# THE EFFECTS OF LARGE AMOUNTS OF THIAMIN ON SCORBUTIC AND NON-SCORBUTIC ANIMALS

by

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# INTRODUCTION

The History of Beriberi and the Discovery of Thiamin

Accounts of beriberi reach into the ancient history of the Orientals. Macgowan, (1) in his "History of China", states that beriberi was recognised by the Chinese in 2697 B.C.

Its presence, however, was not recognised in the western world until the seventeenth century. In 1645 A.D. Jacobus Bontius (2) observed the presence of dry beriberi in Java. One hundred and fifty years later Rogers (3) described wet beriberi. Wet and dry beriberi were considered two separate diseases until 1835, when Malcolmson (4) noticed that cases of one kind often changed to the other. From such observations he concluded that there possibly were several forms of the same disease. Hirota (5) described infantile beriberi in 1898.

In 1884, Takaki, (6) the Surgeon General of the Japanese Navy, found that changing the rations would do much to eradicate beriberi from the navy personnel. The substitution of moderate amounts of meat, legumes and barley for a portion of the rice reduced the incidence of the disease from 23 to 40 per cent to less than $\frac{1}{2}$ per cent.

Though Takaki was one of the earliest advocates of the dietary theory of the origin of the disease, it was the work of Eijkman (7) a Dutch physician and chemist in Java, that appears to have most effectively endorsed the theory. Eijkman showed that a paralytic condition resembling beriberi could be developed in fowls by feeding them upon an exclusive diet of polished rice. He showed that the disease did not develop if the fowls were fed unpolished rice, or polished rice plus rice polishings.

In this way he came to believe that the disease was due to the deficiency of some substance present in the whole grain but absent from the starchy portion.

Fletcher (8) published his studies of the disease in Kuala Lumpur in 1905. He conducted his experiments in an insane asylum and found that over 50 percent of the patients fed polished rice for longer than a month developed beriberi while none of the patients fed brown rice for varying lengths of time developed it.

Fraser and Stanton (9) published results similar to those of Fletcher in 1909. Their work was done in railroad labor camps in the Malay States.

Other names woven into the story of the discovery of the B vitamins are those of Funk and Vedder. Funk (10) is credited with aiding in the projection of the deficiency theory as the cause of beriberi, scurvy, rickets and pellagra and with giving the name "vitamine" to the substances necessary for the prevention of each disease. The work of Vedder (11) was concerned principally with eradication of beriberi from the Philippines.

Stepp, 1909, (12) found that mice lived satisfactorily on a diet of bread and milk but found that if the bread was previously extracted with alcohol, the diet would not maintain them. The addition of the alcoholic extract restored the diet.

Hopkins, 1912, (13) found that rats could not survive on a diet containing purified protein, carbohydrate, fat and minerals but that the addition of milk (25 per cent of the diet) with its "accessory factors" resulted in satisfactory nutrition. Osborne and Mendel, 1913, (14) and McCollum and Davies, 1915, (15) made similar reports. In 1916, McCollum (16) proposed the name water soluble B for the substance necessary for the prevention of beriberi.

Mitchell, 1919, (17), Emmett and Luroe, 1920, (15) and Smith and Hendrick, 1926, (19) were among the first to observe that the water soluble fraction of rice polishings appeared to contain a complex rather than a single vitamin factor. It was found that an autoclaved extract was growth promoting but no longer anti-neuritic.

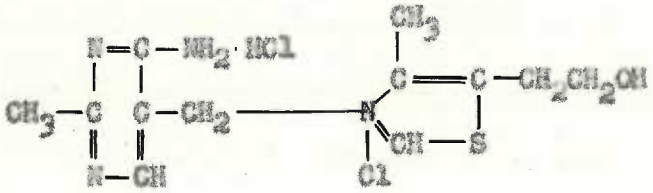
Jansen and Donath, 1926, (20) isolated the vitamin in crystalline form as the hydrochloride.

In 1927 the British Accessory Food Factors Committee (21) approved a system of nomenclature in which vitamin B was used for the complex and vitamin $B_1$ for the anti-neuritic vitamin. Other members of the B complex were given separate subscripts.

The composition of vitamin $B_1$ was determined by Windaus and coworkers, 1932, (22). They reported the composition to be $C_{12}H_{17}N_4OS$.

Williams, 1935, (23) synthesized it as the bromide hydrobromide.

At present the anti-neuritic vitamin is obtained chiefly as the hydrochloride, the structural formula for which according to Williams is:



(24).

It was he who also suggested the name "thiamin" which has been universally accepted (21).

The Physiological Action of Thiamin

While the symptoms of beriberi are many the primary physiological action of thiamin responsible for preventing the disease is unknown.

Thiamin demonstrates physiological significance in tissue respiration. Lohmann and Schuster (25) have shown that co-carboxylase, necessary for the decarboxylation of pyruvic acid in tissue respiration is the pyrophosphoric acid ester of thiamin hydrochloride.

Degeneration of the myelin sheath of peripheral nerves and lesions in ganglion cells of the brain, spinal cord and dorsal roots are seen in thiamin deficiency. (26)(27)

Hypertrophy of the right heart is a very frequent finding in thiamin deficiency especially if edema is also present. (28)(29) The cause is unknown but it may be due to water retention of muscle which takes place in the absence of thiamin. Drury (30) observed severe bradycardia during deprivation.

The vitamin has a pronounced effect upon appetite (31) and late stages of deficiency are characterized by atony of the stomach and intestinal tract (32).

Thiamin is necessary for normal adrenal glands, (33) hypertrophy resulting from deficiency. The islands of Langerhans hypertrophy during deficiency (34)(35). Production of sex hormones is affected (36)(37). The need for thiamin has been found to increase during lactation. (38). The thymus, thyroid, pituitary glands and liver are said to atrophy during deficiency (39).

Thiamin may play a part in the transmission of nervous impulses. According to one theory of nerve mediation the charge of acetyl choline liberated by an impulse which arrives at a synapse must exert its effect and be removed before a succeeding impulse can be effective (40).

Choline esterase may have a role in the removal of the acetyl choline liberated. There is evidence to the effect that thiamin may conserve choline esters and therefore potentiate their effects by inhibiting the action of choline esterase (41). Choline esterase activity is high in beriberi pigeons (42). Thiamin administration decreases this activity (43).

Symptoms of Thiamin Deficiency

The symptoms of thiamin lack in man vary with the duration and degree of deficiency.

1. In adults the onset of symptoms may be vague and insidious. Fatigue and loss of strength are among the first subjective manifestations. Stiffness of the legs is often observed. These symptoms are soon followed by headache, insomnia, nervousness, dizziness, dyspnea, loss of appetite, loss of weight, dyspepsia, anemia, tachycardia and tenderness of calf muscles. After a variable period of time the major symptoms of beriberi appear and these may be divided into three groups:

a - symptoms referable to involvement of the nervous system called dry beriberi. Here, peripheral neuritis is the most conspicuous manifestation and is associated with weakness, leg cramps, burning of ankles and soles of the feet and increased ankle and knee jerks in the early stages, which, as the disease progresses, decrease and finally disappear. Anesthesia and numbness and atrophy of skin and muscle follow involvement of the nerve. Sphincter control is maintained until the late stages. The vagus nerve is often affected.

b - symptoms associated with generalised edema called wet beriberi. The edema is most conspicuous in the feet and legs and gradually ascends up the body. There are often effusions into the pericardium, pleura, peritoneal cavity and lungs. There is atrophy of the muscle cells, reduction of fat and swelling and loss of striation, with edema fluid in the muscles.

c - acute symptoms depending upon cardiac involvement and congestion of viscera. Acute cardiac failure is often the cause of sudden death in thiamin deficiency. The heart is hypertrophied and dilated and appears to have lost contractile power. Other viscera may show congestion.

2. In infants, who are very susceptible, the symptoms of thiamin deficiency are similar to those of wet beriberi. The onset is sudden, associated with rigidity of the body, whining, constipation, diminished urinary excretion, weakness, edema, cardiac enlargement, cyanosis and rapid and irregular pulse. If the infant is not cured promptly he may die suddenly.

3. A variety of conditions have been observed in association with thiamin deficiency. Chronic alcoholism, pregnancy, diabetes, pellagra, pernicious anemia, etc. are often accompanied by thiamin lack. This lack may be due to inadequate food intake, vomiting, improper assimilation or utilization due to the primary disease but the effects are the same as in the uncomplicated cases (39).

The History of Scurvy and the Discovery of Ascorbic Acid

The earliest accounts of scurvy refer to it as a plague. Hippocrates spoke of men in the army who suffered from leg pains, gangrene of the gums, and loss of teeth. There is an account of its breaking out during Lent when the men ate no meat but partook of a species of eel which they believed had eaten dead people and therefore led to the loathsome disease.

It was a scourge among the Egyptians of the thirteenth century. Reference is made to the lividity and spongy condition of the gums among the Crusaders and to the fact that "barber surgeons" had to cut away dead flesh so that people might chew their food. Black spots about the legs, weakness and general debility were accompanying features.

Scurvy has probably existed in Northern Europe and Asia ever since the first inhabitation by man. It was especially prevalent in Prussia and among the Swedes.

The early colonists of North America were much afflicted with scurvy. The French met with very high mortality during the Canadian winters and the English in New Foundland debated the wisdom of abandoning settlement because of it.

It appears that no war was omitted from this type of sickness. It was prevalent during the Revolutionary and Civil Wars and the last World War.

Scurvy was early associated with life at sea. The long voyages of the fifteenth and sixteenth centuries were often failures due to the incapacitation of the crews by it. One of the earliest accounts of such experiences is that of Vasco de Gama, 1497. However, these long expeditions with inevitable out breaks of scurvy undoubtedly did much to turn attention to its

dietary origin. In contrast to the earliest accounts of scurvy at sea the expedition of Captain Cook, 1772-1775, lost no men from it. An important contributing factor for the success of the British Navy between 1779-1813 was due to the addition of lime and lemon juices to the diet of the men (44).

In 1895 Theobald Smith reported a hemorrhagic condition in guinea pigs restricted to a cereal diet (45).

In 1912 Holst and Frölich (46) recognized the similarity between this disease in guinea pigs and scurvy in human beings. They observed that a diet causing scurvy in man likewise produced it in guinea pigs and that substances having a curative effect in one were equally efficacious in the other.

The work of Chick and Hume (47), Cohen and Mendel (48), Harden and Zilva (49) resulted in the final acceptance of scurvy as a deficiency disease.

In 1919, Drummond (50) designated the antiscorbutic substance as vitamin C.

Cohen and Mendel (48), Le Mar, Campbell and Sherman (51) developed general technics and experimental diets which resulted in quantitative measurement of antiscorbutic activity.

Szent-Györgyi 1928 (52) isolated a reducing substance from such sources as oranges, lemons and suprarenal cortex. He considered it to be a hexuronic acid $C_6H_8O_6$.

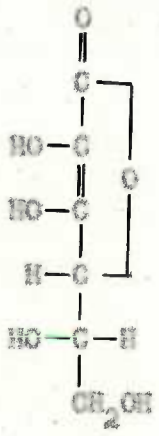
In 1930 Tillmans and Hirsch (53) correlated the reducing power of various foods with their vitamin C content.

Waugh and King 1932 (54) isolated crystalline vitamin C which proved to be identical with the "hexuronic" acid first isolated by Szent-Györgyi (52) and Kendall (55) from adrenal glands.

Synthesis of the vitamin was accomplished by Reichstein, Grussner and Oppenauer 1933 (56).

The structural formula of vitamin C was first reported by Haworth, Hirst and co-workers (57). Karrer (58) Micheel and Kraft (59), Euler and Klussmann (60) soon made confirmatory reports.

The structural formula for vitamin C is:



for which Haworth and Szent-Gyorgyi (61) suggested the name ascorbic acid to connote its antiscorbutic nature.

The Physiological Action of Ascorbic Acid

Very little is known relative to the manner in which ascorbic acid exerts its physiological action

It is functionally significant in relation to the formation of mesenchymal intercellular substance, such as collagenous connective tissue material, osteoid tissue, bone, and dentine of the teeth. It is necessary for maintenance of the integrity of the blood vessels.

Under normal conditions the fibroblast appears to lie in a ground substance within which fibrils are formed which in turn form collagen. These fibrils appear to be cemented together by a translucent substance. It is believed that the formation and cementing of these fibrils during the development of collagen is controlled by ascorbic acid.

In scorbutic guinea pigs fibroblasts and ground substance are formed but fibrils and collagen are not.

Whether ascorbic acid exerts its most direct effect upon the fibroblasts or the intercellular material is undecided but in the primary response of scorbutic animals to ascorbic acid the newly formed material is in close proximity to the fibroblasts. The fibroblasts do not seem to be changed in appearance until the late stages of scurvy (62).

Tissues characterised by high metabolic activity have a high ascorbic acid content (63). Individual tissues of young animals are richer in ascorbic acid than the corresponding tissues of older animals and the ascorbic acid requirement of the growing animal is greater than that of the mature animal (64) (65).

Bonner and Axtmann (66) indicate that ascorbic acid serves as a powerful growth stimulant for young plant embryos.

Ascorbic acid is necessary for the red blood cell forming tissues (67).

A prescorbutic state often occurs wherein impairment of physiological functions other than growth are indicated first. This border line of deficiency in animals results in greater sensitivity to injury from diptheria toxin (68) (69), and the dextrose tolerance of such animals is lowered (70) (71). Resistance against infections is lowered in the prescorbutic and scorbutic conditions (72). Cormia (73) reports that ascorbic acid will inhibit cutaneous reactions of arsphenamine in sensitised skin.

Beneficial effects have been reported by ascorbic acid administration in cases of lead poisoning (74).

There are accounts of activating and inhibiting effects of ascorbic acid on enzymes in vitro. Whether these effects are of physiological importance is still to be proved.

The cytochrome-indophenol oxidase system has been shown to act as a catalyst for the aerobic oxidation of ascorbic acid and evidence indicates that this system may be responsible for the slow aerobic oxidation of the vitamin in excised animal tissues (75) (76).

There is no clear cut evidence to support the theory that ascorbic acid serves as a hydrogen-transport agent in animal tissues. Hopkins and Morgan (77), and Borsook (78) in support of this theory in relation to plants, and emphasise that glutathione plays a role in the cycle.

Green and Richter (79) reported that ascorbic acid acts as an inhibitor in the adrenalin-adrenochrome oxidation system in heart tissue.

Lemberg, Corlis-Jones and Norris (80) Barron and Harrer (81) have correlated the coupled oxidation of ascorbic acid and hemochromogens.

Reher (82) has reported a close relationship between ascorbic acid

and guinea pig complement. The oxidation reduction behavior of complement was found to be dependent largely upon the ascorbic acid content of plasma. Complement activity is dependent upon its being in the reduced state.

Ascorbic acid has been shown to be an essential factor in wound healing. It is necessary for normal proliferation of connective tissue and bridging of the wound (83)(84).

Symptoms of Ascorbic Acid Deficiency

The degree of ascorbic acid deficiency is fairly well correlated with the severity of symptoms and the locations of the lesions are largely influenced by growth and stress (21).

Animals at the borderline of deficiency show a lowered resistance to toxins, infections, and lessened general resistance (70)(71)(72)(73).

As the state of deficiency increases general weakness and lassitude become apparent. The first obvious effect of severe depletion is capillary fragility and the presence of petechiae (85).

As the depletion progresses the hemorrhages become more massive and affect larger vessels. Skeletal lesions are present as indicated by x-ray studies (86).

In very severe depletion hemorrhages are very extensive and skeletal and gingival lesions are extensive and severe (21).

The individual lesions of scurvy may be described as follows:

Cutaneous lesions due to ascorbic acid deficiency aside from petechia (85) include hyperpigmentation of the skin, dilation of the hair follicles and fragmentation and loss of hair (85).

Skeletal lesions are most common in the costrochondral junctions, distal and proximal ends of the femur, proximal end of the tibia and in the wrist bones. In the area of the lesion the formation of bone ceases

and the osseous part becomes rarified. At the costochondral junction there is replacement of the normal junction by a zone of collagen poor connective tissue in which is imbedded fragments of calcified cartilage matrix free of osteoid tissue. It has been shown that in ascorbic acid deficiency the osteoblasts revert to fibroblasts when unable to form osteoid tissue and attempt to form a fibrous union between diaphysis and epiphysis, (the "Rustmark") within which lie calcified fragments of cartilage matrix and bony trabeculae. These lesions are often accompanied by hemorrhages which may be subperiosteal or within the bone. Connective tissue fibers show fragility. There is a watery zone about the older osteoblasts. There is a weakening of the periosteal attachment and great proliferation of connective tissue cells in an attempt to strengthen it (21).

The teeth of adults with scurvy show resorption of dentine. The resorption begins about Tome's canals. Degeneration of the odontoblasts and hyperemia and atrophy of the pulp occur. These lesions first occur in the apex of the tooth and at the division of the root canal (21). Fish and Harris reported defects in enamel and cement also (87).

When the teeth are present lesions occur in the gingiva, especially about abnormal teeth. The gums become swollen, and bleed. Loosening of the teeth often occurs. There is destruction of the epithelium, often followed by ulceration (21).

Fragmentation of striated muscle fibers occurs in severe scurvy. There is great effort to repair the damage by proliferation of sarcolemma. There may be replacement of muscle fibers by connective tissue poor in collagen (21).

Bloody tumors of the conjunctiva and ecchymosis of the eyelids may appear (21).

Effusions into the serous cavities are common. There may be edema. Enlargement and dilation of the heart may occur (21).

Atrophy of the bone marrow occurs with replacement of amyloid resembling material (88)(89).

The digestive tract is usually not seriously affected; there may be some hemorrhagic area (21). The adrenals are swollen and hypertrophied in early scurvy but atrophic in late scurvy (90). The lymphatic tissues and glands of internal secretion atrophy to some extent.

