Hess (23), the retention of calcium and phosphorus should not be determined on the basis of intake and excretion. He believes that "calcium is not only absorbed from the intestinal canal, but is excreted into it. It is evident, therefore, that the calcium in the feces represents not only the dietary calcium which has failed to be absorbed, but likewise the calcium which has been absorbed and not utilized."

It is known that 25 to 30 per cent of the phosphorus of the body is contained in tissues other than bone, which implies that some of the phosphorus which is excreted may originate from the tissues.

Retention and excretion of these minerals cannot give the true measure of their utilization. Therefore, we are forced to depend upon optimum amounts in the diet as the best assurance against shortage.
Bone Formation

The theories concerning bone formation are numerous. The advancement of these theories can be traced chronologically. Perhaps the oldest theory was that held by the early histologists who believed bone formation to be the result of a specific secretory action of the osteoblasts. A most scientific and reliable information on calcification of bone was given in the Harvey lecture (56) by Wells in 1911. At that time Wells concluded that, "Calcium is carried in the blood in amounts not far from the saturation point, held in solution by the colloid and the carbon dioxide, and existing probably in the form of an unstable double salt of calcium bicarbonate and dicalcium phosphate. In ossification, the deposition is initiated by a process of colloid adsorption causing a concentration of this double salt in the hyaline matrix which is to be calcified, and which has a strong affinity for calcium salts."

From the beginning of calcification the calcium seems to be deposited as carbonate and phosphate in about the same ratio as in mature bone. "Hyaline cartilage possesses an affinity for calcium which is not exhibited to an equal degree by other tissues."

Various ideas were derived later from this conclusion. Three main views were summarized and revived by Watt (57) in 1923. "(a) That the salts are deposited in the matrix by a precipitation in situ from the interaction of soluble salts in the blood and the tissues; (b) That these salts are excreted or secreted either with the matrix or into the matrix by the bone cells; (c) That a complex combination
salt known as calcium carbonophosphate, carried in solution in the blood, is thrown out of solution in the bone matrix by a change of carbon dioxide content of the tissue, and after precipitation, is finally converted into the two components, calcium carbonate and neutral calcium phosphate in the exact proportion found in the mature bone.

From the microscopic study of the calcium and phosphorus precipitation in various media, Watt himself has been able to evaluate the previous theories. In the fresh bone of various animals examined in various ways, Watt was unable to find any microscopic evidence of the salts in forms similar to the calcium phosphate or calcium carbonate. This indicates that the bone salts are not deposited in the matrix by simple precipitation, resulting from changes of carbon dioxide in the tissue.

The theory that the salts furnished by the blood are taken by the bone cells and secreted by them seems to be reasonable in view of the reversible action of the osteoblasts, either in absorbing calcium salts or giving them up.

Later in 1925 the precipitation theory was supplanted by the belief that calcium and phosphorus are deposited as insoluble salts from the supersaturated fluid bathing the cartilage. Holt, Le Mer and Chown (58) in the analyses of serum found that the normal blood is more than 200 per cent supersaturated with tricalcium phosphate, and it is probable that the other body fluids, including urine, are similarly supersaturated with this salt. This great reservoir for
calcium and phosphorus become available for the production of solid tricalcium phosphate only in tissues where some agent is present which accelerates precipitation.

Howland, Marriott and Kramer (59) in 1926 have stated that they could show how calcium and phosphorus are deposited as tricalcium phosphate by calculating the ratio of residual calcium and phosphorus existing in the normal bone. They assumed that some of the calcium in bone exists as calcium carbonate and some magnesium as tertiary magnesium phosphate. By excluding the amount of calcium carbonate and magnesium phosphate, the residual calcium and phosphate were obtained. The Ca : P ratio of various bones and in the bones of various normal individuals had the range of 1.90 to 1.97, as compared to the Ca : P ratio of 1.94 in tricalcium phosphate.

However, two years later, (1928), Shear and Kramer (60) had failed to find any evidence of tricalcium phosphate appearing in bone through their physiochemical analysis. Nevertheless, calcium phosphate was found in serum as colloid, which, when precipitated, formed varying proportions of soluble calcium acid phosphate and calcium hydroxide. Precipitation occurs only when the calcium and phosphorus are supersaturated in the serum.

In 1929 Shear, Washburn and Kramer (61) contended that if the ratio of Ca : P of normal bones is 1.94, it may be as well assumed that the calcium and phosphorus are deposited as $2 \text{CaHPO}_4 + \text{CaO}$ as to assume that it is deposited as tricalcium phosphate, as was suggested by Howland and his coworkers.
More recently, (1931), Kramer, Shear and Siegel (62) reported the results of an extended series of experiments which support their theory. Sera of the rachitic rats all showed a Ca\times P product of 27 or lower. Before the product rose to 40, practically no healing occurred. Normal sera of children or animals all gave a Ca\times P product of 50 or above. These results are remarkably paralleled by observation of the calcification of rachitic cartilage when it is immersed in a solution similar in kind and concentration to blood serum.

In the same year, the question "In what way are the bones formed?" was attacked from another angle. Roseberry, Hastings and Morse (63), from the suggestion of Taylor and Sheard (64) in presenting 3 Ca_3 (PO_4)_2 \cdot Ca CO_3 as the formula for bone substance, furthered the study of bone composition by the use of x-ray spectrograms. From their results there is evidence that calcium salts in bone exist in crystalline form, of the apatite series, which had been so found chemically by Gassmann in 1929 (65). No evidence is found that calcium acid phosphate or calcium carbonate exist in bone as such. With this, they suggest the formula for bone as follows: Ca CO_3 \cdot N Ca_3 (PO_4)_2 where N is not less than 2 nor greater than 3.

