

MORPHOLOGICAL EFFECTS OF A
DIETARY-INDUCED HYPERPARATHYROIDISM
ON THE PARATHYROIDS, KIDNEYS,
AND TIBIAS OF RATS

Thesis for the Degree of Ph. D.
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Charlotte Marie Dienhart
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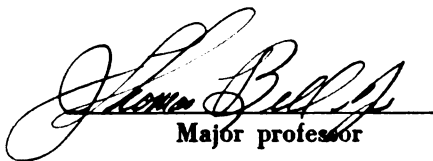
**Morphological Effects of a
Dietary-Induced Hyperparathyroidism
on the Parathyroids, Kidneys,
and Tibias of Rats**

presented by

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**has been accepted towards fulfillment
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By

CHARLOTTE MARIE DIENHART

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
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1960

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A handwritten signature, likely "John B. Kelly", is written over a horizontal line.

ABSTRACT

The parathyroids, kidneys and tibias of non-pregnant, pregnant, and lactating Long-Evans hooded rats maintained on a low calcium, vitamin D-free diet for 1-20 days were examined for morphological changes and compared to tissues from animals which had been fed a stock diet. Significant cellular hypertrophy of the parathyroids occurred and reached a peak on the tenth day. This hypertrophy was directly correlated with the length of the dietary period. After 10 days the glands showed a slight regression in size but were still enlarged to a significant degree. Renal metastatic calcifications, degenerating tubular epithelial cells and thickened glomerular capsules were found in the kidneys of all animals on the experimental diet. The degree of damage was directly correlated with the time of the diet period. The tibias, after the third dietary day, showed a progressive disruption of the normal calcification process manifested chiefly by a narrowing of the epiphyseal plates and a reduction in the hypertrophied cartilage cell zones and numbers of bony spicules. The greatest degree of change in all tissues appeared in the pregnant group.

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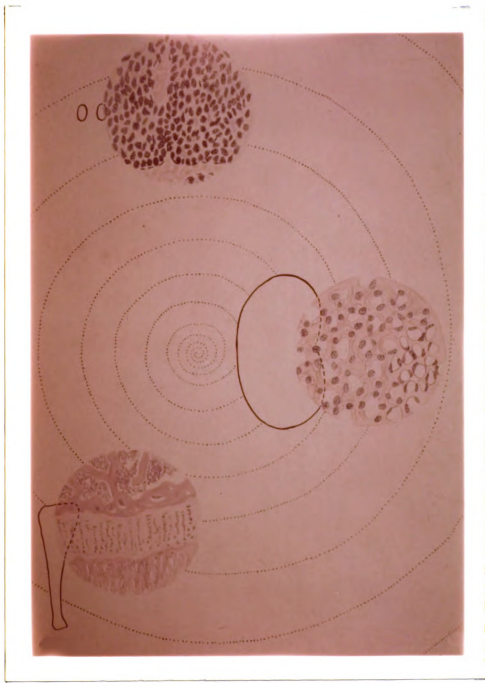
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INTRODUCTION

The function of the parathyroid glands in controlling the calcium ion concentration in the blood plasma has led to the investigation of the mechanisms by which parathyroid hormone and vitamin D, mediated by citrate, maintain the constancy of the serum calcium level.

Most investigators support either one of two hypotheses as to the site of action of the hormone: (1) that it acts on the kidney to promote the excretion of phosphate in the urine, or (2) that the main site of action is the bone. Thus it may be that the hormone controls resorption of phosphate by the kidney, which affects the serum calcium indirectly, and also is responsible for the direct mobilization of calcium by extra-renal mechanisms.

Investigations have been made into the various conditions which cause an alteration in the serum calcium level. Those which have a tendency to lower this level (low calcium diets, lack of vitamin D, pregnancy, kidney diseases, administration of phosphates and oxalates) have been shown to induce hyperparathyroidism with morphological changes occurring in the parathyroids variously designated as a general hypertrophy or hyperplasia.

In addition, other organs of the body, such as bone and the soft tissues, concomitantly exhibit altered morphology in hyperparathyroid animals. Therefore, experimental

hyperparathyroidism has been utilized to study the relationships between morphology and function in regard to the role of the parathyroids in controlling calcium and phosphorus metabolism.

Although the literature contains the results of various studies in which some histological work was done, there remains the question of a systematic and more detailed clarification and correlation of morphological changes over a prescribed experimental period under well-defined physiological conditions.

This study was designed to contribute information about histological and histochemical changes occurring in the parathyroid, kidney, and bone of the female rat in experimental hyperparathyroidism produced by a low calcium, vitamin D-free diet. The morphology of these structures in non-pregnant, pregnant, and lactating hyperparathyroid animals will be compared to their normal controls.

REVIEW OF LITERATURE

I. INTRODUCTION

Although the presence of the parathyroids as entities distinct from the thyroid had been established by Sandstrom in 1880 (Rich, et al., 1958), it was in 1898 that Welsh recorded what were probably the first histological observations made on these glands. In addition, he described the position, general characteristics, and vascular supply of the parathyroids in man, ox, sheep, rabbit, cat, and ferret. Welsh noted their close resemblance to other epithelial organs and described the two classic cell types in the human--the principal and the oxyphile--that are still recognized. His published work included several photomicrographs which illustrated his findings.