Ascorbic acid deficiency is aggravated by certain diseases among which are pneumonia, typhoid fever and tuberculosis.(84)

# EXPERIMENTAL WORK

## SECTION I

### Preliminary Observations and Outline of Work

It was at first casually observed that scorbutic guinea pigs fed large amounts of thiamin were likely to die more quickly than those without thiamin. This led to an investigation of the effects of thiamin when administered to scorbutic and non scorbutic guinea pigs.

Several scorbutic diets were tried but since guinea pigs thrive on a diet of Olympic rabbit pellets and green leaves or Olympic rabbit pellets and supplements of synthetic ascorbic acid, the pellets were chosen for the stock diet, and given ad. lib.

These pellets contain alfalfa meal, ground barley, yellow corn meal, ground oats, wheat middlings, wheat shorts, wheat bran, cocoanut oil, soy bean oil meal, linseed oil meal, molasses, dried beet pulp, limestone, salt and vitamins A and D in feeding oil.

Thiamin as thiamin hydrochloride (Mallinkrodt) was used in aqueous solution and given by dropper.

Crystalline ascorbic acid (Lilly) was used in aqueous solution and given by dropper.

At the beginning of the work general observation and weight records were kept for groups of male guinea pigs maintained on the following daily diets for varying periods:

Group 1 received Olympic rabbit pellets and 50 mg. ascorbic acid.

Group 2 received Olympic rabbit pellets, 50 mg. ascorbic acid and 25 mg. thiamin hydrochloride.

Group 3 received Olympic rabbit pellets.

Group 4 received Olympic rabbit pellets and 25 mg. thiamin hydrochloride.

The effects of these diets on weight are summarized in the following corresponding tables:

Table 1 - normal diet

| animal number | wt. gms. initial | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|---|---|---|---|---|---|---|---|
| 1 | 728 | 798 | 826 | 826 | 854 | | |
| 2 | 728 | 756 | 798 | 826 | 812 | 826 | 854 |
| 3 | — | 434 | 448 | 504 | 546 | | |
| 4 | 484 | 532 | 581 | 581 | 672 | | |
| 5 | 343 | 343 | — | 406 | 448 | 462 | |
| 6 | 294 | — | 364 | 378 | | | |

Table 2 - normal diet plus thiamin

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 812 | 854 | 882 | 882 | 882 | | |
| 2 | 812 | 826 | 840 | 828 | 814 | | |
| 3 | 776 | 805 | 833 | 819 | 778 | | |
| 4 | — | 474 | 483 | 518 | 560 | | |
| 5 | 364 | 364 | 378 | 434 | 448 | 476 | |
| 6 | 798 | 777 | 763 | 798 | | | |
| 7 | 728 | 770 | 798 | 798 | | | |

Table 3 - scorbutic diet

| animal number | wt. gms. initial | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|---|---|---|---|---|---|---|---|
| 1 | 700 | 672 | 686 | 700 | 693 | 658 | 609 |
| 2 | 700 | 714 | 728 | 714 | 658 | 620 | |
| 3 | 532 | 602 | 616 | 602 | 574 | | |
| 4 | 504 | 504 | 588 | 520 | 500 | | |
| 5 | 392 | 385 | 406 | 427 | 488 | 476 | |
| 6 | 378 | — | 371 | 336 | | | |
| 7 | 350 | 301 | 266 | | | | |
| 8 | 336 | 343 | 308 | | | | |
| 9 | 336 | 329 | 266 | | | | |

Table 4 - scorbutic diet plus thiamin

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 686 | 742 | 749 | 777 | 588 | | |
| 2 | 588 | 644 | 658 | 574 | 570 | | |
| 3 | 588 | 644 | 672 | 588 | 490 | | |
| 4 | 574 | 574 | 588 | 623 | 574 | | |
| 5 | 518 | 553 | 553 | 511 | 518 | | |
| 6 | 490 | 504 | 546 | 462 | | | |
| 7 | 364 | 371 | 336 | | | | |
| 8 | 350 | 280 | — | | | | |
| 9 | 350 | 364 | 336 | | | | |
| 10 | 336 | 336 | 294 | | | | |

## DISCUSSION

The first 5 guinea pigs of group 1, which received pellets plus ascorbic acid, gained an average of 123 gms. during the first 4 weeks, while the similarly numbered animals in group 2, receiving pellets plus ascorbic acid and thiamin gained an average of 45 gms. during the same length of time. It is regretted that the effects of the daily administration of 25 mg. of thiamin hydrochloride to animals receiving more than 50 mg. of ascorbic acid daily were not obtained. The first 5 guinea pigs in group 3, receiving pellets alone gained an average of 17 gms. during the first 4 weeks while the similarly numbered animals in group 4 receiving pellets plus thiamin lost an average of 43 gms. during the same length of time.

It was found that older guinea pigs are much less susceptible than younger pigs to ascorbic acid deficiency. Guinea pigs weighing 700 gms. or more usually did not show signs of scurvy on the scorbutic diet until about 4 weeks. Guinea pigs weighing around 400 gms. usually showed definite signs of scurvy within 3 weeks on the scorbutic diet while pigs weighing 300 gms. usually showed signs of scurvy within 2 weeks. The fact that older animals are less susceptible to ascorbic acid deficiency than are younger animals has been previously reported and confirmed (64) (65).

It was observed that decreased food intake, weight loss, tenderness of the limbs and lameness, all occurred at about the same time in the animals fed the scorbutic diet. The animals fed the scorbutic diet plus 25 mg. thiamin hydrochloride did not seem to show signs of scurvy appreciably earlier than the animals on the scorbutic diet alone, with the exception of weight loss, but after the disease was apparent thiamin caused its more rapid progress. Some of the scorbutic animals fed thiamin lost complete use of the hind legs and dragged themselves about on the front legs as if

completely paralyzed in the hind legs.

It was found that the hemorrhages in the scorbutic guinea pigs fed thiamin were more massive than those in the animals on the scorbutic diet alone. Hemorrhages were never found in the animals fed on the normal diet or on the normal diet plus thiamin.

Longenecker, Fricke and King (91) showed that ascorbic acid synthesis increased in rats when chloretone and various other substances are administered. If chloretone administration increases the utilization of ascorbic acid, its administration to guinea pigs which do not synthesize it should aggravate the condition of scurvy.

The procedures and data of further study on the effects of the four diets listed, and of chloretone on hemorrhages in guinea pigs are recorded in Section II.

It was found that gross bone deformities appeared in animals fed the scorbutic diet and the scorbutic diet plus thiamin. The wrist joints were especially affected. In some of these animals the front paws took positions similar to those of the flippers of a seal. The costochondral junctions were found to be considerably enlarged in severe scurvy. The procedures and data of further work on the effects of the four diets on bone structure are recorded in Section III.

Since the bones in the scorbutic animals showed gross pathology, it was considered important to determine the calcium and phosphorus excretions in relation to food intake of animals on each of the four diets. Procedures and data for this phase of the work are recorded in Section IV.

Scorbutic guinea pigs fed thiamin showed signs of increased restlessness. There were various signs of nervousness and the actions of the animals were discoordinated for a time after thiamin administration. There were jerking movements of the body, eye blinks, jaw movements, etc.

It was observed that scorbutic guinea pigs fed thiamin for a short time (10 days) usually died. They often died during or immediately after a ten day period of thiamin therapy and were very ill with scurvy. In some cases the guinea pigs lived for several months if continuously given ample green food, starting immediately after the period of thiamin administration. The animals appeared to improve for a time after resumption of the normal diet, ate well, and began to gain weight. Eventually, however, though they continued to appear hungry and tried to eat they began to lose weight. It seemed that the strength of the chewing muscles progressively decreased and the animals became unable to exert force when chewing, though they continually tried to eat. In conjunction with apparent weakening of the jaw muscles there was decreased control of these muscles and the animals would spend long periods with jaws constantly opening and closing in a rapid chewing motion. In some animals this condition of constant chewing became permanent until death. When these animals died they were extremely emaciated, but on examination it was found that there were no longer any scorbutic hemorrhagic areas. Because of these observations and of other possible signs of possible nerve involvement, the study of peripheral nerves and skeletal muscle was undertaken. Procedures and a progress report on the study of peripheral nerve and skeletal muscle are recorded in Section V.

Because thiamin administration appeared to aggravate the scorbutic condition including bone pathology in guinea pigs which cannot synthesize ascorbic acid, it was decided to investigate its effects on bone formation in rats which can synthesize it. Since chloretone has been found to greatly increase the production of ascorbic acid in rats (91) the effect of chloretone administration to rats in conjunction with thiamin, was studied.

## SECTION II

### The Effects of Large Amounts of Thiamin and Chloretone on Scorbutic and Non-scorbutic Guinea Pigs

A. In order to determine the effects of large amounts of thiamin on the production of hemorrhage in guinea pigs, groups of male animals weighing 420-430 gms. were placed on the following daily diets for 5 weeks:

Group 1 received Olympic rabbit pellets and 50 mg. ascorbic acid.

Group 2 received Olympic rabbit pellets, 50 mg. ascorbic acid and 25 mg. thiamin hydrochloride.

Group 3 received Olympic rabbit pellets.

Group 4 received Olympic rabbit pellets and 25 mg. thiamin hydrochloride.

After 5 weeks the animals were killed and examined for hemorrhages. The effects of each diet on the production of hemorrhage are indicated by a representative animal from each group shown in Plates I and II.

B. In order to determine the effects of large amounts of thiamin and thiamin plus chloretone upon scorbutic and non-scorbutic guinea pigs, male animals weighing 420-430 gms. were maintained on the following daily diets for 5 weeks:

Animal No. 1 received Olympic rabbit pellets, 20 mg. chloretone and a daily supplement of 25 mg. thiamin hydrochloride during the last 10 days of the 5 week period.

Animal No. 2 received Olympic rabbit pellets and a daily supplement of 25 mg. thiamin hydrochloride during the last 10 days of the 5 week period.

Animal No. 3 received Olympic rabbit pellets.

Animal No. 4 received Olympic rabbit pellets, 100 mg. ascorbic acid, 25 mg. thiamin hydrochloride, and 20 mg. chloretone.

After 5 weeks the animals were killed and examined for hemorrhages. The effects of each diet are shown in Plate II.

# DISCUSSION

Animals maintained on a daily diet of Olympic rabbit pellets and green leaves or on pellets plus a daily supplement of 50 mg. ascorbic acid have never shown any signs of hemorrhage.

Animals fed on a daily diet of Olympic rabbit pellets, 50 mg. ascorbic acid and 25 mg. of thiamin hydrochloride have never shown signs of hemorrhage. It is possible that long continued use of massive doses of thiamin with a normal diet would bring about this scorbutic condition.

Animals fed on Olympic rabbit pellets have never failed to develop hemorrhages. The time necessary to produce hemorrhages largely depends upon the age of the animal and its diet previous to the experiment.

Animals maintained on Olympic rabbit pellets and 25 mg. thiamin hydrochloride develop more massive hemorrhages in a given time than those maintained on the scorbutic diet alone. The effects of thiamin seem to be about as great if its administration is begun after the first signs of scurvy develop.

The animal maintained on the scorbutic diet and chloretone during the 5 week period and fed 25 mg. thiamin hydrochloride during the last 10 days developed the most massive hemorrhages of any animal in a given time.

The animal maintained on Olympic rabbit pellets, 100 mg. ascorbic acid, 25 mg. thiamin hydrochloride and 20 mg. chloretone throughout the 5 week period showed no signs of hemorrhage at the end of the period.

It appears that chloretone increases the need for ascorbic acid and that when the extra requirement due to thiamin administration is superimposed the scorbutic condition is aggravated.

It is of interest to note that the administration of large amounts of ascorbic acid will prevent the combined deleterious effects of large amounts of thiamin and chloretone.

PLATE I

2 4 3 1

1. Animal on normal diet.
2. Animal on normal diet plus thiamin.
3. Animal on scorbutic diet.
4. Animal on scorbutic diet plus thiamin.

PLATE II

1 2 3 4

1. Leg from guinea pig fed Olympic rabbit pellets, thiamin and chloretone.
2. Leg from guinea pig fed Olympic rabbit pellets and thiamin.
3. Leg from guinea pig fed Olympic rabbit pellets.
4. Leg from guinea pig fed Olympic rabbit pellets, ascorbic acid, thiamin and chloretone.

## SECTION III

### A Study of the Effects of Thiamin on Bone Development in Normal and Scorbutic Guinea Pigs

As has been stated (21) the histological changes of bone in scurvy are most marked where growth in length is rapid. The bones most likely to show typical changes are the ribs.

Since the costochondral junctions were chosen for study in this part of the work a description of the classical picture of a junction from a scorbutic animal will be given:

The columns of cartilage cells in the proliferative cartilage normally tend to be linear whereas in scurvy they become irregular. In scurvy, the cells composing the columns are smaller than normal, the rows are usually shorter and are separated by irregular masses of cartilage matrix.

These columns of cartilage matrix are fewer, more irregular and larger than normal. They tend to form an irregular framework through which it is difficult for capillaries to penetrate.

Normally, with the exception of a few masses of cartilage reserved for framework and which are changed to bone, the removal of cartilage matrix is quite prompt and is followed by replacement by marrow tissue.

Scurvy appears to interfere with the mechanism of removal of cartilage matrix. Since this mechanism is inhibited in scurvy the irregular masses composing the scorbutic lattice increase due to growth and to the advance of the zone of provisional calcification, and encroach upon the marrow tissue. The scorbutic lattice may therefore be much thickened. This thickening persists until the growth process in the cartilage cells is arrested.

This scorbutic lattice appears to be rarified and fractures easily.

The fractures increase in extent and number as the scorbutic condition increases in severity. In the late stages this lattice is disorganised and appears as if churned, and instead of maintaining a general longitudinal direction, the particles of broken matrix are compressed laterally and may penetrate into the weakened places near the cartilage shaft junction.

In scurvy, osteoblasts fail to form bone in a normal manner and none of the matrix is found in the process of being converted to bone. The formation of new trabeculae is therefore inhibited. Older trabeculae, that were formed previous to the scorbutic process may be present but are separated from the lattice by a zone which is devoid of newly formed trabeculae. This zone of rarefaction appears to extend entirely across the bone.

The scorbutic process, also causes the resorption of preformed bone, seemingly by a reversal of the normal bone building function of the osteoblasts. The osseous framework of the entire bone becomes attenuated and in the portions immediately adjacent to the cartilage there is also resorption of bone from the cortex. In the angle between the cortex and the lattice there may be no bone at all, merely a layer of fibrous tissue and the cartilage shaft junction is likely to be the site of peculiar distortions.

The marrow in scorbutic bone is extensively changed. Hematiopoietic tissue is largely replaced at the ephiphyseal end by a loose meshed fibrous tissue "the Grenzmark" containing few fibroblasts and much intercellular material which stains like mucin and resembles embryonic connective tissue (92).

Plate III diagramatically compares the normal costochondral junction with those demonstrating varying degrees of scurvy (44).

In order to determine the effects of large amounts of thiamin on bone

formation in scorbutic and non-scorbutic guinea pigs, male animals weighing 420-430 gms. were placed on the following daily diets for 5 weeks:

Animal No. 1 received Olympic rabbit pellets and 50 mg. ascorbic acid.

Animal No. 2 received Olympic rabbit pellets, 50 mg. ascorbic acid and 25 mg. thiamin hydrochloride.

Animal No. 3 received Olympic rabbit pellets.

Animal No. 4 received Olympic rabbit pellets, and 25 mg. thiamin hycrochloride.

After 5 weeks the animals were killed and histological sections of the costochondral junction of the third right rib of each pig were made (93). While sections were prepared from only one animal maintained on each diet, the animal showed symptoms characteristic of the diet and was otherwise considered representative.

The effects of each diet on bone formation are indicated by photographs of the sections of the costochondral junctions prepared and shown in Plates IV - XIX.

## DISCUSSION

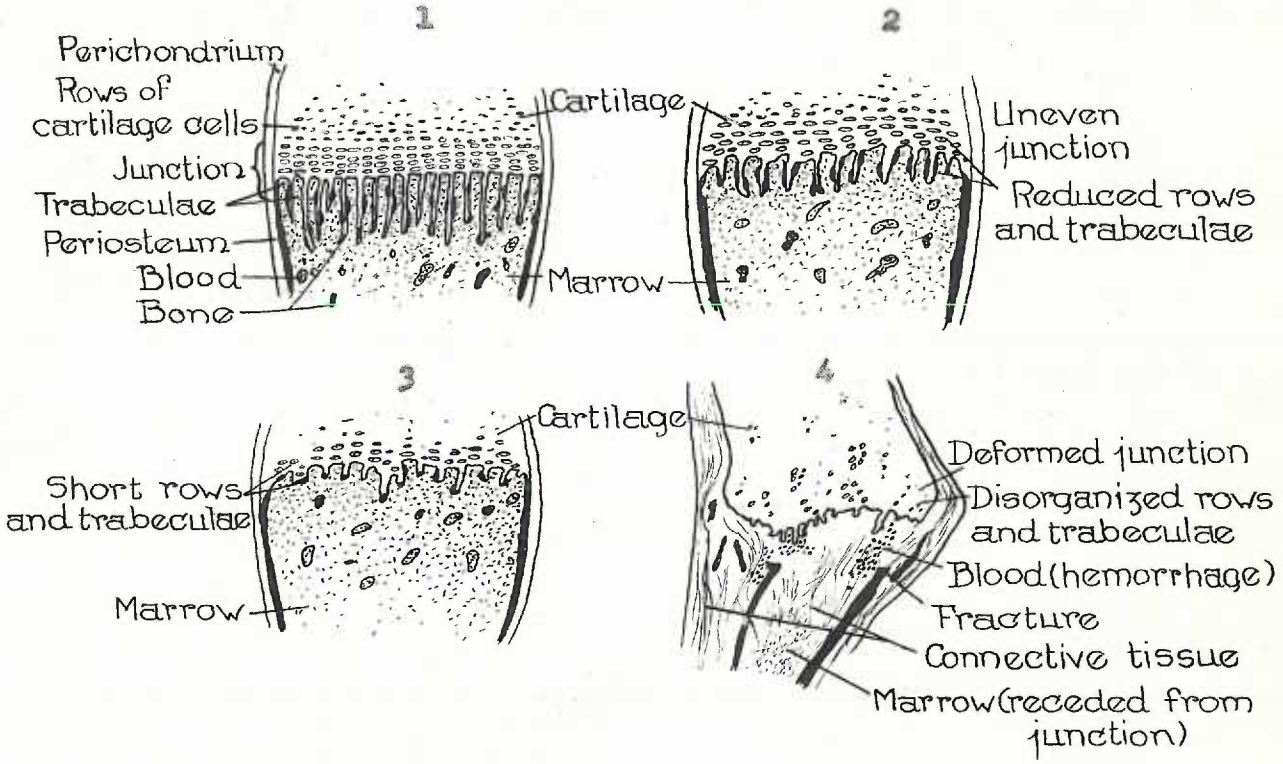
The cartilage cells in the normal bone are arranged in orderly rows, quite regularly spaced. The cartilage trabeculae between them are quite regular and present a fairly even transverse plane. The cartilage bone junction is even with no bulging. The cartilage matrix lattice is thin. Bone trabeculae and bone are seen in close proximity to the cartilage.