Later in the year Bogart and Hastings (66) give further proof of the above formula by chemical analysis of the treated and untreated bones as well as by the various kinds of commercial calcium phosphate compounds. They found that treatment, especially ashing,
resulted in considerable loss of carbonate unless special precautions are taken. All bone preparations give a ratio approximating that for tertiary calcium phosphate. The Ca₃PO₄ : CaCO₃ ratio is approximately 2, giving the assurance that the inorganic substance of bone is a crystalline salt, with the formula of CaCO₃ · N Ca₃ (PO₄)₂ where N approximates the value of 2.

In 1932 another new theory was formulated by Kay (67). An enzyme, phosphatase is found to be present in animal tissues, but particularly active in the ossifying cartilage and young growing bones. The osteoblasts, the hypertrophic cartilage cells and certain cells of the inner section of the periosteum in a growing bone contain, or can secrete a very active phosphatase. Upon hydrolysis, the salts of phosphoric esters, brought to the ossifying zone by the blood stream, cause a local increase in concentration of phosphate ions. When the soluble products of calcium phosphate, such as calcium acid phosphate, tri calcium phosphate or some other basic compounds, present in the circulating plasma, come in contact with the phosphate ions, deposition takes place. This deposition of calcium phosphate is brought about in or in the neighborhood of the cells which secrete the enzyme.

"It has been found, however, that in rickets, in spite of impaired calcification of the bones, phosphatase is present in increased, rather than in decreased, amounts." (68). These two conflicting ideas are merely theories, both of which need further experimental proof.
To date, 1933, the latest development in the theory of bone formation is reported by Benjamin (68) based on the old conception of the specific affinity of cartilage for calcium. In the present study, phosphorus has been shown to be combined with calcium in a complex form, capable of adsorption by barium sulfate. Furthermore, the cartilage, only in the region of the junction of epiphysis and metaphysis in which calcification normally occurs, has the property of adsorbing this calcium-phosphorus complex. She concludes, therefore, that "Normal calcification of bone is brought about by a process of adsorption of the calcium-phosphorus complex and that this form, and not ionic or other forms of calcium, is the type primarily involved in calcification".

So far this theory has not been further developed.

While the development of theories regarding bone formation brings us to the present points of view cited above, this study does not deal with questions as to the methods of deposition or the forms in which calcium and phosphorus are found in the bony tissues. These views are merely presented in an attempt to show the progress made on this problem.
A. Standardization of the Recipe

To provide a typical Chinese meat-bone dish for analysis, the recipe and method of cooking were tested until the finished product resembled the native homemade dish known as sweet and sour spareribs. The standardized recipe for an individual serving is as follows:

- **Pork spareribs** - 150 to 180 gms.
- **Cooking solution (total)** - 250 cc.
  - **Rice vinegar** - 75 cc.
  - **Soybean sauce** - 10 cc.
  - **Distilled water** - 150 cc.
  - **Sugar** - 10 gms.
  - **Salt** - 1 gm.
  - **Cornstarch (for thickening)** - 5 gms.
  - **Cold distilled water** - 15 cc.

Pork spareribs is the only kind of meat used in this dish. Cornstarch and distilled water were used in cooking the samples in order to eliminate any other possible source of calcium and phosphorus.

**Chinese Vinegar** - To make more typical, imported Chinese rice vinegar and soybean sauce were used. This vinegar is an acidified product of rice resulting from the process of fermentation. The commercial product is made in larger scale. A sweetened vinegar is obtainable for those desiring such variation.
Below is a recipe treasured by the prospective grandmother while preparing for her daughter an essential dietary ingredient to be used during lactation.

Rice (partly polished) - - - - 6 lbs.
Cold water - - - - - - - - - 20 lbs.
Salt - - - - - - - - - - - 2 oz.
Concentrated vinegar or - - 10 lbs.
Mother of vinegar - - - - 6 oz.

Method - Brown the raw rice in large kettle. Pour in cold water slowly. Add salt and vinegar. Seal in jar and keep in warm place. Ready to be used in three months.

Soybean Sauce - Soybean sauce is well known as the chief seasoning in Chinese cookery. Since it is made from fermented soybean the question has long been raised whether or not it furnishes additional calcium and phosphorus in the Chinese diet. It is generally a commercial product made in heated rooms with special apparatus. It takes much time and attention to go through the processes so only a few people make it at home. The following home recipe is obtained from the "Chinese Encyclopedia of Daily Necessities". The accuracy of this recipe is doubtful.

Yellow soybean - 1 vol. Sugar - 1/10 of 1 vol.
Wheat flour - - 1 vol. Mixed spices
Salt - - - - - 1 vol. Water - - - 2 to 3 vols.
Method - Soak soybean in water for a day. Cook two to three hours until brown. Add water whenever necessary. Let stand over night, then mix wall with flour in large vessel. Cover with mat or cloth and keep in warm place for four or five days to allow fermentation to take place. Add spices, salt and two to three volumes of cold water. Stir every other day until fermentation is completed at two to five months. Add sugar and cook to boil. Allow sediment to settle after which the clear solution is poured off.

B. Sampling of Materials

In preparing samples for this experiment, the spareribs of one side of the animal were purchased from the market. The middle six ribs were chosen and each cut into six 1 1/2 inch pieces. Alternate pieces of each rib were taken to make three rather uniform samples. Two of these samples were cooked, in small enamel kettles of like size and shape, over electric plates, starting with medium temperature. The meat was browned slightly and then the cooking solution was added, covered and cooked at low temperature with little stirring until the meat was tender and came readily off of the bone. This took from 50 to 60 minutes. The remaining liquid was thickened with the cornstarch paste. After it boiled again the heat was turned off and the contents allowed to stand for 15 minutes, the time considered in waiting to be served. The total time for preparation was 80 minutes, 75 of which the meat and bone were
in contact with the solution.

One of these two cooked products was labeled "cooked" sample. The meat was separated from the bone with a knife and fork, then washed five times with hot distilled water and drained. All visible pieces of bone were collected and treated likewise. The solution was carefully poured into a large, clean bottle together with the washings from the meat, bone and utensils used.

The other sample was served to a Chinese student, who ate it in the native fashion, that is, he picked up a piece of bone and meat with chop sticks and, with his front teeth, bit off the meat and then put the bone into his mouth, sucked or chewed out the absorbed solution from the bone marrow. Frequently small pieces of bone tissue were broken off which he might swallow. The remaining solution is always used up as gravy on rice, a required accompaniment to such a dish in the ordinary dinner. All bone residues were collected for analysis and labeled "cooked-chewed" sample.

For the "raw" sample, the bone was separated as completely as possible from all the meat fibers. All bone samples were cracked slightly with great care before drying. Each sample was divided into two portions as checks for each other.

Below is a list of all the samples in the prepared meat-bone dish analyzed.
C. Preparation of Samples for Analysis

All bone and meat samples were dried in an oven at 100°C for three to four hours. Then they were put in separate sacks for extraction in Soxhlet extractors. Hot alcohol was first used for four hours and followed by ether for the same length of time. The ether was removed from the samples by heating at 35 to 40°C. The samples were placed in desiccators and weighed. They were heated and weighed until a constant weight was obtained. Each sample was ashed in a porcelain dish in a muffle furnace at 700 to 800°C. This took five to six hours. They were weighed after being cooled in desiccators, then heated again and weighed until a constant weight was obtained.

Under a watch glass a little distilled water was added to the bone ash, which was then transferred to a beaker of suitable size. The dish was washed several times with water and lastly with HCl. Another 10 cc. of concentrated HCl were added to insure complete solution. The mixture was stirred thoroughly until the ash was completely dissolved, then boiled for five minutes and filtered through ashless paper into a volumetric flask. Bone samples were made up to 500 or 1000 cc. and meat
samples to 250 cc.

The same procedure was used for the cooking solutions after they were evaporated on a steam bath and dried in an oven, except that no extraction with alcohol and ether was made.

D. Methods of Analysis

The methods used for calcium and phosphorus determinations in this study were modifications of the directions given by the United States Bureau of Standards (69) and the Association of Official Agricultural Chemists (70). The modified methods used by the writer are similar to the routine procedure used in the chemistry laboratory at Oregon State College.

The following is a detailed description of the procedure followed in this laboratory:

Method of Calcium Determination - Calcium was precipitated as calcium oxalate \( \text{CaC}_2\text{O}_4 \) with ammonium oxalate \( (\text{NH}_4)_2\text{C}_2\text{O}_4 \). The approximate amount of calcium in the aliquot portion of the ash solution was estimated. Thus, sufficient amount of \( (\text{NH}_4)_2\text{C}_2\text{O}_4 \) would be added to insure the complete precipitation of the calcium in solution. Due to the high percentage of calcium in the bone samples, 25 cc. aliquot portions were taken, whereas 50 cc. portions were used from the meat and cooking solution samples.

For all determinations, the aliquot portions were drawn with pipettes, the accuracy of which had been checked. The aliquots were then placed in 250 cc. beaker. Ten cubic centimeters of concentrated HCl were added. The solution was diluted to about 100 cc. and a piece of litmus paper was dropped
in. \( \text{NH}_4\text{OH} \) was added very slowly until the solution became alkaline. The \( \text{NH}_4\text{Cl} \) formed from HCl and \( \text{NH}_4\text{OH} \) combines with the magnesium to form a double salt, magnesium ammonium chloride. This mixture is soluble, leaving no \( \text{MgC}_2\text{O}_4 \) to precipitate with the \( \text{CaC}_2\text{O}_4 \).

A drop of methyl red was added to the solution and acetic acid dropped in until the solution was pink. Methyl red changes to a pink color at a pH of 3.8, at which pH, magnesium and phosphates do not precipitate with calcium oxalate.

The solution was then heated to 85° to 95° C., 25 cc. of saturated \( (\text{NH}_4)_2\text{C}_2\text{O}_4 \) were added, with constant stirring, for the precipitation of calcium as \( \text{CaC}_2\text{O}_4 \). The beaker was covered and set aside over night to allow complete precipitation and the growth of crystals.

Filtering was done in a Gooch crucible with purified asbestos. The cover glass and beaker were rinsed five times with distilled water, after all the precipitate was transferred.

The precipitate and crucible were then washed three times. The crucible was returned to the beaker and 100 cc. of distilled water added for a reaction medium.

The solution was titrated with standardized N/10 potassium permanganate \( (\text{KMnO}_4) \). The Ca determination was done at 75° to 80° C., a temperature at which \( \text{KMnO}_4 \) oxidized best. Ten cubic
37

centimeters H₂SO₄ (1 : 1) were used to dissolve the CaC₂O₄ and to form an acid medium which hastens KMnO₄ reaction. The crucible was washed with distilled water several times before it was taken out. The solution was then diluted to about 150 to 200 cc. KMnO₄ was added from a burette not more than 10 cc. per minute. The solution was stirred constantly with a glass rod or thermometer to hasten the reaction. No addition was made before the color completely disappeared. The solution was reheated if temperature fell below 60° C. The last 1 cc. was added drop-wise. The first permanent pink color indicated the end point. The reading of the burette was taken with the help of a flash light.