The bone removed from the animal receiving pellets, ascorbic acid and thiamin show a slight over all appearance of abnormality as compared to the normal. There appears to be slight disorganization of cartilage cells and slight evidence of bulging at the junction.

The bone from the animal receiving only pellets shows shorter rows of disorganized cartilage cells. There is a tendency towards circular groups. The cartilage trabeculae between the cells are shorter than normal. The cartilage shaft junction is irregular. The scorbutic lattice is thick. There is resorption of bone especially in the area immediately adjacent to the cartilage and bone and bone trabeculae are absent from this area. Macroscopic deformity is apparent (scorbutic rosary).

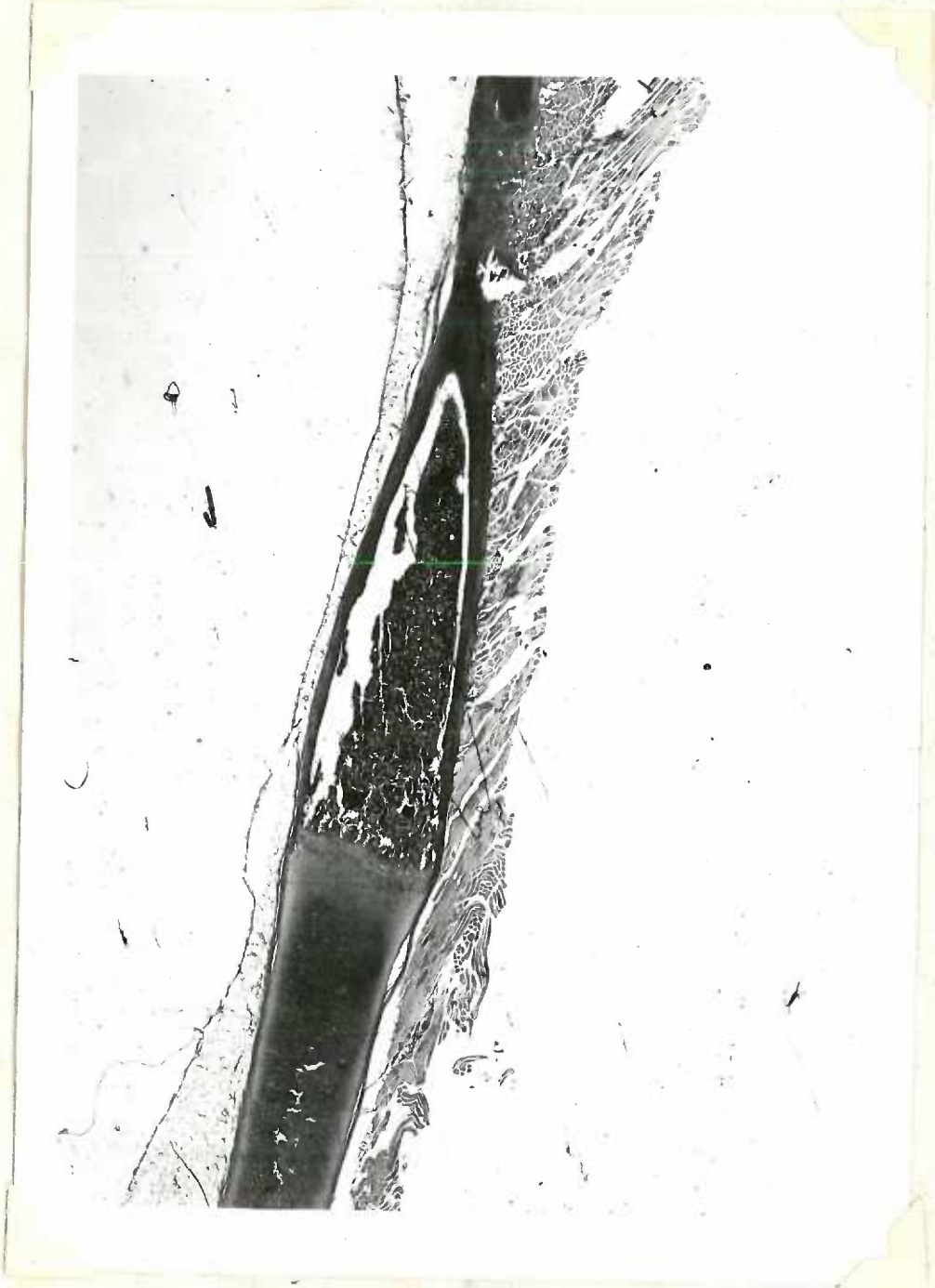
The bone from the animal receiving pellets plus thiamin pictures the cartilage cells arranged in longer, and more regular rows than shown in the case of scurvy alone. However, the deeper cartilage cells appear to be less numerous, suggesting the arrest of proliferation. These deeper cells are smaller than normal. The scorbutic lattice is very thick with a much churned appearance. Longitudinal direction of the lattice particles is lost and a more general lateral direction is assumed. There has been great resorption of bone near the junction. The area near the junction is devoid of bone and bone trabeculae. Only a connective tissue cover appears to remain. The junction shows greater deformity than that from the animal fed pellets alone.

PLATE III



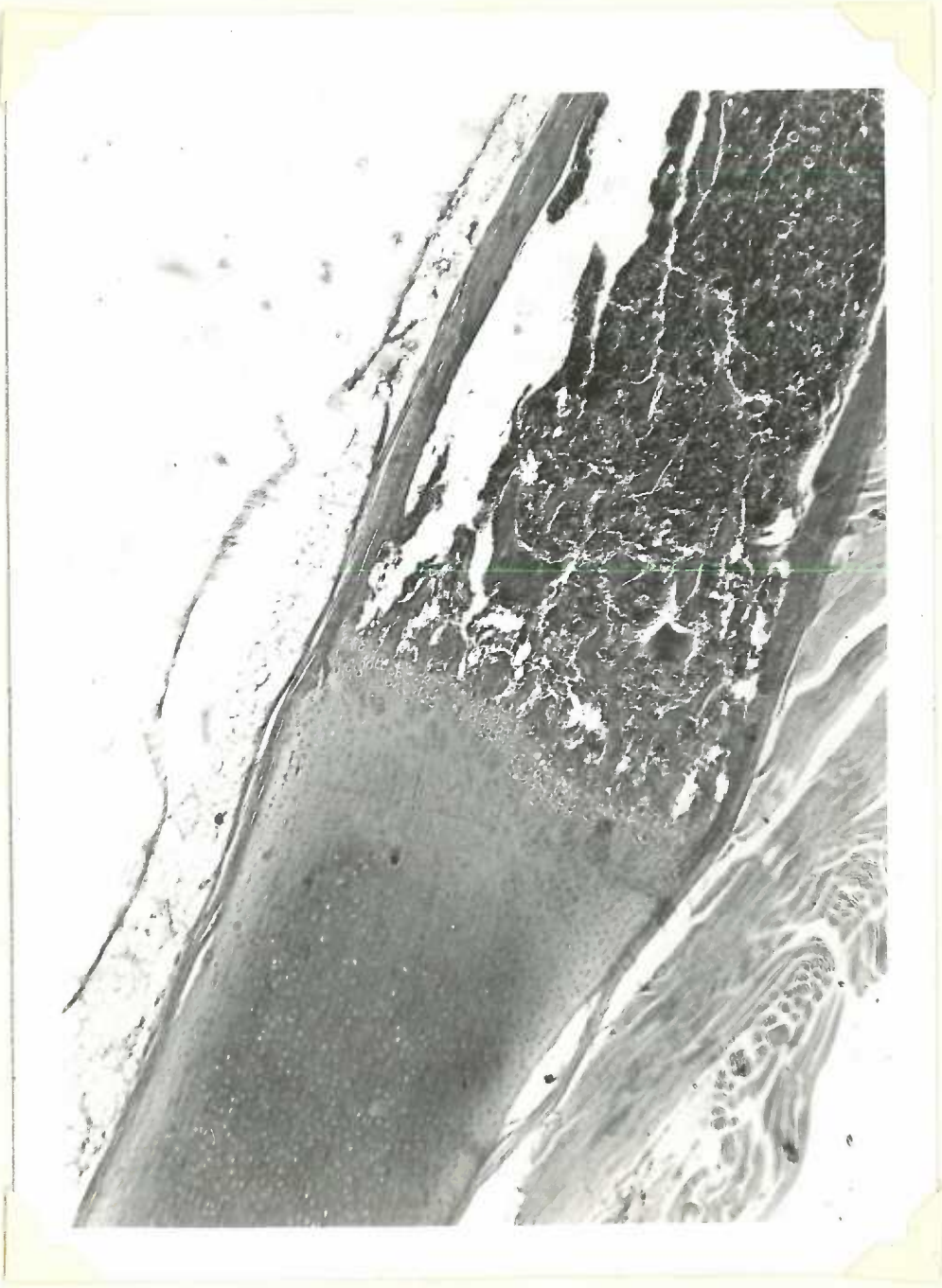

1. Diagram of normal rib-junction.
2. Diagram of rib-junction to illustrate incipient scurvy.
3. Diagram of rib-junction to illustrate definite scurvy.
4. Diagram of rib-junction to illustrate acute scurvy.

PLATE IV



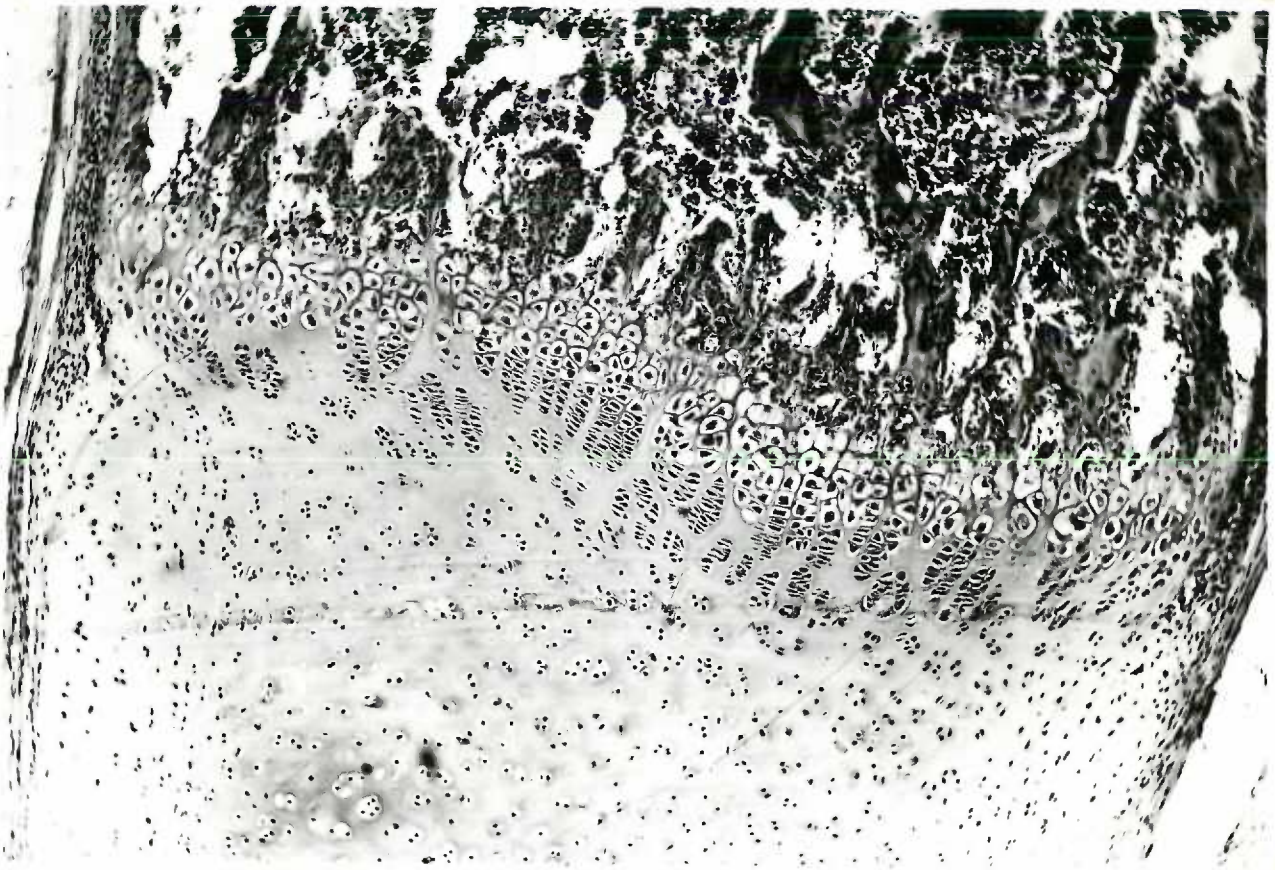
Costochondral junction from guinea pig fed Clympic rabbit pellets plus ascorbic acid

PLATE V



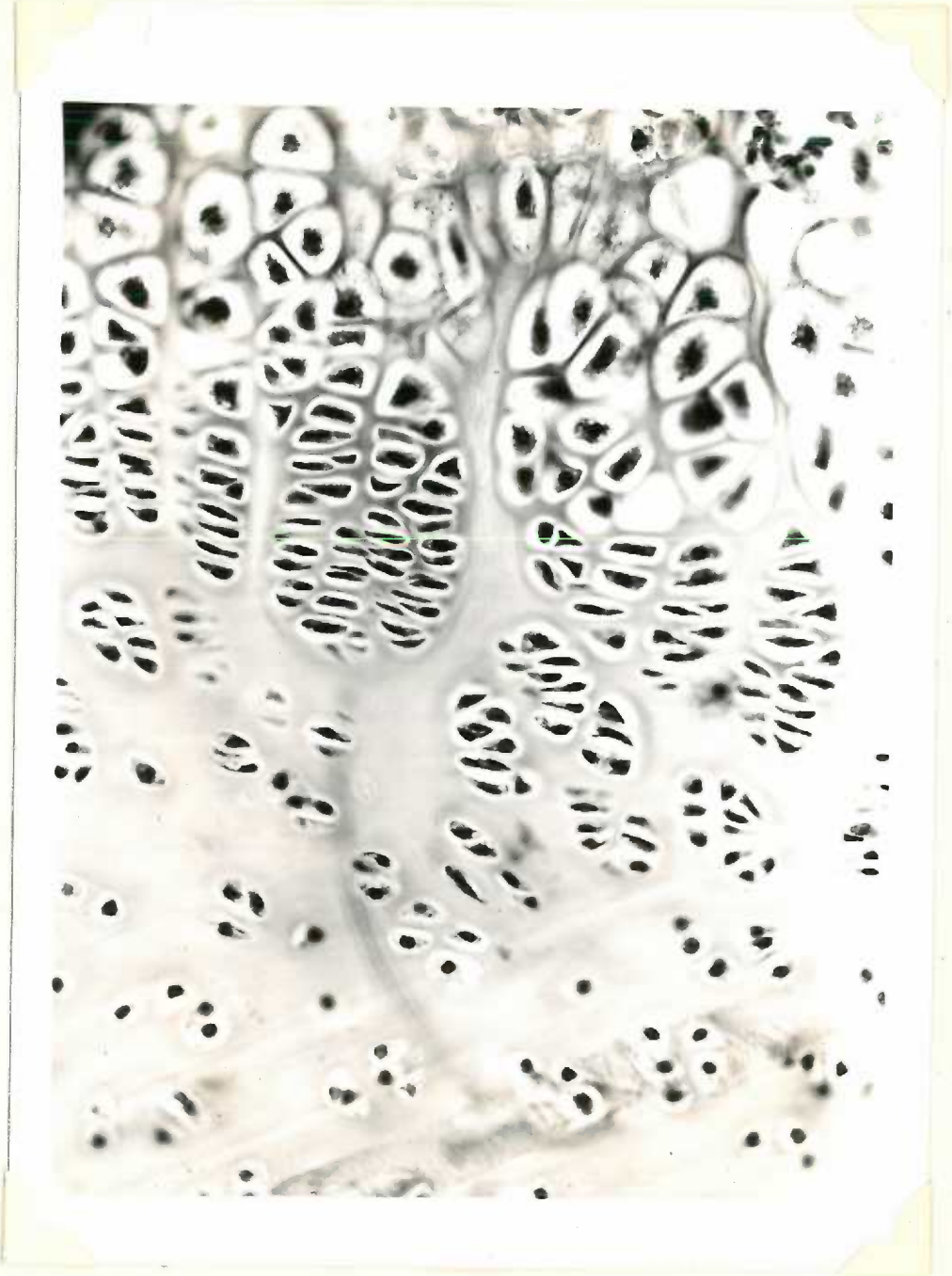
Costochondral junction from guinea pig fed Olympic rabbit pellets plus ascorbic acid