Reactions - According to Kolthoff (71), the reaction takes place slowly at the beginning. This is called the incubation period at which KMnO₄ is reduced to MnO₂. As soon as MnO₂ salt has formed, it hastens the reaction. The color of KMnO₄ disappeared almost instantly. This was called the induction period. Toward the end point, MnO₂ decomposed with great speed forming MnC₂O₄.

CaC₂O₄ + H₂SO₄ → CaSO₄ + H₂C₂O₄

2KMnO₄ + 5H₂C₂O₄ + 3H₂SO₄ → K₂SO₄ + 2MnSO₄ + 10CO₂ + 8H₂O

Calculation - 1 cc. N/10 KMnO₄ = 0.002 gm. Ca.

1. No. cc. KMnO₄ used × N. of KMnO₄ × 0.002 gm. =
   0.1000 N.
   gm. of Ca. in 1 aliquot portion
2. Ca. in 1 aliquot portion \( \times \) No. of aliquot portions in whole sample = Total gm. of Ca. in sample

3. Total gm. of Ca. in sample \( \times \) 100 = o/o of Ca. in material gm. of material in sample

4. Total gm. of Ca. in sample \( \times \) 100 = o/o of Ca. in ash gm. of ash in sample

**Method of Phosphorus Determination**

Phosphorus was precipitated as ammonium phosphomolybdate \((\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3\) with ammonium molybdate solution \((\text{NH}_4)_2\text{MoO}_4\).

The volume of this solution added to the corresponding aliquot portion of the sample was based on the percentage of phosphorus in the sample. For percentages of P. below 5%, 20 to 25 cc. freshly filtered molybdate solution were added; for percentages between 5 and 20%, 30 to 35 cc. were added or approximately 75 cc. for each decigram of phosphorus pentoxide present.

The solution was pipetted into a beaker and diluted to about 100 cc., a small piece of litmus was dropped in to indicate when the solution had been made alkaline with \(\text{NH}_4\text{OH}\) and then slightly acid with \(\text{HNO}_3\). This precaution prevents the precipitation of iron. The solution and the molybdate reagent were heated on water baths to 40 to 45° C., 75 cc. of molybdate reagent were added to each aliquot sample to insure complete precipitation. The mixture was stirred constantly during the addition. The beaker was then covered and kept at 40 to 45° C. for half an hour. It should stand for an additional hour and a half or two hours before filtering.
The phosphomolybdate precipitate was washed with a 1% solution of HNO₃ to remove any ammonium molybdate deposited in the precipitate and to prevent peptization taking place. It was then washed with 1% KNO₃ to remove the HNO₃. The KNO₃ was used in place of water as the latter tends to allow the colloid formation.

In titration the yellow precipitate was dissolved by excess amount of standardized approximately N/10 NaOH until a clear solution was obtained. In this titration, phenolphthalein was used as the indicator as it changes color at a pH corresponding to the equivalent point in this titration. The crucible was washed several times and removed. The excess amount of NaOH was back titrated with standardized N/10 HCl until the pink color disappeared.

**Reaction for Titration**

\[
2 \text{(NH}_4\text{)}_3 \text{PO}_4 \cdot 12 \text{MoO}_3 + 46 \text{NaOH} \rightarrow 2(\text{NH}_4\text{)}_2 \text{HPO}_4
\]

\[
\text{(NH}_4\text{)}_2 \text{MoO}_4 + 23 \text{Na}_2 \text{MoO}_4 + 22\text{H}_2\text{O}
\]

**Back Titration**

\[
\text{NaOH} + \text{HCl} \rightarrow \text{NaCl} + \text{H}_2\text{O}
\]

**Calculation - Based on excess amount NaOH used**

1 cc. of N/10 NaOH = 0.000 135 gm. phosphorus

1. No. cc. of NaOH used \times \frac{N. \ of \ NaOH}{0.000 \ 135 \ gm.} = \frac{0.000 \ 135 \ gm.}{0.1000 \ N.}

   gm. P. in 1 aliquot part

2. P. in 1 aliquot portion \times \text{No. of aliquot portions in whole sample} = \text{total gm. P. in sample}
3. Total gm. of P. in sample \times \frac{100}{\text{gm. of material in sample}} = \% \text{ of P. in material} \\

4. Total gm. of P. in sample \times \frac{100}{\text{gm. of ash in sample}} = \% \text{ of P. in ash} \\

Most of the reagents used were made in sufficient amounts to supply the entire experiment. Distilled water blanks were run in each set of determinations following the same procedure as the samples. The amount of calcium and phosphorus of the blanks was subtracted from the amount of each sample.

The results reported were averages of two or more determinations in which each determination checked with the other within \pm 2\%.
RESULTS

Calcium and Phosphorus in the Cooking Solution

To check the possible source of calcium and phosphorus from vinegar, soybean sauce and a combination of ingredients in the cooking solution used in the recipe, determinations were made of the quantities of calcium and phosphorus contributed to the cooked samples from such ingredients. The analysis was carried out according to the methods described. Aliquot portions of 25 and 50 cubic centimeters were used for these determinations. The results are recorded in Table I.

The total calcium of the cooking solution was only 2 milligrams (0.002 gm.) more than the sum of the calcium from vinegar and soybean sauce. The total phosphorus was considerably higher than the sum of it in the two ingredients. This may be due to experimental error because of the minute quantity of phosphorus in each determination. Regardless of this fact, the calcium and phosphorus in the cooking solution were taken into consideration in the final results.