PLATE VI



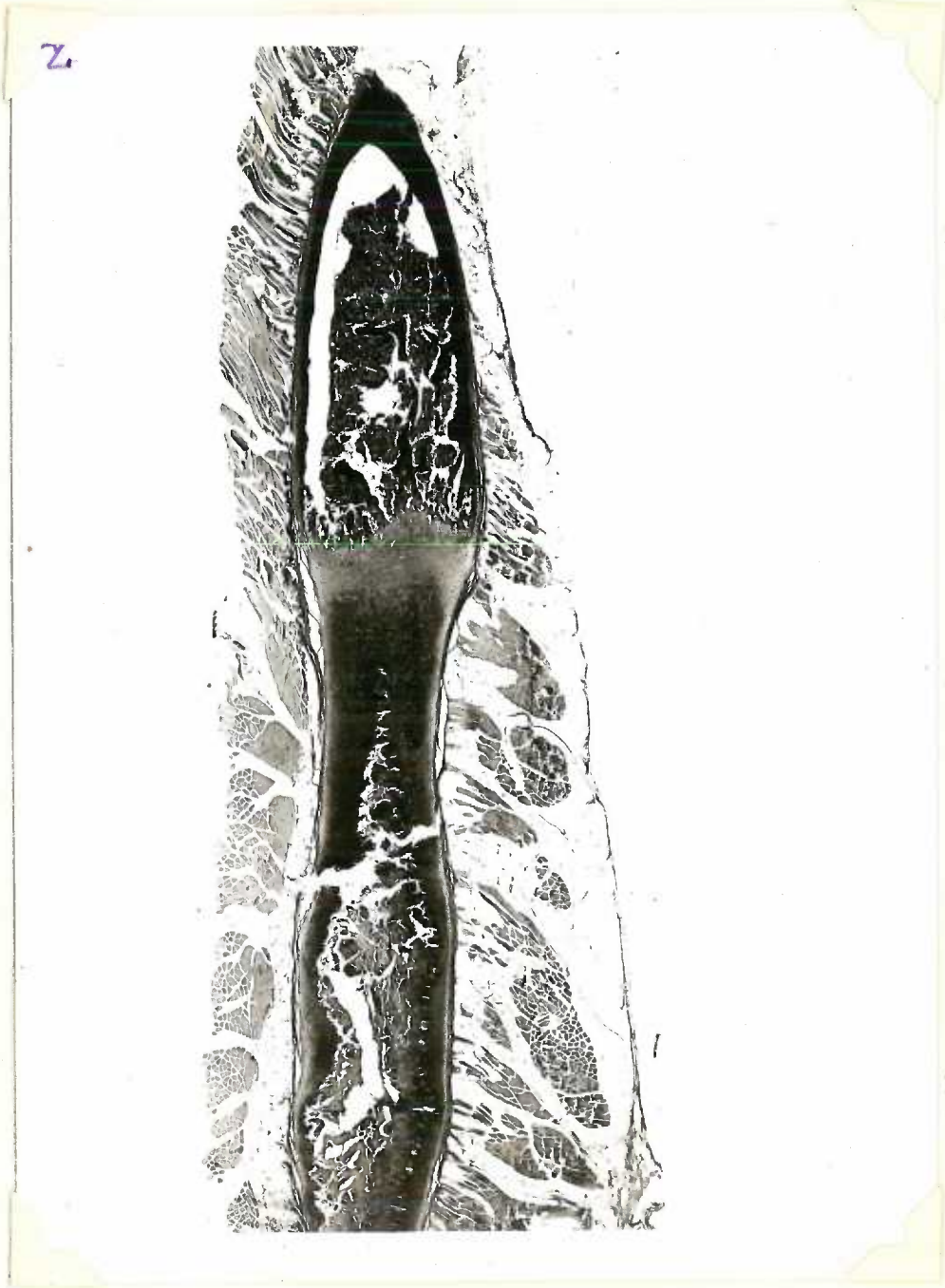
Costochondral junction from guinea pig fed Olympic rabbit pellets plus ascorbic acid

PLATE VII



Cartilage cells at costochondral junction of guinea pig fed Olympic rabbit pellets plus ascorbic acid

PLATE VIII



Costochondral junction from guinea pig fed
Olympic rabbit pellets, ascorbic acid and thiamin

PLATE IX

Costochondral junction from guinea pig fed Olympic rabbit pellets, ascorbic acid and thiamin

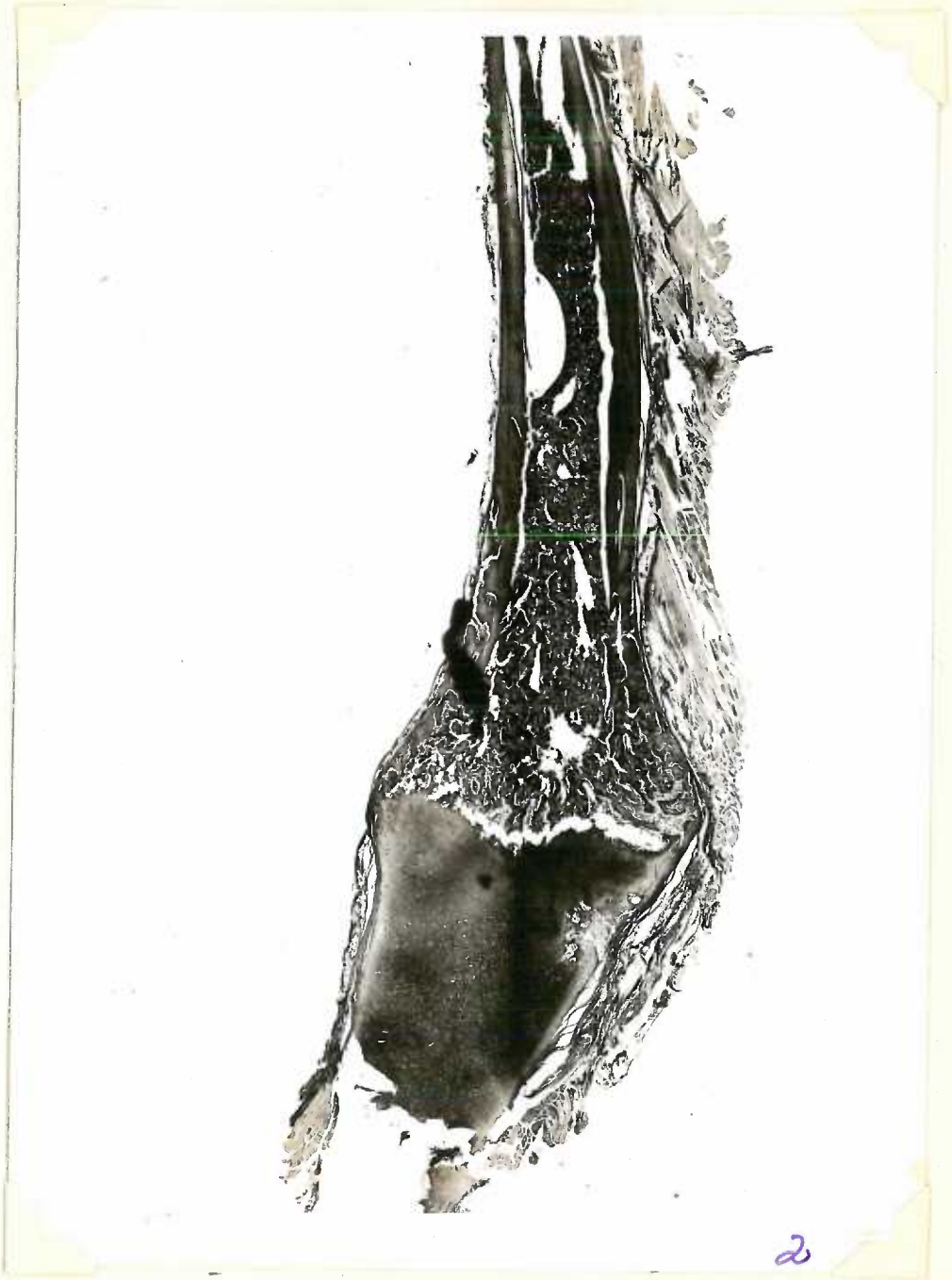
PLATE X

Costochondral junction from guinea pig fed
Olympic rabbit pellets, ascorbic acid and thiamin

PLATE XI

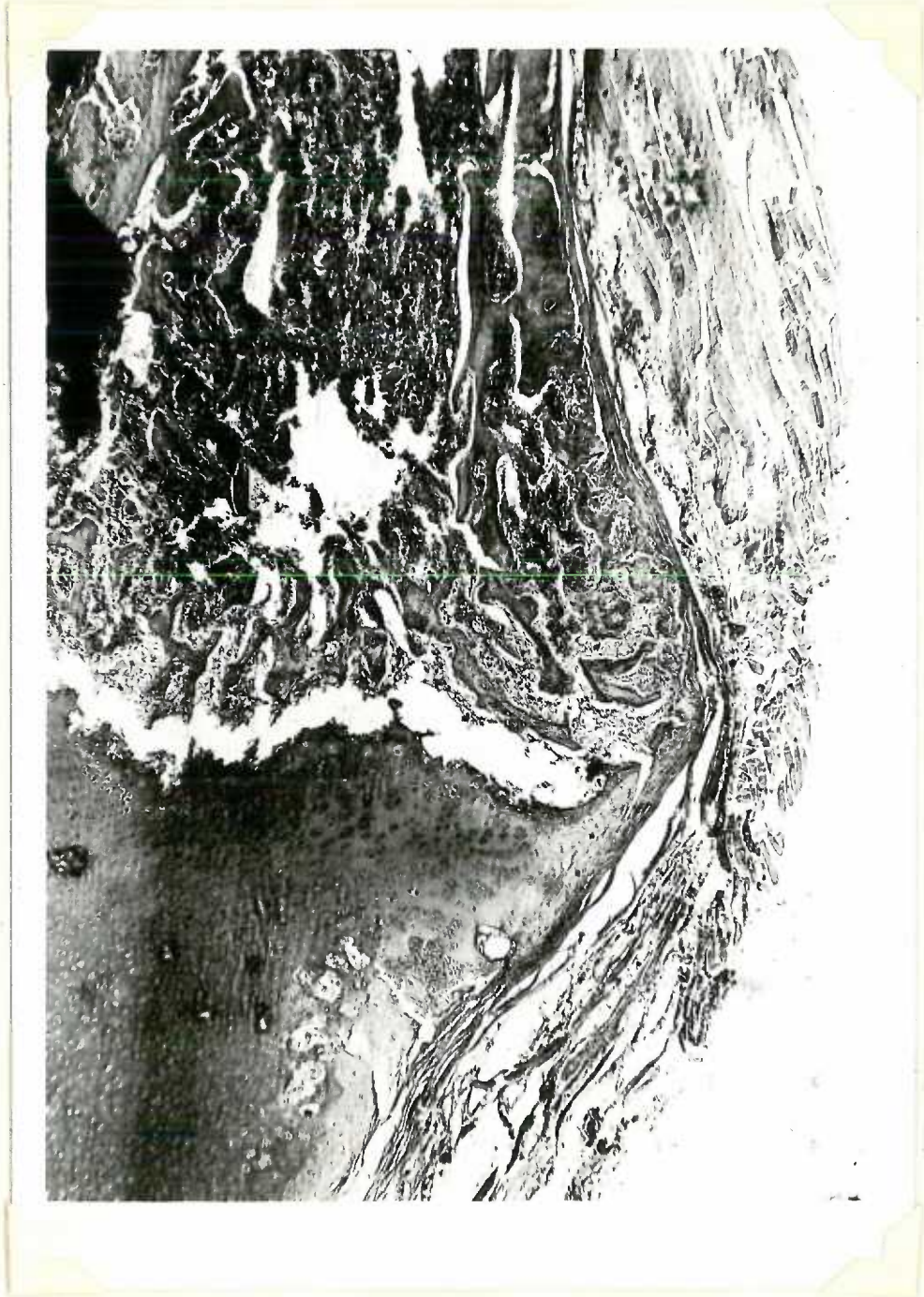
Cartilage cells at costochondral junction of guinea pig fed Olympic rabbit pellets, ascorbic acid and thiamin

PLATE XXI



Costochondral junction from guinea pig fed Olympic rabbit pellets

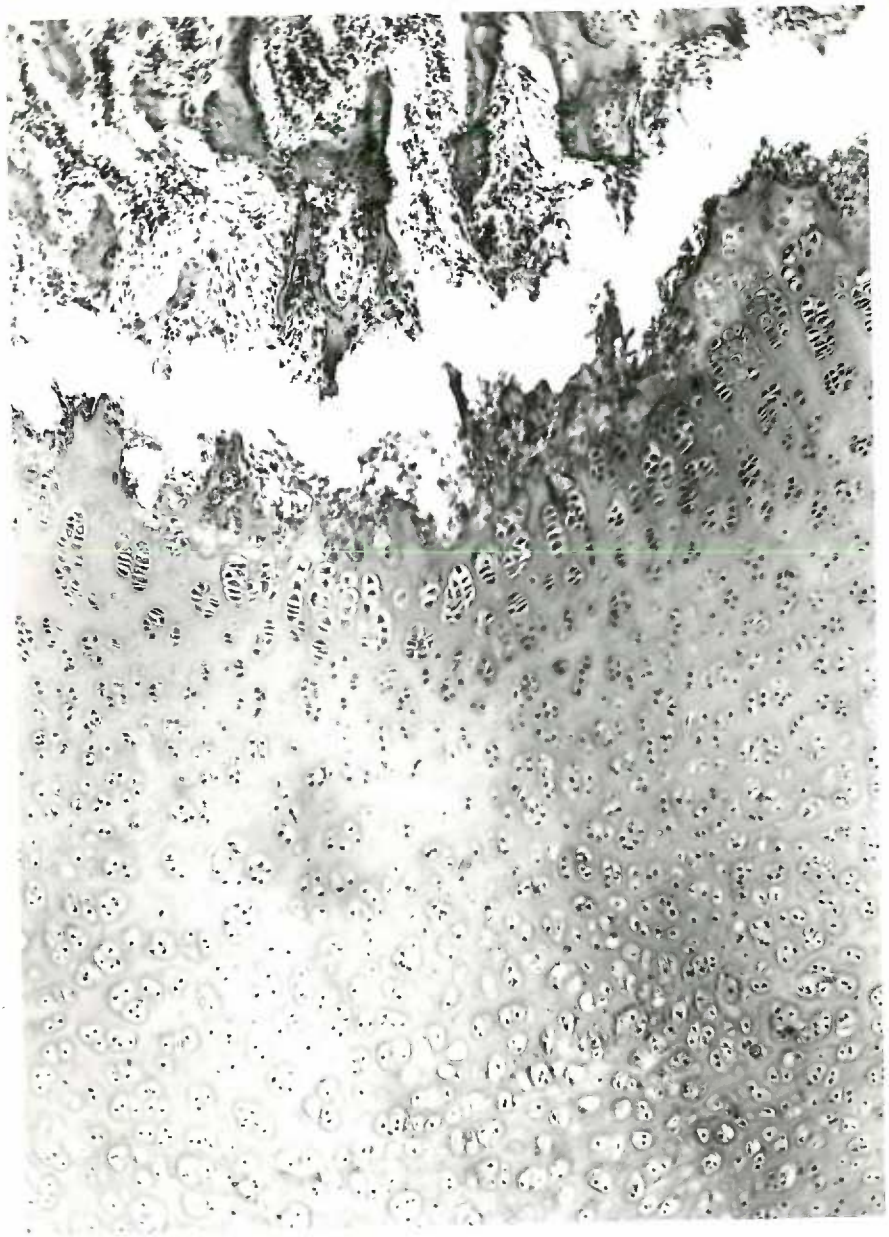
PLATE XIII



Costochondral junction from guinea pig fed

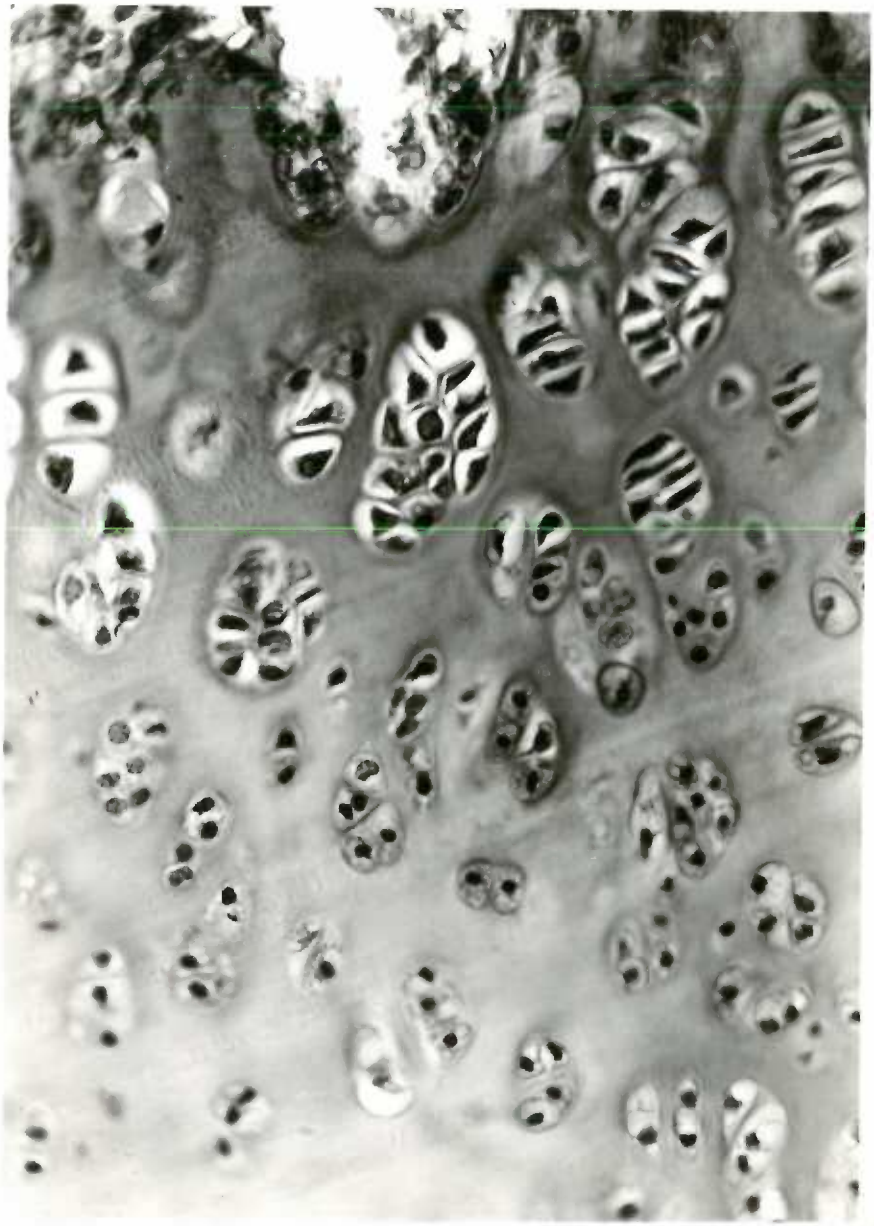
Olympic rabbit pellets

PLATE XIV



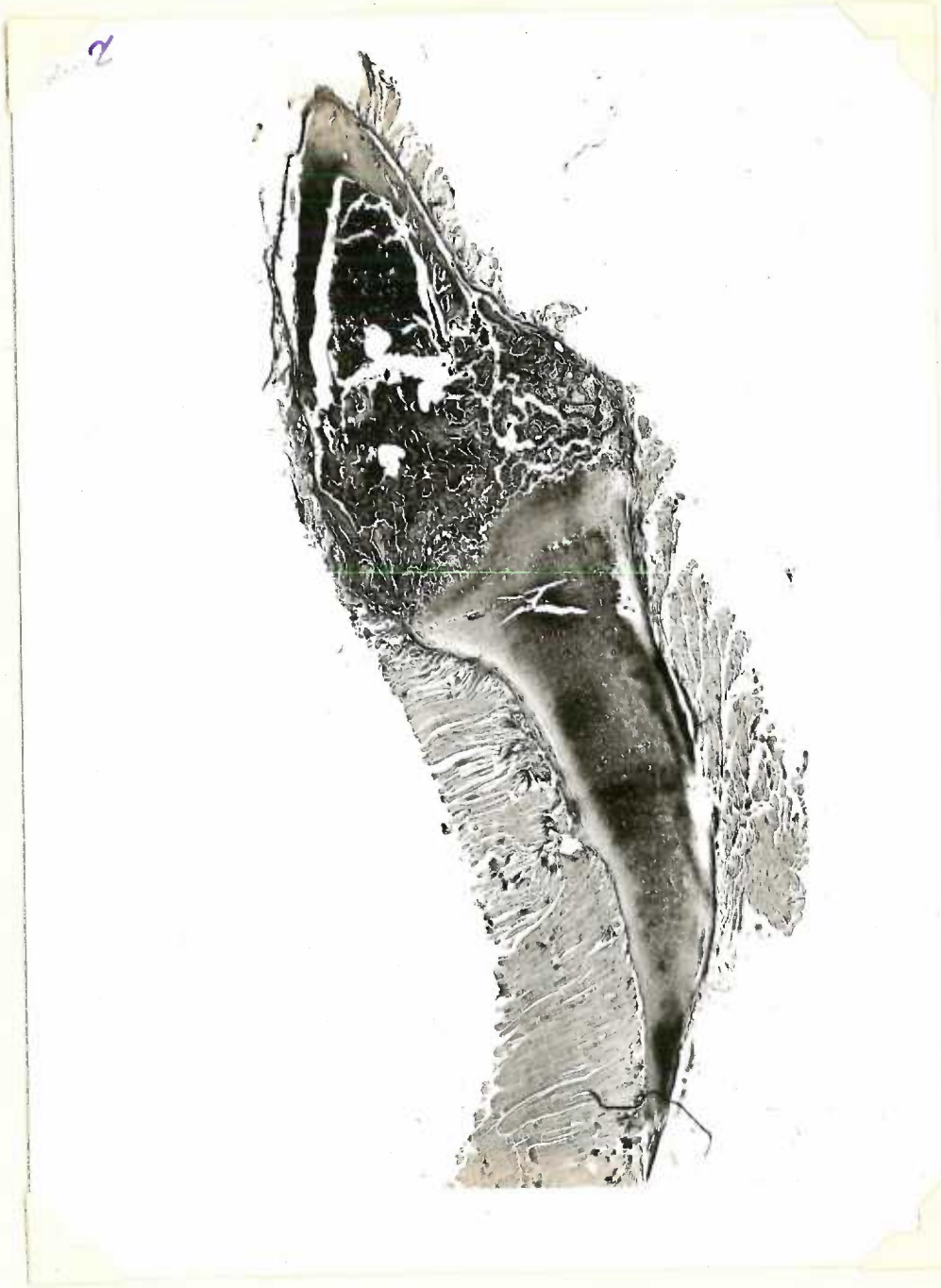
Costochondral junction from guinea pig fed Olympic rabbit pellets

PLATE XV



Cartilage cells at costochondral junction of guinea pig fed Olympic rabbit pellets

PLATE XVI



Costochondral junction from guinea pig fed Olympic rabbit pellets, plus thiamin

PLATE XVII

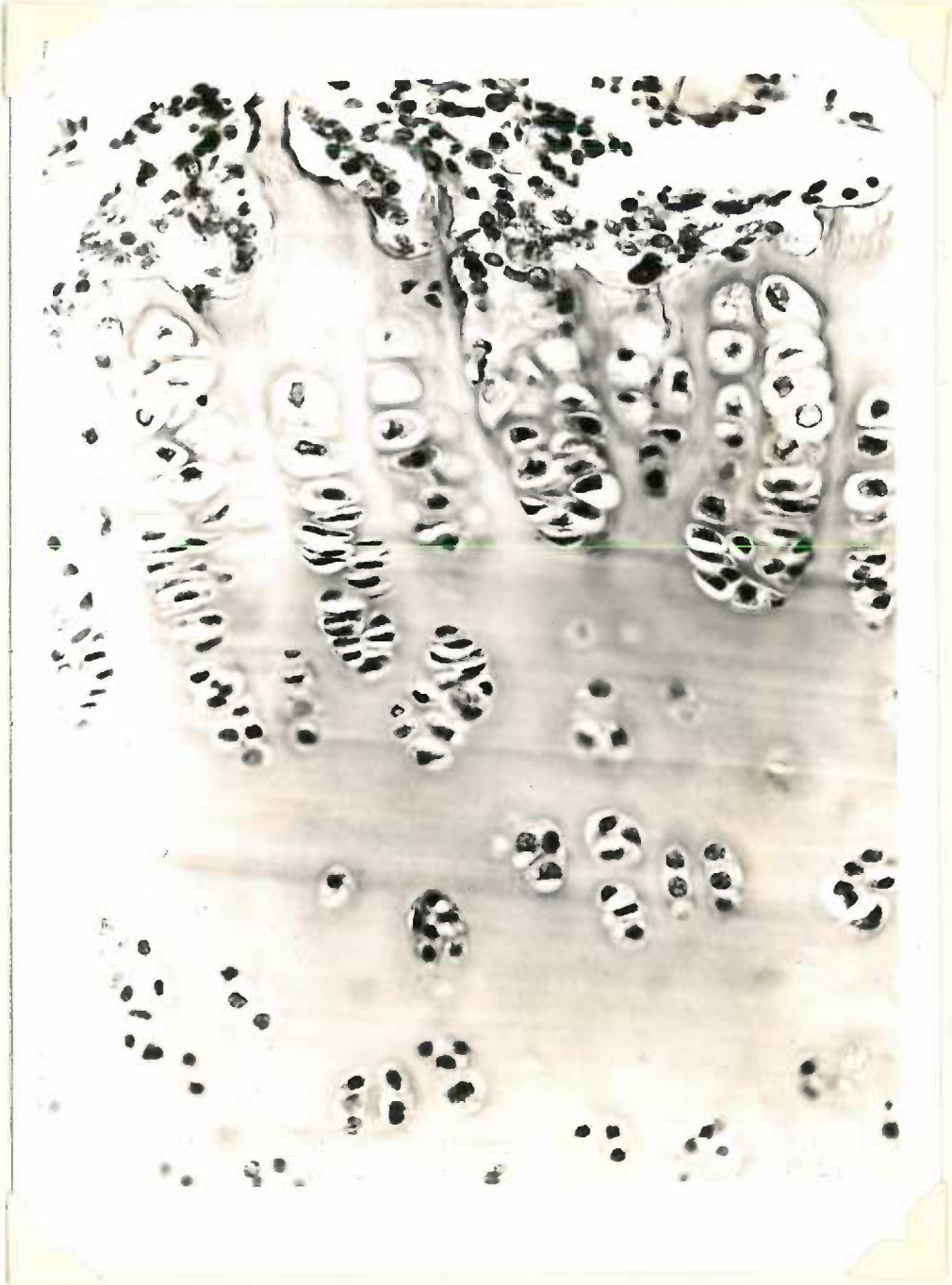
Costochondral junction from guinea pig fed
Olympic rabbit pellets, plus thiamin

PLATE XVIII

Costochondral junction from guinea pig fed

Clympic rabbit pellets plus thiamin

PLATE XIX



Cartilage cells at costochondral junction of guinea pig fed Olympic rabbit pellets plus thiamin

## SECTION IV

### Phosphorus and Calcium Excretion in Normal and Scorbutic Guinea Pigs with and without Thiamin Therapy

Since thiamin administration to scorbutic guinea pigs appears to cause increased bone destruction it was considered important to make studies of phosphorus and calcium excretion in both normal and scorbutic guinea pigs with and without thiamin administration.

Urinary and fecal phosphorus and calcium determinations were made on the excreta from male guinea pigs fed on the following diets for various lengths of time:

No. 1. Olympic rabbit pellets and 50 mg. of ascorbic acid.

No. 2 Olympic rabbit pellets, 50 mg. ascorbic acid and 25 mg. thiamin hydrochloride.

No. 3 Olympic rabbit pellets.

No. 4 Olympic rabbit pellets and 25 mg. thiamin hydrochloride.

Animals were maintained in individual metabolism cages (Plate XX). Each cage was constructed of a 7 inch glass funnel and 1/4 inch wire mesh. The cage floor consisted of a circle of the same mesh, topped by another circle of 1/16 inch wire mesh. A thin film of wet glass wool was stretched across the lower end of the funnel. The stem of the funnel dipped into a 25 cc. graduated cylinder containing 2 cc. concentrated hydrochloric acid. The cage was supported by a wooden stand and a weighted wooden slab served as a cage top. Water and food were supplied from containers wired to the cage wall.

At the end of each 24 hour collection period the funnel was carefully rinsed with dilute hydrochloric acid and emptied into the graduated cylinder. Feces were collected and placed in 20 percent sulfuric acid.

Modifications of the methods for the determination of phosphorus and calcium developed by Fisk and Subarrow (93) and Clark and Collip (94) respectively, were used as follows:

A. Preliminary preparation of urine;

Each 24 hour sample of urine was diluted to 25 cc. with distilled water and well mixed. A 1 cc. sample of the diluted urine was pipetted into a 12 x 1 inch pyrex test tube graduated with a 25 cc. mark. 1 cc. of concentrated nitric acid was added. The mixture was boiled until frothing ceased. 1 cc. of concentrated sulfuric acid was then added and boiling was continued until there was no further evolution of red fumes. This was followed by the addition of 1 cc. of perchloric acid (60 percent) and the mixture was boiled until colorless. When cool, 10 cc. of water were pipetted into the tube followed by the successive additions of 4 cc. of concentrated ammonium hydroxide and 2 drops of brom cresol purple. The contents of the tube were then shaken gently to mix, neutralized by further addition of concentrated ammonium hydroxide and diluted to the 25 cc. mark.

1. Urinary phosphorus;

A 2 cc. sample of the above digest was pipetted into a 100 cc. volumetric flask. 5 cc. of a standard solution containing 0.4 mg. phosphorus were pipetted into a second 100 cc. flask. To the contents of each flask were added 10 cc. ammonium molybdate solution (2.5 percent ammonium molybdate in 5 N sulfuric acid) and 2 cc. of amidol solution (1 percent 2, 4-diamino phenol dihydrochloride in 20 percent sodium bisulfite). The contents of the flasks were diluted to 100 cc. with distilled water and allowed to stand 10 minutes for color development. Samples of the solution were then compared in a photoelectric colorimeter which had been zeroed to water.

2. Urinary calcium;

2 cc. of the diluted digest were pipetted into a graduated 15 cc. centrifuge tube. 2 cc. of 4 percent ammonium oxalate solution and 1 cc. of distilled water were added and the contents of the tube were thoroughly mixed. The mixture was allowed to stand for 1 hour, then centrifuged for 5 minutes at 1000 - 1500 revolutions perminute. The supernatent fluid was poured off and the tube was allowed to stand inverted on filter paper for 5 minutes. The mouth of the tube was then wiped and the sides of the tube were washed with 3 cc. of 2 percent ammonium hydroxide from a wash bottle. The mixture was centrifuged and drained as before. 2 cc of 1 N sulfuric acid were then blown into the tube from a pipette. The tube was placed in a boiling water bath for 1 minute then its contents were immediately titrated with $N/_{100}$ potassium permanganate.

B. Preliminary preparation of feces;

Each 24 hour sample was diluted to 1000 cc. with 10 percent sulfuric acid. 10 cc. of this material were pipetted into a small crucible and dried over a low flame. The residue was then mixed with 1 gm. of sodium nitrate and the crucible was placed in a furnace and its contents ashed. After ashing, 10 cc. of 15 percent hydrochloric acid were added to the residue and mixed well. After standing over night the contents of the crucible were transferred to a 100 cc. volumetric flask with careful rinsing. 2 drops of brom cresol purple were added, the solution was neutralized with concentrated ammonium hydroxide, diluted to the mark and filtered.

1 - 2. Fecal phosphorus and calcium;

The filtrate prepared in B was used for the determination of fecal phosphorus and calcium. The same procedures described for the determination of urinary phosphorus and calcium were used.

The effects of the four diets on phosphorus and calcium excretion are summarized in the following corresponding tables:

## PHOSPHORUS EXCRETION

### Table Ia - Effect of Normal Diet on Daily Urinary Phosphorus Excretion

| Animal Number | FIRST WEEK Wt. gms. | Food gms. | P. mgs. | P. mgs. / food gms. | SECOND WEEK Wt. gms. | Food gms. | P. mgs. | P. mgs. / food gms. | THIRD WEEK Wt. Gms. | Food gms. | P. mgs. | P. mgs. / food gms. | FOURTH WEEK Wt. Gms. | Food gms. | P. mgs. | P. mgs. / food gms. | FIFTH WEEK Wt. Gms. | Food gms. | P. mgs. | P. mgs. / food gms. | SIXTH WEEK Wt. Gms. | Food gms. | P. mgs. | P. mgs. / food gms. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *343 | 21 | 25.67 | 1.22 | 343 | 25 | 22.07 | .88 | 348 | 25 | 26.01 | 1.03 | 406 | 25 | 25.08 | .99 | 448 | 31 | 25.17 | .81 | 462 | 33 | 29.47 | .88 |
| 2 | - | - | 21.08 | - | 434 | - | 20.87 | - | 448 | - | 20.89 | - | 504 | 33 | 30.88 | .92 | 546 | 35 | 23.35 | .67 | | | | |
| 3 | 434 | 15 | 17.53 | 1.16 | | | | | | | | | | | | | | | | | | | | |
| 4 | 434 | 23 | 25.90 | 1.12 | | | | | | | | | | | | | | | | | | | | |
| 5 | 434 | 17 | 24.37 | 1.43 | | | | | | | | | | | | | | | | | | | | |
| **Table IIa - Effect of Normal Diet Plus Thiamin on Daily Urinary Phosphorus Excretion** | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | 364 | 21 | 18.73 | .87 | 364 | 23 | 17.24 | .76 | 378 | 27 | 34.39 | 1.27 | 434 | 25 | 17.05 | .69 | 448 | 26 | 34.42 | 1.314 | 476 | 33 | 31.26 | .95 |
| 7 | - | - | 19.84 | - | 476 | - | 19.01 | - | 483 | - | 20.87 | - | 518 | 30 | 21.82 | .72 | 560 | 35 | 21.87 | .63 | | | | |
| **Table IIIa - Effect of Scorbutic Diet on Daily Urinary Phosphorus Excretion** | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | 392 | 22 | 27.92 | 1.26 | 385 | 23 | 30.16 | 1.28 | 406 | 23 | 44.60 | 1.95 | 427 | 27 | 29.94 | 1.09 | 448 | 31 | 40.50 | 1.27 | 476 | 25 | 53.26 | 2.11 |
| 9 | - | - | 19.82 | - | 504 | - | 23.62 | - | 504 | - | 29.34 | - | 588 | 29 | 33.36 | 1.75 | 520 | 11 | 20.17 | 1.86 | | | | |
| 10 | 756 | 29 | 23.53 | .811 | 761 | 39 | 25.84 | .66 | 796 | 39 | 34.20 | .87 | 796 | 33 | 25.84 | .78 | 872 | 31 | 26.60 | .85 | | | | |
| **Table IVa - Effect on Scorbutic Diet Plus Thiamin on Daily Urinary Phosphorus Excretion** | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | 427 | 24 | 27.90 | 1.15 | 427 | 26 | 22.44 | .87 | 448 | 31 | 33.06 | 1.07 | 490 | 29 | 28.91 | 1.00 | 490 | 33 | 28.23 | .85 | 490 | 32 | 29.60 | .92 |
| 12 | - | - | 23.06 | - | 490 | - | 24.62 | - | 504 | - | 22.15 | - | 546 | 25 | 36.19 | 1.87 | 462 | 8 | 27.15 | 3.29 | | | | |
| 13 | 518 | 25 | 28.12 | 1.22 | 476 | 26 | 28.12 | 1.10 | 511 | 31 | 31.92 | 1.02 | 511 | 29 | 26.60 | .9174 | 532 | 32 | 25.81 | .80 | | | | |
| 14 | 616 | 29 | 20.52 | .70 | 616 | 33 | 31.90 | .96 | 658 | 33 | 28.68 | .87 | 665 | 26 | 25.81 | .99 | 644 | 25 | 28.12 | 1.14 | | | | |
| **Table IIIb - Effect of Scorbutic Diet on Daily Fecal Phosphorus Excretion** | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | 756 | 29 | - | - | 761 | 39 | 100.60 | 2.58 | 796 | 39 | 121.21 | 3.17 | 796 | 33 | 118.84 | 3.60 | 782 | 31 | 246.05 | 7.93 | 714 | 12 | 9.07 | .75 |
| 15 | | | | | | | | | 406 | 19 | 30.43 | 1.60 | 350 | 9 | 24.34 | 2.70 | | | | | | | | |
| 16 | | | | | | | | | 490 | 25 | 27.39 | 1.09 | 448 | 8.5 | 15.21 | 1.79 | | | | | | | | |
| **Table IVb - Effect of Scorbutic Diet Plus Thiamin on Daily Fecal Phosphorus Excretion** | | | | | | | | | | | | | | | | | | | | | | | | |
| 13 | 518 | | | | 476 | 28 | 80 | 2.58 | 511 | 31 | 70.30 | 2.27 | 511 | 29 | 82.42 | 2.65 | 532 | 32 | 90 | 2.81 | 532 | 31 | 25.7 | .83 |
| 14 | 616 | | | | 616 | 33 | 105.45 | 3.19 | 658 | 33 | 76.4 | 2.31 | 665 | 26 | 100.60 | 3.86 | 644 | 25 | 133.33 | 5.33 | 588 | 6 | 33.28 | 5.55 |
| 17 | | | | | | | | | 448 | 26 | 24.54 | .93 | 406 | 4.5 | 12.17 | 2.70 | | | | | | | | |
| 18 | | | | | | | | | 406 | 17 | 21.3 | 1.25 | 427 | 9. | 33.4 | 3.71 | | | | | | | | |