The titratable acidity of the cooking solution was determined colorimetrically with standard sodium hydroxide. Determinations were made for the solution as used in cooking and its 1/10 dilution. The results gave a normality of the cooking solution, range from 0.1495 to 0.1515 providing a 100% ionization.

Since the nature of acids in the rice vinegar is not
### TABLE I

**Calcium and Phosphorus in the Cooking Solution**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Sample</th>
<th>Ash (gms)</th>
<th>Calcium (gms)</th>
<th>Average calcium (gms)</th>
<th>Phosphorus (gms)</th>
<th>Average Phosphorus (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice Vinegar 1</td>
<td>75 cc.</td>
<td>0.7234</td>
<td>0.0032</td>
<td></td>
<td></td>
<td>0.0065</td>
</tr>
<tr>
<td>Rice Vinegar 2</td>
<td>75 cc.</td>
<td>0.7257</td>
<td>0.0030</td>
<td>0.0031</td>
<td>0.0063</td>
<td>0.0064</td>
</tr>
<tr>
<td>Soybean Sauce 1</td>
<td>10 cc.</td>
<td>2.8013</td>
<td>0.0224</td>
<td></td>
<td></td>
<td>0.0080</td>
</tr>
<tr>
<td>Soybean Sauce 2</td>
<td>10 cc.</td>
<td>2.7805</td>
<td>0.0228</td>
<td>0.0231</td>
<td>0.0078</td>
<td>0.0079</td>
</tr>
<tr>
<td>Cooking Solution 1</td>
<td>25 cc.</td>
<td>0.4901</td>
<td>0.0027</td>
<td></td>
<td>0.00464</td>
<td></td>
</tr>
<tr>
<td>Cooking Solution 2</td>
<td>25 cc.</td>
<td>0.4696</td>
<td>0.0027</td>
<td>0.00275</td>
<td>0.0050</td>
<td>0.00482</td>
</tr>
<tr>
<td>Cooking Solution in Recipe</td>
<td>250 cc.</td>
<td></td>
<td>0.0275</td>
<td></td>
<td>0.0482</td>
<td></td>
</tr>
</tbody>
</table>
definitely known, the percentage of ionization is undetermined. By the use of hydrogen electrode method, the pH value is 3.20 to 3.24 within the range of common vinegar.

**Solubility of Tricalcium Phosphate in Water and Cooking Solution**

To test the effect of the cooking solution on tricalcium phosphate, a large amount of this compound was taken and dried in the oven to take out the absorbed moisture. After being coked in a desiccator, four samples were weighed out. Two of these samples were cooked in 2/3 (167 cc.) of the cooking solution described in the recipe, for one hour. The other two samples were kept in same amount of water as the cooking solution for the same length of time.

Due to the pasty consistency of the solution resulting from the cooked cornstarch, the solution could not be filtered out through the filter paper or fine asbestos in the Gooch crucible, with suction. Centrifuging was used and the clear supernatant filtrate was drawn out with a pipette. The precipitates were washed and centrifuged twice. The filtrate and washings were put into a volumetric flask and made up to 1000 cubic centimeters.

Calcium and phosphorus determinations were made on aliquot portions of 25 cubic centimeters each. The results are shown in Table II. The percentages of solubility of both elements in the cooking solution were decidedly higher than those of the water samples. In the case of calcium, 8.09% of the element
TABLE II
The Solubility of Ca$_3$(PO$_4$)$_2$ in Water and Cooking Solution*

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Ca$_3$(PO$_4$)$_2$ in sample (gms.)</th>
<th>Calculated amt. of Ca in sample (gms.)</th>
<th>Calculated amt. of P. in sample (gms.)</th>
<th>Amt. of Ca obtained from sample (gms.)</th>
<th>Amt. of P obtained from sample (gms.)</th>
<th>Per cent solubility of Ca</th>
<th>Per cent solubility of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 1</td>
<td>0.8424</td>
<td>0.3262</td>
<td>0.5161</td>
<td>0.0264</td>
<td>0.0332</td>
<td>8.09</td>
<td>6.43</td>
</tr>
<tr>
<td>Water 2</td>
<td>1.6035</td>
<td>0.6208</td>
<td>0.9832</td>
<td>0.0272</td>
<td>0.0629</td>
<td>4.38</td>
<td>6.39</td>
</tr>
<tr>
<td>Cooking Solution 1</td>
<td>1.2969</td>
<td>0.5019</td>
<td>0.7950</td>
<td>0.1536</td>
<td>0.1049</td>
<td>30.60</td>
<td>13.19</td>
</tr>
<tr>
<td>Cooking Solution 2</td>
<td>1.6333</td>
<td>0.6332</td>
<td>1.0011</td>
<td>0.1904</td>
<td>0.1449</td>
<td>30.12</td>
<td>14.47</td>
</tr>
</tbody>
</table>

*The amounts of Ca and P in the cooking solution were subtracted from the total amounts of these elements obtained from the Ca$_3$(PO$_4$)$_2$ after it was cooked in the solution.
dissolved in water, whereas 30.60% dissolved after cooking. In the solution phosphorus seemed to show less difference. The solubility in water was 6.43% and in cooking solution 14.47%.

The results agree with the statement of Cameron and Seidall (72) that tricalcium phosphate is insoluble or only slightly soluble in large proportion of water but more soluble in a dilute acid medium. Comey (73) collected information concerning solubility of tricalcium phosphate found that very small quantities of salts of alkali metals increase the solubility in water, and that tricalcium phosphate is also more soluble when the water contains starch, glue or other animal substances. According to such statements, this cooking solution provides a favorable condition for the solubility of tricalcium phosphate.

The difference between the percentage of calcium dissolved in water samples I and II suggested that these elements might not be completely dissolved in the amount of washing used. However, it is evident that by cooking in this vinegar-soybean sauce solution, larger quantities of these elements were actually obtained.

**Solubility of Calcium and Phosphorus in Bone in the Cooking Solution**

Since it is determined that solubility of tricalcium phosphate is considerably greater in acid solution than in water, it
may be assumed that there would be larger amounts of calcium
and phosphorus dissolve from bone cooked in acid solution than
in pure water.

Conney (73) stated that the solubility of tricalcium phos-
phate varied according to the forms of materials used. He
quoted from Maly and Donath that in 100,000 parts of water,
2.36 parts gelatinous calcium phosphate, 2.56 parts ignited,
and 3.00 parts from bone dust can be dissolved. Lassaigue,
in a similar study, found that one liter of water containing
one volume of carbon dioxide in 12 hours at 100 C. dissolves
0.75 grams precipitated tricalcium phosphate, 0.166 grams from
bone ash, and 0.30 grams from bone buried 20 years.

Recognizing the possibility of such variations, it seemed
desirable to attempt to determine the solubility of calcium
and phosphorus in bone when cooked in an acid medium.

Samples were prepared as described. In this particular
determination the meat was removed from the bone and weighed
before cooking. After the cooking process, all visible
pieces of bone were collected for analysis. The result is shown
in Table III.

The difference in the percentage of calcium and phosphorus
in the raw and cooked bone samples is seemingly too small to
give a significant result. Since this difference is constant,
a certain loss resulting from cooking may be assumed. From the
mathematical estimation, based on the percentage of the raw bone,
a sample of 45.50 grams of bone should yield 4.3539 grams
TABLE III
Loss of Calcium and Phosphorus in Bone After Cooking

<table>
<thead>
<tr>
<th>Bone Sample</th>
<th>Total Material</th>
<th>Raw Bone Ash gms.</th>
<th>Calcium gms.</th>
<th>Phosphorus gms.</th>
<th>% in Raw Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw 1</td>
<td></td>
<td>5.3387</td>
<td>2.0428</td>
<td>1.0245</td>
<td></td>
</tr>
<tr>
<td>Raw 2</td>
<td></td>
<td>5.0719</td>
<td>1.9064</td>
<td>0.9638</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>151.50</td>
<td>41.20</td>
<td>10.4106</td>
<td>3.9492</td>
<td>9.59</td>
</tr>
<tr>
<td>Cooked 1</td>
<td></td>
<td>5.2706</td>
<td>1.9648</td>
<td>1.0090</td>
<td></td>
</tr>
<tr>
<td>Cooked 2</td>
<td></td>
<td>5.5136</td>
<td>2.0228</td>
<td>1.0485</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>165.30</td>
<td>45.40</td>
<td>10.7842</td>
<td>3.9376</td>
<td>8.78</td>
</tr>
</tbody>
</table>

Theoretical yield from 45.4 gms. bone: 4.3539 2.2019

Loss in 45.4 gms. of bone after cooking: 0.3663 0.1444 -0.31 -0.32

Loss in 100 gms. of bone after cooking: 0.8068 0.3181
calcium, and 2.2019 grams phosphorus. Comparing this theoretical yield to the actual amounts of 3.9876 grams calcium and 2.0575 grams phosphorus, obtained from the cooked sample, a loss of 0.3663 grams calcium and 0.1444 grams phosphorus is evident.

From the above, it seemed possible to interpret the results obtained from the prepared meat-bone dish. Experimental records of the meat and the solution after cooking are shown in Tables IV and V.

All bone samples, raw, cooked and cooked and chewed, were also analyzed. Since there was no way to get the weight of the bone separate from the meat before it was cooked, no comparison of the percentage of calcium and phosphorus in the bone samples could be made. These results, therefore, are excluded.

Table IV gives the record of the determinations on both raw and cooked meat. From nearly equal amounts of raw material, the meat after it was cooked had an extracted dry weight about two times greater than that of the raw. Consequently, the ash of the first was about four times greater than the latter, in spite of the fact that considerable amount of tissue fibers had gone into the solution, as a result of the cooking. The comparison of the amounts of calcium and phosphorus are equally striking. In the cooked meat sample, there were 0.4507 grams of calcium and 0.2699 grams of phosphorus, as compared to 0.0278 grams of calcium and 0.0981 grams of phosphorus in the raw meat
### TABLE IV

Comparison of Calcium and Phosphorus in Meat (Raw and after Cooked in Solution)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Meat &amp; Bone (gms.)</th>
<th>Extracted Meat (gms.)</th>
<th>Total ash (gms)</th>
<th>Calcium in ash (gms)</th>
<th>Phosphorus in ash (gms)</th>
<th>% in ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat 1</td>
<td>6.5850</td>
<td>0.2531</td>
<td>0.0163</td>
<td>0.0564</td>
<td>6.44</td>
<td>22.29</td>
</tr>
<tr>
<td>Raw</td>
<td>6.1585</td>
<td>0.1620</td>
<td>0.0115</td>
<td>0.0417</td>
<td>7.09</td>
<td>25.74</td>
</tr>
<tr>
<td>Total</td>
<td>172.9</td>
<td>12.7435</td>
<td>0.4151</td>
<td>0.0278</td>
<td>0.0361</td>
<td>6.69</td>
</tr>
<tr>
<td>Cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat 1</td>
<td>13.1607</td>
<td>1.1308</td>
<td>0.2988</td>
<td>0.1758</td>
<td>26.43</td>
<td>15.54</td>
</tr>
<tr>
<td>Cooked</td>
<td>9.0527</td>
<td>0.5563</td>
<td>0.1519</td>
<td>0.0941</td>
<td>27.30</td>
<td>16.91</td>
</tr>
<tr>
<td>Total</td>
<td>173.8</td>
<td>22.2204</td>
<td>1.6871</td>
<td>0.4507</td>
<td>0.2699</td>
<td>26.71</td>
</tr>
</tbody>
</table>
TABLE V