*represents weekly average of daily determinations

## CALCIUM EXCRETION

### Table Ian - Effect of Normal Diet on Daily Urinary Calcium Excretion

| Animal Number | FIRST WEEK Wt. gms. | Food gms. | Ca. mgs. | Ca. mgs. Food gms. | SECOND WEEK Wt. gms. | Food gms. | Ca. mgs. | Ca. mgs. Food gms. | THIRD WEEK Wt. gms. | Food gms. | Ca. mgs. | Ca. mgs. Food gms. | FOURTH WEEK Wt. gms. | Food gms. | Ca. mgs. | Ca. mgs. Food gms. | FIFTH WEEK Wt. gms. | Food gms. | Ca. mgs. | Ca. mgs. Food gms. | SIXTH WEEK Wt. gms. | Food gms. | Ca. mgs. | Ca. mgs. Food gms. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | * 343 | 21 | 25.46 | 1.12 | 343 | 25 | 25.10 | 1.004 | 343 | 25 | 28.22 | 1.12 | 406 | 25 | 22.91 | .91 | 448 | 31 | 24.68 | .79 | 462 | 33 | 24.42 | .74 |
| 2 | - | - | 31.79 | - | 434 | - | 24.75 | - | 434 | - | 27.89 | - | 504 | 33 | 28.95 | .87 | 546 | 35 | 30.12 | .86 | | | | |
| 3 | 434 | 15 | 7.50 | 0.50 | | | | | | | | | | | | | | | | | | | | |
| 4 | 434 | 23 | 30.00 | 1.30 | | | | | | | | | | | | | | | | | | | | |
| 5 | 434 | 17 | 21.87 | 1.28 | | | | | | | | | | | | | | | | | | | | |

### Table IIan - Effect of Normal Diet Plus Thiamin on Daily Urinary Calcium Excretion

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 364 | 21 | 22.12 | 1.05 | 364 | 23 | 25.27 | 1.09 | 378 | 27 | 39.08 | 1.44 | 343 | 25 | 28.34 | 1.13 | 448 | 26 | 30.74 | 1.18 | 476 | 33 | 30.64 | .92 |
| 7 | - | - | 28.04 | - | 474 | - | 28.00 | - | 483 | - | 22.47 | - | 516 | 30 | 24.75 | .82 | 560 | 35 | 29.11 | .80 | | | | |

### Table IIIan - Effect of Scorbutic Diet on Daily Urinary Calcium Excretion

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 392 | 22 | 25.13 | 1.14 | 385 | 23 | 33.66 | 1.46 | 406 | 28 | 39.56 | 1.72 | 427 | 27 | 21.28 | .78 | 448 | 31 | 36.70 | 1.18 | 476 | 25 | 29.09 | 1.50 |
| 9 | - | - | 25.40 | - | 504 | - | 21.62 | - | 504 | - | 27.00 | - | 538 | 29 | 28.74 | .99 | 520 | 11 | 18.58 | 1.68 | | | | |
| 10 | 756 | 29 | 58.45 | 2.01 | 761 | 39 | 68.74 | 1.76 | 796 | 39 | 68.74 | 1.76 | 796 | 33 | 53.10 | 1.60 | 782 | 31 | 62.60 | 2.01 | | | | |

### Table IVan - Effect of Scorbutic Diet on Daily Urinary Calcium Excretion

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 427 | 24 | 20.76 | .86 | 427 | 26 | 28.38 | 1.09 | 448 | 31 | 38.01 | 1.22 | 490 | 29 | 31.81 | 1.07 | 490 | 33 | 35.40 | 1.07 | 490 | 32 | 27.21 | .85 |
| 12 | - | - | 23.24 | - | 490 | - | 28.22 | - | 504 | - | 22.99 | - | 546 | 23 | 31.70 | 1.37 | 462 | 8 | 28.15 | 3.38 | | | | |
| 13 | 518 | 23 | 30.62 | 1.33 | 476 | 26 | 84.21 | 3.00 | 511 | 31 | 76.36 | 2.45 | 511 | 29 | 63.70 | 2.20 | 532 | 32 | 67.49 | 2.10 | | | | |
| 14 | 616 | 29 | 44.37 | 1.53 | 616 | 33 | 75.62 | 2.29 | 658 | 33 | 58.49 | 1.77 | 665 | 26 | 54.25 | 2.08 | 644 | 25 | 38.58 | 1.54 | | | | |

### Table IIIbb - Effect of Scorbutic Diet on Daily Fecal Calcium Excretion

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 756 | 29 | - | - | 761 | 39 | 75.00 | 1.92 | 796 | 39 | 80 | 2.06 | 796 | 33 | 90 | 2.71 | 782 | 31 | 125 | 4.03 | 714 | 12 | 176 | 14.66 |
| 15 | | | | | | | | | 406 | 19 | 325 | 17.10 | 350 | 9 | 150 | 16.67 | | | | | | | | |
| 16 | | | | | | | | | 490 | 25 | 328 | 13.00 | 448 | 8.5 | 225 | 26.46 | | | | | | | | |

### Table IVbb - Effect of Scorbutic Diet Plus Thiamin on Daily Fecal Calcium Excretion

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 518 | 23 | 110 | 4.35 | 476 | 23 | 100 | 3.60 | 511 | 29 | 90 | 3.10 | 532 | 32 | 150 | 4.70 | 532 | 31 | 275 | 8.80 | | | | |
| 14 | 616 | 29 | 109 | 3.76 | 616 | 33 | 94 | 2.48 | 658 | 33 | 127 | 3.85 | 665 | 26 | 150 | 5.76 | 595 | 6 | 225 | 37.50 | | | | |
| 17 | | | | | | | | | 448 | 26 | 375 | 14.42 | 406 | 4.5 | 325 | 72.22 | | | | | | | | |
| 18 | | | | | | | | | 406 | 17 | 225 | 13.27 | 427 | 9 | 250 | 27.77 | | | | | | | | |

* represents weekly average of daily determinations

# DISCUSSION

Salter and Aub (96) have reported that the well known cessation of growth in bones in scurvy is accompanied by a failure of calcium deposition. Bahrdt and Edlstein (97) have found a decrease of calcium and phosphorus in bones of infants dead with scurvy. Bayer (98) has reported that in scurvy calcium and phosphorus do not deposit in bone until ascorbic acid is given. Baumann and Howard (99), Chaney and Blunt (100) and Salter (101) have reported deposition of calcium in bone in healing scurvy.

Baumann and Howard (99) have reported a slight increase in urinary calcium excretion in scurvy. Nagayama and Muneshiso (102) have reported an increase in both phosphorus and calcium excretion in scurvy. They found the increase of calcium excretion to be greatest in the feces and the increase of phosphorus excretion to be greatest in the urine.

According to the data summarized in the tables of this section, thiamin does not appear to increase the urinary phosphorus excretion in guinea pigs fed pellets plus ascorbic acid or in guinea pigs fed pellets alone.

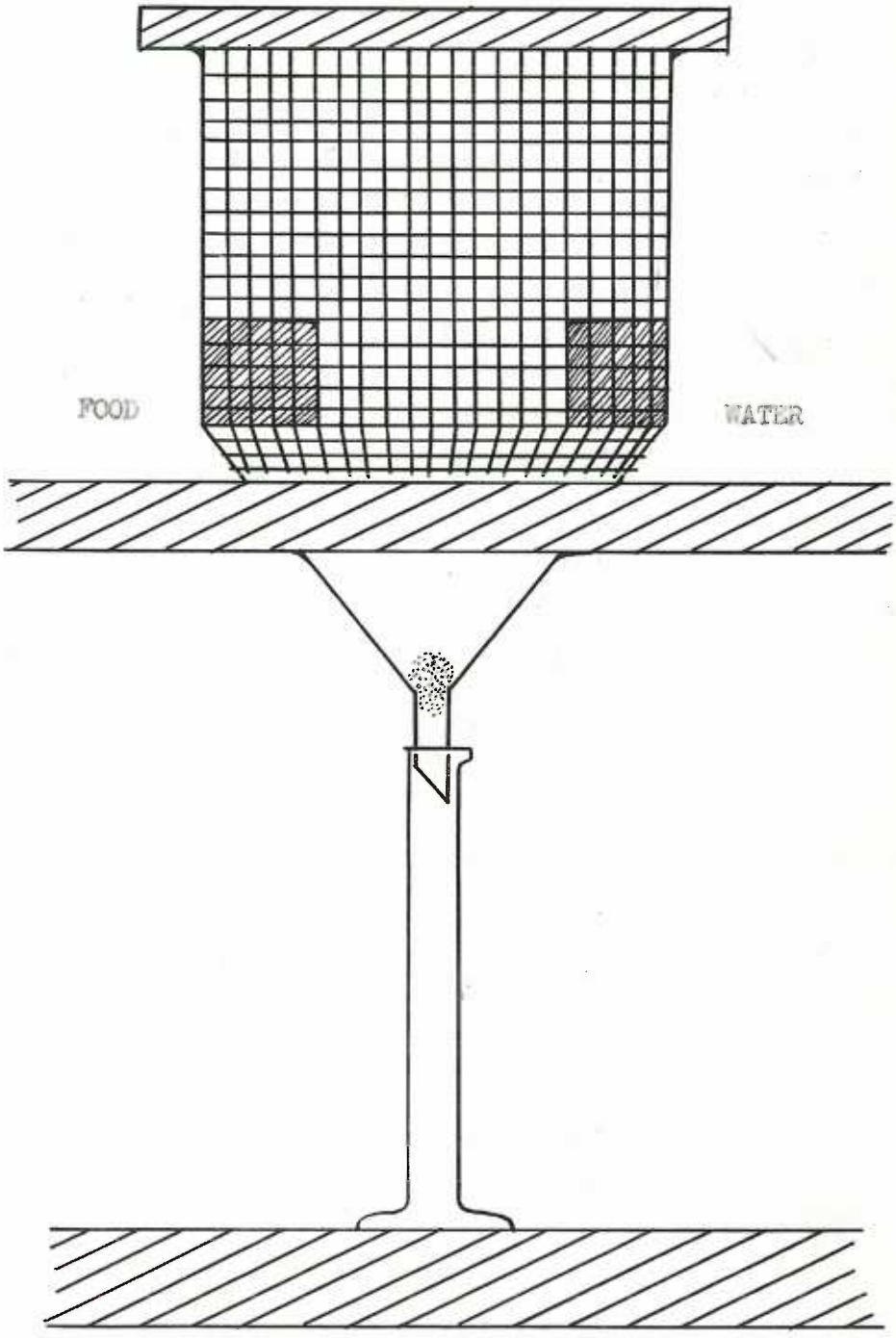
Thiamin does not appear to increase the urinary calcium excretion in guinea pigs fed pellets plus ascorbic acid or in guinea pigs fed pellets alone.

Thiamin does not appear to appreciably increase the fecal phosphorus excretion in guinea pigs fed pellets alone.

Thiamin does cause a marked increase of fecal calcium excretion in guinea pigs fed pellets alone. Data relative to the effects of thiamin on fecal calcium excretion in guinea pigs fed pellets plus ascorbic acid should be obtained.

Olympic rabbit pellets contain a calcium average of 8 mgs. per gm. and a phosphorus average of 6.2 mgs. per gm. (103). Guinea pigs have been maintained for several months in apparent good condition on a diet of these pellets plus ascorbic acid supplement. However, data of Tables III bb and IV bb indicate that guinea pigs fed pellets alone or pellets plus thiamin for several weeks show a negative calcium balance.

PLATE XX




METABOLISM CAGE

## SECTION V

### The Effects of Scurvy and Scurvy Plus Thiamin Upon Muscle and Peripheral Nerves

Freund and co-workers (104) (105) (106) observed the presence of pain, tenderness, hyperflexia and muscular dystrophy among cases, during their study of rheumatoid arthritis. These signs suggested the possibility of nerve involvement and special attention was given to the investigation of the nervous system in conjunction with the study of arthritis. It was found that pathological lesions were consistently present in the peripheral nerve trunks (sciatic, femoral, brachial plexus) of the cases studied.

The characteristic findings were represented by an inflammatory type of nodule in the perineurium of the nerve trunks. Histologically each nodule consisted of two or three zones, a central zone of necrosis which might or might not be present, an intermediate zone of proliferating mesenchymal cells and a peripheral ring like zone of inflammation containing lymphocytes and plasma cells.

Further investigation also revealed that similar inflammatory nodules were present within the muscle. Besides these inter muscular nodules hydropic degeneration, edema, loss of striation, marked swelling or shrinkage and atrophy of muscle fibers was found.

Since scorbutic guinea pigs also consistently demonstrate the symptoms of pain, tenderness and hyperflexia, it was decided that the study of muscle and nerve preparations from such animals and from scorbutic animals fed thiamin should be undertaken in an attempt to determine the presence or absence of pathological states in these tissues.

Histological sections made with hematoxylin and Van Gieson staining techniques were prepared. The tissue was taken from the right sciatic nerve and adjacent muscle of male guinea pigs maintained on Olympic rabbit pellets for 5 weeks, and from pigs maintained on Olympic rabbit pellets for 5 weeks plus a daily supplement of 2.5 mg. of thiamin hydrochloride in aqueous solution fed by dropper.

The nerves from the scorbutic guinea pigs showed signs of myelin degeneration as did the nerves from scorbutic pigs fed thiamin. This finding if confirmed would indicate that thiamin in the absence of ascorbic acid does not contribute toward maintenance of the myelin sheath. The muscle fibers from the scorbutic animals fed thiamin were unusually small.

Further work on these problems is in progress and the preparation of histological sections by the Marchi method is planned.

# SECTION VI

## The Effects of Large Amounts of Thiamin and Chloretone on Rats Receiving Rachitogenic and Rickets-Healing Diets

The bone pictures of the costochondral junctions which indicate increased bone destruction in scorbutic guinea pigs fed thiamin suggested that thiamin might affect the course of rickets in animals.

Longenecker, Fricke and King (91) showed that ascorbic acid synthesis is increased in rats when chloretone is administered. This suggests that chloretone administration to rats increases the need for ascorbic acid.

Our evidence indicates that chloretone administration with probable ascorbic acid depletion aggravates the symptoms in scorbutic guinea pigs. This is especially apparent in increased hemorrhage and in enlargement of the costochondral junctions. The bone effects of chloretone in guinea pigs, which do not synthesize ascorbic acid, raised the question of the effects on calcification, of chloretone, in rats which do synthesize ascorbic acid.

A series of experiments was planned in an attempt to learn the separate and combined effects of thiamin and chloretone on the processes of decalcification and calcification in rats.

Each phase of the experiment was carried out on a group of 5 young rats.

The Steenbock rachitogenic diet number 2965 was used. Thiamin in aqueous solution, chloretone in corn oil and cod liver oil were fed by dropper.

The animals were maintained on the rachitogenic diet for 21 days during which time marked decalcification of the leg bones occurred.

Following this period the animals were given 3.3 U.S.P. units of vitamin D. in 6 daily feedings, except four groups of 5 rats kept for negative controls. After 2 more days, the animals were killed and the metaphyses of the longitudinally sectioned radii and ulnae examined, after staining in silver nitrate, for the recalcification brought about by the administration of vitamin D. The vitamin effect was determined by the commonly employed "line test" of Parks et al (107).

A. The effects of large amounts of thiamin and chloretone on decalcification in rats.

Four groups of animals were fed as follows for 21 days:

Group 1 received only the rachitogenic diet.

Group 2 received the rachitogenic diet plus a daily supplement of 10 mg. thiamin hydrochloride.

Group 3 received the rachitogenic diet plus a daily supplement of 5 mg. chloretone.

Group 4 received the rachitogenic diet plus daily supplements of 10 mg. thiamin hydrochloride and 5 mg. chloretone.

After the 21 day period the animals were killed and the bones examined.

The results are summarized in the following corresponding tables:

Table 1 The Effects of the Rachitogenic Diet on Decalcification (negative controls)

| animal number | sex | initial wt. gms. | wt. at end of 21 days | wt. gain gms. | Results |
|---|---|---|---|---|---|
| 1 | | 47 | 54 | 7 | |
| 2 | | 40 | 46 | 6 | most decalcification |
| 3 | | 46 | 49 | 3 | in this group |
| 4 | | 45 | 52 | 7 | |
| 5 | | 46 | 48 | 2 | |

total gain, gms. 25
ave. gain, gms. 5

Table 2 The Effects of Thiamin on Decalcification (negative controls)

| animal number | sex | initial wt. gms. | wt. at end of 21 days | wt. gain gms. | Results |
|---|---|---|---|---|---|
| 1 | | 49 | 51 | 2 | |
| 2 | | 47 | 52 | 5 | moderate decalcification |
| 3 | | 45 | 52 | 7 | in this group |
| 4 | | 47 | 54 | 7 | |
| 5 | | 41 | 49 | 8 | |

total gain, gms. 29
ave. gain, gms. 5.8

Table 3 The Effects of Chloretone on Decalcification (negative controls)

| | | | | | |
|---|---|---|---|---|---|
| 1 | | 47 | 54 | 7 | |
| 2 | | 40 | 46 | 6 | least decalcification |
| 3 | | 46 | 49 | 3 | in this group |
| 4 | | 45 | 52 | 7 | |
| 5 | | 46 | 48 | 2 | |

total gain, gms. 25
ave. gain, gms. 5

Table 4 The Effects of Thiamin and Chloretone on Decalcification (negative controls)

| | | | | | |
|---|---|---|---|---|---|
| 1 | | 45 | 47 | 2 | |
| 2 | | 46 | 50 | 4 | moderate decalcification |
| 3 | | 45 | 52 | 7 | in this group |
| 4 | | 49 | 45 | 5 | |
| 5 | | 45 | 51 | 6 | |

total gain, gms. 24
ave. gain, gms. 4.8

B. The effects of thiamin and chloretone administration during the rachitogenic period.

Four groups of animals were fed on the rachitogenic diet during the 31 day period. During the 10 day healing period all rats were fed 3.3 U.S.P. units of vitamin D in 8 daily feedings. During the rachitogenic period the rats in each group received supplements as follows:

Group 1 control, no supplement.

Group 2 received 10 mg. thiamin hydrochloride daily.

Group 3 received 5 mg. chloretone daily.

Group 4 received 10 mg. thiamin hydrochloride plus 5 mg. chloretone daily.

The results are summarized in the following corresponding Tables.

Table 1 Control

| animal number | sex | initial wt. gms. | 21 day wt. | gain wt. gms. | wt. gms. 31 days | wt. gain gms. | total gain gms. | line test assay |
|---|---|---|---|---|---|---|---|---|
| 1 | | 44 | 52 | 8 | 58 | 6 | 14 | + + |
| 2 | | 46 | 51 | 5 | 57 | 6 | 11 | M.H. * |
| 3 | | 44 | 49 | 5 | 54 | 5 | 10 | + + + |
| 4 | | 50 | 59 | 9 | 61 | 2 | 11 | + + |
| 5 | | 45 | 51 | 6 | 55 | 4 | 10 | + + |
| | | | | | | | | 2.25 ** |
| | | total gain, gms. | | 33 | | 24 | 56 | |
| | | ave. gain, gms. | | 6.6 | | 4.6 | 13.2 | |

* Metaphyseal healing.
** Represents average healing on a scale of 0 to 4; the latter figure represents complete healing.

Table 2 The Effects of Thiamin Administration During the Decalcification Period

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | 44 | 52 | 7 | 55 | 3 | 10 | M.H. |
| 2 | | 45 | 49 | 4 | - | - | - | died |
| 3 | | 43 | 51 | 8 | 55 | 4 | 12 | + + + |
| 4 | | 47 | 55 | 8 | 59 | 4 | 12 | + + + |
| 5 | | 47 | 53 | 6 | 60 | 7 | 13 | M.H. |
| | | | | | | | | 3.00 |
| | | total gain, gms. | | 33 | | 18 | 47 | |
| | | ave. gain, gms. | | 6.6 | | 3.6 | 9.4 | |

Table 3 The Effects of Chloretone Administration During the Decalcification Period

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | 41 | 47 | 6 | 47 | 0 | 6 | + + + |
| 2 | | 55 | 62 | 7 | 63 | 1 | 8 | M.H. |
| 3 | | 44 | 47 | 3 | 49 | 2 | 5 | + + + + |
| 4 | | 46 | 50 | 4 | 52 | 2 | 6 | + + + + |
| 5 | | 46 | 49 | 3 | 47 | 2 | 1 | + + + + |
| | | | | | | | | 3.7 |
| | | total gain, gms. | | 19 | | 3 | 26 | |
| | | ave. gain, gms. | | 3.8 | | .6 | 5.2 | |

Table 2 The Effects of Chloretone Administration During the 31 Day Period

| animal number | sex | initial wt. gms. | 21 day wt. | gain gms. | wt. gms. 31 days | wt. gain gms. | total gain gms. | line test assay |
|---|---|---|---|---|---|---|---|---|
| 1 | | 44 | 57 | 13 | 59 | 2 | 15 | + |
| 2 | | 48 | 55 | 7 | 50 | 5 | 2 | + + + + |
| 3 | | 46 | 57 | 11 | 53 | 4 | 7 | + + + + |
| 4 | | 42 | 46 | 2 | 52 | 6 | 8 | + + + + |
| 5 | | 43 | 46 | 3 | 52 | 6 | 9 | + + + + |
| | | | | | | | | 3.40 |
| | total gain, gms. | | | 39 | | 5 | 41 | |
| | ave. gain, gms. | | | 7.8 | | 1 | 8.2 | |

Table 3 The Effects of Thiamin and Chloretone Administration During the 31 Day Period

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | | 44 | 48 | 4 | 49 | 1 | 5 | + + + + |
| 2 | | 47 | 56 | 9 | 59 | 3 | 12 | + + + + |
| 3 | | 47 | 52 | 5 | 57 | 5 | 10 | + + + + |
| 4 | | 45 | 49 | 4 | 47 | 2 | 2 | + + + + |
| 5 | | 40 | 47 | 7 | 50 | 3 | 10 | + + + + |
| | | | | | | | | 4.00 |
| | total gain, gms. | | | 29 | | 10 | 39 | |
| | ave. gain, gms. | | | 5.8 | | 2 | 7.8 | |

D. The effects of thiamin and chloretone administration during the healing period only.

Three groups of rats were fed the rachitogenic diet during the 31 day period. During the first 8 days of the 10 day healing period each rat received 3.3 U.S.P. units of vitamin D in 8 daily feedings. During the 10 day healing period the groups were fed supplements as follows:

Group 1 received 10 mg. thiamin hydrochloride daily.

Group 2 received 5 mg. chloretone daily.

Group 3 received 10 mg. thiamin hydrochloride, and 5 mg. chloretone daily.

The results are summarized in the following corresponding Tables:

Table 1 The Effects of Thiamin Administration During the Healing Period

| animal number | sex | initial wt. gms. | 21 day wt. | gain wt. gms. | wt. gms. 31 days | wt. gain gms. | total gain gms. | line test assay |
|---|---|---|---|---|---|---|---|---|
| 1 | | 47 | 52 | 5 | 57 | 5 | 10 | + + + + |
| 2 | | 43 | 47 | 14 | 50 | 3 | 17 | + + + |
| 3 | | 43 | 49 | 6 | 54 | 5 | 11 | + + + + |
| 4 | | 37 | 43 | 6 | 46 | 3 | 9 | + + + + |
| 5 | | 45 | 48 | 3 | 56 | 8 | 11 | + + + + |
| | | | | | | | | 3.80 |
| | total gain gms. | | | 34 | | 24 | 58 | |
| | ave. gain gms. | | | 6.8 | | 4.8 | 11.6 | |

Table 2 The Effects of Chloretone Administration During the Healing Period

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | 46 | 52 | 6 | 50 | -2 | 4 | + + |
| 2 | | 46 | 53 | 7 | 53 | 0 | 7 | + + |
| 3 | | 44 | 51 | 7 | 56 | 4 | 11 | + + + |
| 4 | | 45 | 52 | 7 | 52 | 0 | 7 | + + |
| 5 | | 47 | 53 | 6 | 57 | 4 | 10 | + + + |
| | | | | | | | | 2.40 |
| | total gain, gms. | | | 33 | | 6 | 39 | |
| | ave. gain, gms. | | | 6.6 | | 1.2 | 7.8 | |

Table 3 The Effects of Thiamin and Chloretone Administration During the Healing Period

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | 42 | 50 | 8 | 51 | 1 | 9 | + + ++ |
| 2 | | 40 | 42 | 2 | 43 | 1 | 3 | + + + + |
| 3 | | 42 | 47 | 5 | 51 | 3 | 8 | + + + + |
| 4 | | 43 | 48 | 5 | 49 | 1 | 6 | + + + + |
| 5 | | 45 | 51 | 6 | 55 | 4 | 10 | + + + + |
| | | | | | | | | 4.00 |
| | total gain, gms. | | | 26 | | 10 | 36 | |
| | ave. gain, gms. | | | 5.2 | | 2 | 7.2 | |

# DISCUSSION

The results recorded in Section VI are abstracted in the accompanying Table and presented with the discussion.

The Effects of Thiamin and Chloretone Administration to Rats during the Rachitogenic and Healing Periods.

| | | Decalcification Period | Decalcification and Calcification Period | Calcification Period |
|---|---|---|---|---|
| | | A | | |
| Effects of Thiamin and Chloretone on Decalcification | | | | |
| 1. | Control | most decalcification | | |
| 2. | Thiamin | moderate decalcification | | |
| 3. | Chloretone | moderate decalcification | | |
| 4. | Thiamin and Chloretone | least decalcification | | |
| | | B | C | D |
| Effects of Thiamin and Chloretone on Calcification | | | | |
| 1. | Control | * 2.25 | 2.25 | 2.25 |
| 2. | Thiamin | 3.00 | 3.60 | 3.60 |
| 3. | Chloretone | 3.70 | 3.40 | 2.40 |
| 4. | Thiamin and Chloretone | 4.00 | 4.00 | 4.00 |

* These values represent the average healing on a scale of 0 to 4. The latter figure represents complete healing.

The data presented in part A shows that decalcification in rats on a high calcium low phosphorus (rachitogenic) diet was inhibited to a considerable extent by the administration of chloretone which increases ascorbic acid synthesis in these animals.

When thiamin was administered with chloretone to rats on a rachitogenic diet the decalcification was greater than when chloretone alone was administered. This increased decalcification suggests that thiamin administration itself increases the need for ascorbic acid.

When thiamin alone was administered to rats on a rachitogenic diet the amount of decalcification was the same as when chloretone and thiamin were administered together. No explanation is offered for this situation.

The data presented in part B shows that healing in rats on a high calcium low phosphorus (rachitogenic)diet was greatest where both thiamin and chloretone were administered during the decalcification period.

When chloretone was administered alone during the decalcification period the healing was greater than was observed in the group fed thiamin alone.

The group fed thiamin alone during the decalcification period showed more healing than the controls but less healing than the group fed chloretone alone during the decalcification period.

The data presented in part C shows that the administration of both chloretone and thiamin to rats on a rachitogenic diet during both the decalcification and calcification periods gave complete healing which was identical with the results obtained in part B when chloretone and thiamin were given only during the decalcification period.

When thiamin alone was administered during the decalcification and calcification periods, the healing was greater than that in the controls

and greater than when chloretone alone was administered during both periods. This healing was also greater than when thiamin was fed during the decalcification period only.

When chloretone alone was administered during the decalcification and calcification periods, the healing was greater than that for the controls, less than when thiamin alone was administered and less than when chloretone was administered only during the decalcification period.

The data presented in part D shows that when additions were made during the calcification period alone, greatest healing occurred when both thiamin and chloretone were given.

When thiamin alone was administered during the calcification period, the healing was greater than that for the controls or for chloretone administration alone. The healing was also greater than when thiamin was administered during the decalcification period or during both the decalcification and calcification periods.

When chloretone was administered alone during the calcification period the healing was only slightly greater than for the controls, but less than when thiamin was administered alone or when thiamin was administered with chloretone. It was also less than when chloretone was administered during the decalcification period, or decalcification and calcification periods.

The data presented in parts A, B, C and D suggests that ascorbic acid inhibits decalcification in the absence of vitamin D. It also suggests that thiamin increases the demand for ascorbic acid but that in the presence of an adequate supply of ascorbic acid thiamin also inhibits decalcification.

Since chloretone administration during the decalcification period resulted in more healing than when given during the calcification period, it is suggested that the greatest effect of chloretone is due to increased ascorbic acid synthesis which exerts its greatest effect in inhibiting decalcification in the absence of vitamin D, rather than in aiding calcification in the presence of vitamin D.

Since thiamin administration during the decalcification period resulted in less healing than when given during the calcification period it is suggested that thiamin exerts its greatest effect in promoting calcification in the presence of vitamin D.

The fact that administration of both chloretone and thiamin during both decalcification and calcification periods shows greater effect than when one is administered alone suggests that the effects of chloretone and thiamin are more or less additive.

## SUMMARY

It was at first casually observed that scorbutic guinea pigs fed thiamin were likely to die more quickly than those without thiamin. This led to an investigation of the effects of thiamin on scorbutic and non-scorbutic animals.

It was observed that decreased food intake, weight loss, tenderness of the limbs and lameness, all occurred at about the same time in the animals fed a scorbutic diet. Animals fed a scorbutic diet plus large amounts of thiamin did not appear to show signs of scurvy appreciably earlier than animals on a scorbutic diet alone, but after the disease was apparent thiamin caused its more rapid progress. Some of the scorbutic animals fed thiamin lost complete use of the hind legs and dragged themselves about on the front legs as if completely paralyzed in the hind legs.

It was found that hemorrhages in the scorbutic guinea pigs fed thiamin were more massive than those on a scorbutic diet alone. Hemorrhages were never found in animals fed on a normal diet or on a normal diet plus thiamin.

Longenecker, Fricke and King (91) showed that ascorbic acid synthesis is increased in rats when chloretone and various other substances are administered. Chloretone administration to guinea pigs which cannot synthesize ascorbic acid was found to aggravate the condition of scurvy.

Gross bone deformities appeared in animals fed a scorbutic diet and a scorbutic diet plus thiamin. The costochondral junctions were found to be considerably enlarged and histological sections showed greatest bone destruction in the bones of scorbutic animals fed thiamin.

Since the bones of scorbutic animals and scorbutic animals fed thiamin showed gross pathology, it was considered important to determine the calcium and phosphorus excretions of normal and scorbutic guinea pigs with

and without thiamin administration. It was found that thiamin does not cause any appreciable increase of urinary calcium or phosphorus in normal or scorbutic guinea pigs. Fecal phosphorus excretions in scorbutic guinea pigs fed thiamin appeared to increase slightly over that of scorbutic pigs and fecal calcium excretion was markedly increased in the scorbutic pigs fed thiamin.

It was observed that scorbutic guinea pigs fed thiamin usually died. They often died during or immediately after a 10 day period of thiamin therapy and were very ill with scurvy. In some cases the guinea pigs lived for several months if continuously given ample green food, starting immediately after the period of thiamin administration. The animals appeared to improve for a time after resumption of the normal diet, ate well, and began to gain weight. Eventually, however, even though they continued to appear hungry and tried to eat they began to lose weight. It seemed that the strength of the chewing muscles progressively decreased and the animals became unable to exert force when chewing, though they continually tried to eat. In conjunction with apparent weakening of the jaw muscles, there was decreased control of these muscles and the animals would spend long periods with jaws constantly opening and closing in a rapid chewing motion. In some animals this condition of constant chewing became permanent until death. When these animals died they were extremely emaciated, but on examination it was found that there were no longer any scorbutic hemorrhagic areas.

Because of these observations and of other possible signs of nerve involvement the study of peripheral nerve and skeletal muscle was undertaken. This work is still in progress.

Because thiamin administration appeared to aggravate the scorbutic condition, including bone pathology in guinea pigs which cannot synthesize

ascorbic acid, it was decided to investigate its effects on bone formation in rats which can synthesise it. Since chloretone has been found to greatly increase the production of ascorbic acid in rats the effects of chloretone administration to rats in conjunction with thiamin, was studied. The results of this study are abstracted in the accompanying Table.

The Effects of Thiamin and Chloretone Administration to Rats during the Rachitogenic and Healing Periods

| | Decalcification Period | Decalcification and Calcification Period | Calcification Period |
|---|---|---|---|
| | A | | |
| Effects of Thiamin and Chloretone on Decalcification | | | |
| 1. Control | most decalcification | | |
| 2. Thiamin | moderate decalcification | | |
| 3. Chloretone | moderate decalcification | | |
| 4. Thiamin and Chloretone | least decalcification | | |
| | B | C | D |
| Effects of Thiamin and Chloretone on Calcification | | | |
| 1. Control | * 2.25 | 2.25 | 2.25 |
| 2. Thiamin | 3.00 | 3.60 | 3.80 |
| 3. Chloretone | 3.70 | 3.40 | 2.40 |
| 4. Thiamin and Chloretone | 4.00 | 4.00 | 4.00 |

* These values represent the average healing on a scale of 0 to 4. The latter figure represents complete healing.

# BIBLIOGRAPHY


1. Macgowan: A History of China. 1879. Quoted from Vedder, E. B. : Beriberi. William Wood and Co., New York, 1913.

2. Bontius, Jacobus: De medicina Indorum libri iii, Cap. 1. De Paralyseos Quadam Specie Quam Indigenae Beriberi Vocant. Lugdun, Batavia, 1645.

3. Rogers, C.: De Hydrope Asthmatico in Ceylonia Grassante Beriberia Dicto Complectens. Edinburgi, 1808.

4. Malcolmson, H. G.: A Practical Essay on the History and Treatment of Beriberi. Madras, 1835.

5. Hirota, Z.: Ueber die durch die Milch, der an Kakke Leidenden Frauen, Verursachte Krankheit der Säuglinge. Central bl. f. innere Med. vol. 19, pp. 385-92, 1898.

6. Takaki, K.: Three Lectures on the Preservation of Health amongst the Personnel of the Japanese Navy and Army. Lancet vol. I, pp. 1369-74, 1451-5, 1520-9, 1906.