Comparison of Calcium and Phosphorus in Solution
Before and After Cooking

<table>
<thead>
<tr>
<th>Solution</th>
<th>Amount Used</th>
<th>Calcium in ash</th>
<th>Phosphorus in ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>First portion after cooking</td>
<td>1.9365</td>
<td>0.0472</td>
<td>0.0504</td>
</tr>
<tr>
<td>Second portion</td>
<td>2.3338</td>
<td>0.0525</td>
<td>0.0638</td>
</tr>
<tr>
<td>Total</td>
<td>250 cc.</td>
<td>0.0997</td>
<td>0.1142</td>
</tr>
<tr>
<td>Solution before cooking</td>
<td>250 cc.</td>
<td>0.0275</td>
<td>0.0482</td>
</tr>
<tr>
<td>Gain after cooking</td>
<td></td>
<td>0.0722</td>
<td>0.0660</td>
</tr>
</tbody>
</table>
sample. Assuming that the meat from nearly equal weights of the corresponding parts of the same piece of spareribs was equal, a considerable gain in solution of these minerals was obtained after the process of cooking.

No explanation can be made for the varying percentage of calcium and phosphorus in the meat ash of both raw and cooked samples. However, the percentage is used to check the accuracy of the result.

Table V gives the record of calcium and phosphorus obtained from the solution after the meat and bone were cooked in it. The total weight of ash in the cooking solution was estimated on the basis of the 25 cubic centimeter portions taken for determination. This might contribute to the unexplainably high amount of ash in the sample. However, in determinations of this study, both calcium and phosphorus were quantitatively higher in the solution after it was cooked than before. The calcium in the cooked solution was 0.0997 grams and 0.1142 grams of phosphorus, as against 0.0275 grams of calcium and 0.0482 grams of phosphorus in the solution before cooking, thus indicating a gain of 0.0722 grams of calcium and 0.0660 grams of phosphorus.

Table VI gives the distribution of the measurable amounts of calcium and phosphorus actually obtained from an individual serving of the "sweet-sour-spareribs". In a 172.9 grams
TABLE VI

Distribution of the Measurable Amounts of Calcium and Phosphorus in an Individual Serving of "Sweet Sour Spare Ribs"

<table>
<thead>
<tr>
<th>Source</th>
<th>Raw Material</th>
<th>Calcium gms.</th>
<th>Phosphorus gms.</th>
<th>Ca : P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat and Bone</td>
<td>172.9 gms.</td>
<td>0.4507</td>
<td>0.2699</td>
<td></td>
</tr>
<tr>
<td>Cooked Solution</td>
<td>250 cc.</td>
<td>0.0997</td>
<td>0.1142</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.5504</td>
<td>0.3841</td>
<td>1.43</td>
</tr>
</tbody>
</table>
(6.5 oz.) sample of meat and bone, cooked with the diluted vinegar solution, according to the method described, 0.5504 grams of calcium and 0.3841 grams of phosphorus are obtained, providing both meat and solution were eaten.
Discussion of Results

Certain difficulties prevented the obtaining of comparative figures for calcium and phosphorus in the raw and cooked bone used in this Chinese meat and bone dish. The identical bone samples, which were to be cooked with the meat in the solution, could not well be weighed before and after cooking, since the meat could not be removed. After cooking, considerable amount of the organic materials of the bone had been dissolved away from the bone, leaving the net weight of the bone smaller. As a result of this, a higher percentage of ash in the total weight of the bone is found in the cooked sample and higher still in the chewed samples.

With reference to the results of the calcium and phosphorus determinations in Table III, there is unquestionable evidence that a certain amount of the minerals has been lost due to cooking. A 45.4 gram sample of raw bone from 165.30 grams of spare ribs showed a loss of 0.3663 grams calcium and 0.1363 grams phosphorus. From a slightly larger amount of spare ribs, namely 172.9 grams, it may be assumed that the weight of the raw bone would be somewhat higher and, therefore, the total loss of calcium and phosphorus would be greater.

These losses must be accounted for. This can be done when we compare the gain of calcium and phosphorus in the meat and solution after cooking. (Tables IV and V)

From the similar weights of spare ribs, the amount of extracted meat in the cooked sample weighed nearly double the weight
of the sample of raw meat, regardless of considerable amount of meat fibers that had broken down into the solution.

More startling still is the greater amount of ash, calcium and phosphorus in the cooked meat.

Since no reported studies could be found regarding the possibility of absorption of calcium and phosphorus by meat during the cooking process, it is assumed that these elements, after being dissolved from the bone, were deposited or adhered to the tissue. Another possible assumption may be made. Due to the salt forming property of the protein, calcium may be used to form calcium proteinate. A large proportion of the calcium and phosphorus was obtained in the cooked meat itself even if the solution was not so carefully conserved or completely eaten.