7. Eijkman, C.: Eine beriberiähnliche Krankheit der Hühner. Arch. Path. Anat. (Virchow's) vol. 149, pp. 187-94, 1897.

8. Fletcher, W.: Rice and Beriberi. Jour. Trop. Med. and Hyg. vol. 12, pp. 127-34, 1909.

9. Fraser, H. and Stanton, A. T.: An Inquiry Concerning the Etiology of Beriberi. Lancet vol. I, pp. 451-5, 1909.

10. Funk, C.: Die Vitamine. J. F. Bergmann, Wiesbaden, 1914.

11. Vedder, E. B.: Beriberi. William Wood and Co., New York, 1913.

12. Stepp, W.: Versuche über Fütterung mit lipoidfreier Ernährung. Biochem. Zeitschr. vol. 22, pp. 453-60, 1909.

13. Hopkins, F. G.: Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries. Jour. Physiol. vol. 44, pp. 425-60, 1912.

14. Osborne, T. B. and Mendel, L. B.: The Influence of Butterfat on Growth. J. Biol. Chem. vol. 16, pp. 423-37, 1913.

15. McCollum, E. V. and Davis, M.: The Essential Factors in the Diet during Growth. J. Biol. Chem. vol. 23, pp. 231-46, 1915.

16. McCollum, E. V. and Kennedy, Cornelia: The Dietary Factors Operating in the Production of Polyneuritis. J. Biol. Chem. vol. 24, pp. 491-502, 1916.

17. Mitchell, H. H.: On the Identity of the Water-Soluble Growth Promoting Vitamins and the Antineuritic Vitamins. J. Biol. Chem. vol. 40, pp. 399-413, 1919.

18. Emmett, A. D. and Luros, G. O.: Water Soluble Vitamines. J. Biol. Chem. vol. 43, pp. 265-86, 1920.

19. Smith, M. L. and Hendrick, E. G.: Some Nutrition Experiments with Brewers Yeast. Public Health Rep. vol. 41, pp. 201-7, 1926.

20. Janson, B. C. P. and Donath, W. F.: Isolation of Anti-Beriberi Vitamins. Jededeel. v. d. dienst. volksgezondh. in nederl. Indie vol. 16, pp. 186-99, 1927.

21. The Vitamins. American Medical Association, Chicago, Ill. 1939.

22. Windaus, A., Schmoche, R. T., Rukkopf, H., Laquer, F. and Schultz, T.: Crystalline Antineuritic Vitamin from Yeast. Z. physiol. Chem. vol. 204, pp. 123-8, 1932.

23. Williams, R. R.: Structure of Vitamin $B_1$. J. Am. Chem. Soc. vol. 58, pp. 1063-4, 1936.

24. Cline, J. K., Williams, R. R., Ruehle, A. E. and Waterman, R. E.: Studies of Crystalline Vitamin B. Identification of the Pyrimidine Portion. J. Am. Chem. Soc. vol. 59, pp. 530-3, 1937.

25. Lohmann, L. and Schuster, F.: Uber den Gehalt der Kartoffeln an Vitamin $B_1$ and $B_2$ (Komplex) und sein Beeinflussung durch verschiedine Dungung. Biochem. Ztschr. vol. 294, pp. 188-90, 1937.

26. Zimmerman, H. M. and Burach, E.: Lesions of the Nervous System Resulting from Deficiency of the B Complex. Arch. Pathol. 13, pp. 207-32, 1932.

27. Wolbach, S. B.: The Pathologic Changes Resulting from Vitamin Deficiency. J. Am. Med. Assoc. vol. 108, p. 7, 1937.

28. Kilie, W. G.: A Contribution to the Pathology of Beriberi. Lancet vol. II, pp. 985-6, 1898.

29. Wenckebach, K. F.: Heart and Circulation in a Tropical Avitaminosis (Beriberi). Lancet vol. II, pp. 265-8, 1928.

30. Drury, A. N., Harris, L. J. and Jandsley, C.: Vitamin B Deficiency in the Rat. Biochem. J. vol. 24, pp. 163-49, 1930.

31. Carr, Walter G.: Some Effects of Water-Soluble Vitamins Upon Nutrition. J. Biol. Chem. vol. 44, pp. 255-282, 1920.

32. Cowgill, G. R. and Gilman, A. S.: Physiology of the Vitamins. The Effect of Lack of the Vitamin B Complex on the Secretion of Gastric Juice in Dogs with Gastric Pouches. Arch. Int. Med. vol. 53, pp. 58-70, 1934.

33. McCarrison, R.: Studies in Deficiency Disease. Oxford Medical Publications, Henry Proude and Hodder and Stoughton, London, 1921.

34. Wolbach, S. B.: The Pathologic Changes Resulting from Vitamin Deficiency. J. Am. Med. Assoc. vol. 108, pp. 7-13, 1937.

35. Ogata, T. S.: Referat über die Reiserkrankung des Geflügels. (Kakkeähnliche Krankheit oder Polyneuritis Gallinarum). Japanische Path. Gesellsch. vol. 10, p. 1, 1920.

36. Evans, H. M. and Bishop, K. S.: On the Relations between Fertility and Nutrition; the Ovulation Rhythm in the Rat on Inadequate Nutritional Regimes. J. Metabolic Research vol. 1, pp. 335-6, 1922.

37. Ueno, J.: Deficiency of Vitamin B and Endocrine Glands of Female White Rats. Jap. J. Obst. and Gynec. vol. 17, pp. 267-78, 1934.

38. Macy, Icie, Outhouse, Julia, Graham, Alice and Long, Louisa M.: The Quantitative Estimation of Vitamin B. J. Biol. Chem. vol. 73, pp. 189-208, 1927.

39. Williams, R. R. and Spies, T. D.: Vitamin $B_1$ and Its Use in Medicine. Macmillan Co., New York, 1938.

40. Glick, D.: The Nature and Significance of Cholinesterase. Biological Symposia. vol. V, 1941.

41. Mina, B. and Agid, R.: Influence de la vitamine $B_1$ sur l'activite de l'acetylcholine. Compt. rend. Acad. Sc. vol. 205, pp. 576-583, 1937.

42. Antopol, W., Glaubach, S., and Glick, D.: Choline Esterase Activity in Blood Serum and Duodenum of Beriberi Pigeons. Proc. Soc. Exp. Biol. and Med. vol. 42, pp. 679-83, 1939

43. Glaubach, S., and Antopol, W.: The Nature and Significance of Cholinesterase. Biological Symposia. vol. V, 1941.

44. Hess, A. F.: Scurvy-Past and Present. Lippencott, Philadelphia, 1920.

45. Smith, Theobald: Swine Erysepelas or Mouse Septaemia Bacilli from an Outbreak of Swine Disease. U. S. Dept. Agriculture. Bureau Animal Industry Ann. Rept. pp. 172, 1895-96.

46. Holst, A. and Frölich, T.: Experimental Studies Relating to Ship Beriberi and Scurvy. J. Hyg. vol. 7, pp. 634-71, 1907.

47. Chick, H. and Hume, E.: The Distribution in Wheat, Rice and Maize Grains of the Substance, the Deficiency of Which in a Diet Causes Polyneuritis in Birds and Beriberi in Man. Proc. Roy. Soc. (London) vol. B, 90, pp. 44-60, 1917.

48. Cohen, B. and Mendel, L. B.: Experimental Scurvy of the Guinea Pig in Relation to the Diet. J. Biol. Chem. vol. 35, pp. 425-53, 1918.

49. Harden, A. and Zilva, S.: Accessory Factors in the Nutrition of the Rat. Biochem. J. vol. 12, pp. 408-15, 1918.

50. Drummond, J. C.: Note on the Role of the Antiscorbutic Factor in Nutrition. Ibid., vol. 13, pp. 77-80, 1919.

51. Le Mer, V. K. Campbell, H. L. and Sherman, H. C.: Quantitative Determination of the Antiscorbutic Vitamin. J. Am. Chem. Soc. vol. 44, pp. 165-72, 1922.

52. Szent-Györgyi, Albert: Observations on the Function of Peroxidase Systems and the Chemistry of the Adrenal Cortex. Biochem. J. vol. 22, pp. 387-1409, 1928.

53. Tillmans, J. and Hirsch, P.: Ueber das Vitamin C. Biochem. Z. vol. 250, pp. 312-20, 1932.

54. Waugh, W. A. and King, C.G.: The Chemical Nature of Vitamin C. Science vol. 75, pp. 357-8, 1932.

55. Kendall, E. C.: A Study of the Chemistry of the Suprarenal Gland. Proc. Staff Meetings Mayo Clinic, vol. 6, pp. 296, 1931.

56. Reichstein, T., Grussner, A. and Oppenauer, R.: Synthesis of d- and l-Ascorbic Acid. Helv. Chim. Acta, vol. 16, pp. 561-5, 1933.

57. Herbert, R. W., Percival, E. H. V., Reynolds, R. J. W., Smith, F. and Hirst, E. L.: Constitution of Ascorbic Acid. J. Chem. Soc. pp. 1270-90, 1933.

58. Karrer, Paul, Schopp, K. and Zehender, F.: Concerning Vitamin C. Helv. Chim. Acta, vol. 16, pp. 1161-3, 1933.

59. Michell, Fritz, and Kraft, Kurt: The Constitution of Vitamin C. Ztschr. physiol Chem. vol. 215, pp. 215-24, 1933.

60. Karrer, Paul, von Euler, H. and Hellstrom, H.: Vitamin C. Arkiv. kemi. mineral. Geol. vol. 11B, p. 6, 1933.

61. Szent-Gyorgyi, Albert, and Haworth, W. N.: "Hexuronic Acid" (Ascorbic Acid) as the Antiscorbutic Factor. Nature vol. 131, p. 24, 1933.

62. Wolbach, S. B. and Howe, P. R.: Intercellular Substances in Experimental Scorbutus. Arch. Pathol. vol. 1, pp. 1-24, 1926.

63. Giroud, Antoine and Rakoto-Ratsimanga, A.: Distribution of Vitamin C in Invertebrates. Bull. Soc. Chim. Biol. vol. 18, pp. 375-83, 1936.

64. Glick, David, and Roskind, G. R.: The Concentration of Vitamin C in the Thymus in Relation to Its Histological Changes at Different Stages of Development and Regression. J. Biol. Chem. vol. 114, pp. 1-7, 1936.

65. Roskind, G. R., and Glick, David: The Vitamin C Concentration of the Corpus Luteum with Reference to the Stage of the Estrus Cycle and Pregnancy. J. Biol. Chem. vol. 113, pp. 27-33, 1936.

66. Bonner, J.: Production of Growth Substance by Rizopus Suinus. Biol. Zentr. vol. 52, pp. 565-82, 1932.

67. Parsons, L. G., and Hawksley, J. C.: The Anhaematopoietic Anaemias. Arch. Dis. Childhood, vol. 8, pp. 117-144, 1933.

68. King, C. G. and Menten, M. L.: The Influence of Vitamin C Level upon Resistance to Diphtheria Toxin. J. Nut. vol. 10, pp. 129-53, 1935.

69. Greenwald, C. K. and Hardo, E.: Vitamin C and Diphtheria Toxin. Proc. Soc. Exper. Biol. and Med. vol. 32, pp. 1157-62, 1935.

70. Sigal, Alex, and King, C. G.: The Influence of Vitamin C Deficiency upon the Resistance of Guinea Pigs to Diphtheria Toxin. J. Pharmacol. and Exper. Therap. vol. 61, p. 1, 1937.

71. Sigal, Alex, and King, C. G.: Vitamin C and Diphtheria Toxin. ibid. vol. 59, pp. 466-473, 1937.

72. Perla, David and Marmorston, Jessie: Role of Vitamin C in Resistance. Arch. Path. vol. 23, pp. 683-712, 1937.

73. Cornia, P. K.: Post-Arshpenamine Dermatitis. J. Inv. Dermatology, vol. 4, pp. 81-93, 1941.

74. Lang, S.: Über die Wirkung der experimentellen Bleivergiftung auf den Ascorbinsaure und Adenosintriphosphorsaregehalt einzelner Organe. Arch. f. exper. Path. u. Pharmakol. vol. 200, pp. 637-640, 1943.

75. Stotz, Elmer, Schultz, M. O., Karrer, C. J. and King, C. G.: The Oxidation of Ascorbic Acid in the Presence of Guinea Pig Liver. J. Biol. Chem. vol. 122, pp. 407, 1938.

76. Stotz, Elmer, Karrer, C. J., Schultze, M. O. and King, C. G.: Tissue Respiration Studies on Normal and Scorbutic Guinea Pig Liver and Kidney. J. Biol. Chem. vol. 120, pp. 129-40, 1937.

77. Hopkins, F. G. and Morgan, E. J.: Some Relations Between Ascorbic Acid and Glutathione, Biochem. J. vol. 30, pp. 446-62, 1936.

78. Borsook, Henry, Davenport, H. W., Jeffreys, C. E. P. and Warner, R. C.: The Oxidation of Ascorbic Acid and Its Reduction in Vitro and in Vivo. J. Biol. Chem. vol. 117, pp. 237-279, 1937.

79. Green, D. E. and Richter, David: Adrenaline and Adrenochrome. Biochem. J. vol. 31, pp. 596-616, 1937.

80. Lemberg, R., Cortis-Jones, B. and Norrie, M.: Coupled Oxidation of Ascorbic Acid and Hemochromogens. Biochem. J. vol. 32, pp. 149-70, 1938.

81. Barron, E. S. G., De Meio, E. H. and Klemperer, F.: Biological Oxidations: V. Copper and Hemochromogens as Catalysts for the Oxidation of Ascorbic Acid, J. Biol. Chem. vol. 112, pp. 625-40, 1936.

82. Baker, E. E., Pillemer, L., Wertheimer, E. and Gradis, H: Ascorbic Acid and Complement Function. J. Immunol. vol. 34, pp. 19-37, 1938.

83. Hunt, A. H.: The Role of Vitamin C in Wound Healing. Brit. Jour. Surg. vol. 28, pp. 436-66, 1941.

84. Hatsell, J. E. and Crowley, E. T.: Vitamin Therapy in the Surgical Patient. Am. J. Surg. vol. 56, pp. 289-99, 1942.

85. Dalldorf, Gilbert: Criterion of Hemorrhagic Diathesis in Experimental Scurvy. J. Exper. Med. vol. 53, pp. 289-97, 1931.

86. Park, E. A., Guild, H. G., Jackson, D. and Bond, M.: The Recognition of Scurvy with Especial Reference to the Early X-Ray Changes. Arch. Dis. Childhood, vol. 10. pp. 265-294, 1935.

87. Fish, E. W. and Jarris, L. J.: The Effects of Vitamin C Deficiency on Tooth Structure in Guinea Pigs. Trans. Roy. Soc. (London) vol. 223 B, pp. 489-510, 1934.

88. Maltier, S. E. and Chew, W. B.: The anemia of Scurvy. Effect of Vitamin C Diet on Blood Formation in Experimental Scurvy of Guinea Pigs. J. Exper. Med. vol. 55, pp. 971-79, 1932.

89. Höjer, J. A.: Studies in Scurvy, Acta Peadiat. (supp) vol. 3, p. 8, 1924.

90. Bessey, O. A., Menten, M. S. and King, C. G.: Pathologic Changes in the Organs of Scorbutic Guinea Pigs. Proc. Soc. Exper. Biol. and Med. vol. 31, pp. 455-60, 1934.

91. Longenecker, Herbert E., Friske, H. H. and King, C. G.: The Effect of Organic Compounds upon Vitamin C Synthesis in the Rat. J. Biol. Chem. vol. 135, pp. 497-510, 1940.

92. Kronmeyer, J.: Practice of Pediatrics. vol. 1, 1944.

93. Wilson, Rubye E.: Decalcification in Vitro. Am. J. Clin. Path. vol. 6, pp. 77-82, 1942.

94. Fisk, C. H., Subarrow, Y.: The Colorimetric Determination of Phosphorus. J. Biol. Chem. vol. 66, pp. 375-400, 1925.

95. Clark, E. P. and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification. J. Biol. Chem. vol. 63, pp. 461-64, 1925.

96. Salter, W. T. and Aub, J. C.: Deposition of Calcium Bone in Healing Scorbutus. Arch. Path. vol. 11, pp. 380-82, 1931.

97. Bahrt, H. and Edelstein, F.: Organlysen bei Barlcuscher Krankheit. Z. Kinderh. Berl. vol. 9, pp. 415, 1913.

98. Bayer, G.: The Chemical and Physical State of the Bones in Experimental Scurvy. Beitr. Path. Anat. vol. 87, pp. 204-08, 1931.

99. Bauman, L. and Howard, C. P.: Metabolism of Scurvy in an Adult. Arch. Int. Med. vol. 9, pp. 665-79, 1912.

100. Chaney, M. S., Blunt, K.: The Effect of Orange Juice on the Calcium, Phosphorus, Magnesium and Nitrogen Retention and Urinary Organic Acids of Growing Children. J. Biol. Chem. vol. 66, pp. 829-45, 1925.

101. Salter, W. T. and Aub, J. C.: Deposition of Calcium Bone in Healing Scorbutus. Arch. Path. vol. 11, pp. 380-2, 1931.

102. Nagayama, T. and Munehisa, T.: The Calcium and Phosphorus Metabolism of Guinea Pigs Fed on a Vitamin Free Diet. J. Biochem. vol. 11, pp. 191-201, 1929.

103. Communications from Sperry Flour Co., San Francisco, Calif., 1944.

104. Freund, Hugo A., Steiner, Gabriel, Leichtentritt, Bruno: Nodular Polymyositis in Rheumatoid Arthritis. Science vol. 101, pp. 202-3, 1945.

105. ibid. _____ Lab. and Clin. Med. vol. 27, pp. 1256-6, 1942.

106. ibid. Peripheral Nerves in Chronic Atrophic Arthritis. Amer. Jour. Path. vol. 18, pp. 865-85, 1942.

107. Hawk, P. B. and Bergeim, Olaf: Practical Physiological Chemistry. P. Blakiston's Son and Co., Inc. Philadelphia. Eleventh Edition, 1937.

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