In the solution, after being cooked, there were also appreciably increased amounts of calcium and phosphorus as compared to the solution before cooking. (Table V). If the calcium and phosphorus are dissolved from the bone at all, no doubt they may first go into the solution. The reason why the amounts of calcium and phosphorus are not higher is well explained by the previous assumption that considerable amounts were taken up by the meat.

There appears to be sufficient evidence presented to conclude that in the process of cooking there is a definite loss of calcium and phosphorus from the bone with a relative increase in these minerals in the meat and the cooking solution.

In the experimental processes there were a number of
unavoidable handicaps and errors, making the interpretation of
the results somewhat difficult. However, special effort has been
taken to minimize experimental errors in the preparation of samples
and in the chemical determinations.

In the preliminary tests on the solubility of tricalcium
phosphate, a wrong procedure was made in washing the precipitates.
According to Sjedall and Comey, tricalcium phosphate is insoluble
or only slightly soluble in cold water. Further washing of the
precipitates then may put into solution a little more of these
elements than should be dissolved in the given volume of water or the
cooking solution. However, the precipitates of both water and
cooking solutions were washed twice with approximately equal
amounts of water and, therefore, the chances of error may be assumed to be
approximately the same.

Nevertheless, the difference in solubility of tricalcium
phosphate in water and the cooking solution was so great (Table II)
that the washing of the precipitates would not interfere with the
conclusion made that the cooking solution favors the solubility of
tricalcium phosphate.

The increased amounts of calcium and phosphorus in meat and
solution after cooking seem, without doubt, to come from the bone,
yet this study does not furnish a direct proof of that fact. Since
the bone and meat are supposed to be cooked together, it is
impossible to get the exact weight of the identical bone before
cooking.
The amounts of calcium and phosphorus of the weighed crude bone raw and after cooking, indeed, gives an indirect proof of the loss. However, the result was obtained in one sample alone, and hence with no statistical assurance of certainty. Furthermore, the incompleteness of this study lies not only in the technical difficulties but also in the experiment itself. Each ingredient used and every process taken in preparation of the sample is an influencing factor.

While the variants involving the cut of pork spare ribs, the approximate size and shape of the pieces of bone, the type of utensil, the temperature, the same added ingredients (vinegar, soybean sauce, etc.) were standardized, yet there were other variants which were beyond such control, namely, the condition and age of the animal and the variations in the bone resulting therefrom.

Even if the age and the nutritional history of the animal were controlled, slight variations made in the ingredients, the cooking conditions and the sampling might effect the result materially.

Unless a statistical study were made on a large number of samples prepared under the standardized conditions, the exact result of this experiment could not be repeated.

In spite of all these difficulties, by checking closely the data on the meat and solution after cooking, this one individual serving of "sweet-sour spare ribs" dish composed of 172.9 grams of bone and meat with 250 cubic centimeters of the vinegar and soybean sauce solution, cooked at low temperature for one hour, furnished 0.5505 grams of calcium and 0.3841 grams of phosphorus. These
amounts gave a Ca : P ratio of 1.43

Although the amounts of calcium and phosphorus obtained give a ratio believed to be favorable for assimilation, the forms in which these elements exist and the physiological availability for human utilization require further experimental study.

According to Sherman (4), the daily allowance for the average man is 0.68 grams of calcium and 1.32 grams of phosphorus. When these elements are adequately furnished, the forms and ratio in which they exist are of less importance.

If these amounts of 0.5504 grams calcium and 0.3841 grams phosphorus obtained from this "sweet-sour sparerib" dish were assimilated as well as those well recognized sources, such as milk and cheese, this special way of cooking meat and bone is one which should be favored and used plentifully in the diet, especially when milk is not provided in adequate amounts.
1. In the Chinese diet, milk and cheese, the well recognized sources of calcium and phosphorus, are not used. A typical Chinese dish, which is called "sweet-sour spare ribs", was analyzed as a possible source of these elements.

2. Pork spare ribs were cut into 1/2 inch pieces and cooked in a rice vinegar, soybean sauce, salt and sugar solution for one hour at a low temperature. Samples of bone, meat, and cooking solution were analyzed for calcium and phosphorus before and after cooking.

3. Tricalcium phosphate is found to be more soluble in the cooking solution with a pH of 3.2 and containing salt and sugar, than in water.

4. Providing the calcium and phosphorus of bone are in some form of tricalcium phosphate, this cooking solution would favor their solubility.

5. From the analysis of a sample of 172.9 grams of meat and bone, total amounts of 0.5504 grams calcium and 0.3841 grams of phosphorus were obtained. Of these amounts, 0.4507 grams of calcium and 0.2699 grams of phosphorus were obtained from the meat, and 0.0997 grams of calcium and 0.1142 of phosphorus from the solution, after cooking. In this particular soybean sauce, only traces of calcium and phosphorus were found.

6. Quantitatively, the amount of calcium obtained in this experiment exceeded the minimum requirement of 0.45 grams, and
approaches the allowance of 0.68 grams per man per day, while
the amount of phosphorus obtained hardly meets half the minimum
requirement. This element, however, is more generously dis-
tributed in foods and it is easier, therefore, to fulfill the
daily requirement.
CONCLUSION

1. Pork spare ribs prepared according to a Chinese method of cooking, furnishes considerable proportion of the daily allowance of calcium and phosphorus for the adult. If these elements are absorbed and utilized, another significant source of calcium and phosphorus has been demonstrated.

2. It is possible that this peculiar method of cookery used extensively by the Chinese may be of particular value in providing adequate amounts of calcium and phosphorus in the Chinese diet.

3. Biological metabolic experiments should be carried on in animals and humans to find out how calcium and phosphorus from this source are absorbed and utilized.
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Correction for incomplete reference.