II. EARLY MORPHOLOGICAL STUDIES

Early experimental work correlated dietary deficiencies and parathyroid tumors with morphological changes in the parathyroid glands. These studies included the observation of enlarged parathyroids in rats fed ox meat and water (Watson, 1905), enlarged cells in human parathyroid tumors (Thompson and Harris, 1908; Kurokawa, 1925), and one of the first detailed studies by Erdheim in 1914 (Minor and Pappenheimer, 1921) of secondary hyperplasia and hypertrophy of the parathyroids in rickets. Marine (1914) found enlarged glands

in fowl fed maize and wheat and thought that this overgrowth might be the result of calcium deficiency.

Relative to body weight, normal female albino rats were found to have larger parathyroids than males (Jackson, 1916; Jackson and P'an, 1932).

Examining human parathyroids from cases of rickets, Pappenheimer and Minor (1921) observed a very definite increase in gland size, but stated that this was due only to cell multiplication and not to an increase in the size of the individual cells. They found no increased vascularity or increase in supporting tissue in these enlarged glands (Minor and Pappenheimer, 1921).

Parathyroctomized rats developed osteomalacia on diets deficient in calcium and vitamin A (Kornechevsky, 1922), while Luce (1923) found that the most pronounced and consistent enlargement of the parathyroids in rats was seen after feeding calcium deficient diets. She designated this increase in size as hyperplasia and not hypertrophy, although she noted no enlarged nuclei or hypertrophied cells and no increase in connective tissue.

Although he found a decrease in mitotic figures with increasing age in rats, Hoskins (1924) found no differences from the normal in the parathyroids of pregnant and lactating rats. However, Kurokawa (1925) described "nodular hyperplasia of the oxyphile cells" in the glands of man during pregnancy.

Depriving rabbits of ultraviolet rays caused enlargement of the parathyroids which was termed a hyperplasia by Grant and Gates (1924).

Numerous osteoclasts were found in the bones of chickens suffering from "leg weakness," denoting a failure of osteogenesis. At the same time there was a continuing and possibly exaggerated resorption of previously formed bone, thus showing a bone picture similar to that seen in hyperparathyroid animals (Pappenheimer and Dunn, 1925). This observation led these investigators to differentiate this "leg weakness" in chickens from rickets in rats whose bones showed large conspicuous osteoblasts, denoting a failure of calcium deposition. Doyle (1925) on the other hand advocated using the presence of enlarged parathyroids as a criterion for judging the presence of rickets in chickens.

Repeated injections of guanidine caused hypertrophy and hyperplasia of the parathyroid cells in rabbits. This finding led to the belief that guanidine metabolism was controlled by these glands (Susman, 1926).

Two different phases of parathyroid change were found in chickens deprived of vitamin D, the first being a period of active hypertrophy and hyperplasia and the second a period of "regression" (Nonidez and Goodale, 1927). During the first phase, the differences from normal were expressed quantitatively as an increase in both the number of cells and in

cell size, with the presence of fairly abundant mitoses. The second phase occurred after three months' deprivation of sunlight and was characterized by a shrinkage of the "epithelial cords." This decrease in volume of the gland was considered by the authors to be small since the glands still appeared larger than those of normal birds. They also noted hyperplasia of the stroma and some "mucous degeneration." When the chickens were placed in sunlight, a marked decrease in parathyroid cell size occurred.

In another study chickens deprived of the shorter wave lengths of light showed hyperplasia and hypertrophy of the parathyroids (Higgins and Sheard, 1928).

After producing severe anemias in rabbits by administering hydroxylamine, Eisler (1928) observed "simple hyperplasia" of the parathyroids. He suggested that this enlargement might be secondary to certain changes in calcium metabolism.

Hueper (1927) found degeneration and necrosis of the tubular epithelium in the kidneys of dogs given parathyroid extract injections. Calcification of the tubular cells with desquamation and formation of solid dark blue casts in the lumen was noted.

The influence of increased doses of parathyroid hormone led to the development of osteitis fibrosa cystica in puppies (Jaffe and Bodansky, 1930a). The bone changes were

not of the type caused by low calcium diets, i.e., an osteoporosis, which is quite different histologically from osteitis fibrosa (Jaffe and Bodansky, 1930b) . These authors did not agree with the theory that parathyroid enlargement observed with bone disease was of a secondary nature appearing as a result of compensatory hypertrophy. They cited Korsakoff and Miva and Støeltner who in 1898 (Jaffe and Bodansky, 1930b) pointed out that dogs on low calcium diets developed osteoporosis, not rickets.

III. EXPERIMENTAL HYPERPARATHYROIDISM

Studies performed under a great variety of stimuli showed that the parathyroids possessed an "inherent power" to increase in size, this increase being caused by general hyperplasia and not hypertrophy of individual cells (Barr and Bulger, 1930).

Bodansky et al. (1930) investigated the association of parathyroid enlargement with bone dystrophies and found that repeated injections of even small doses of parathormone led to bone resorption in young guinea pigs with the severity of the lesions being related to dosage and duration of the experiment. These lesions presented a typical picture of osteitis fibrosa.

In producing experimental hyperparathyroidism in puppies, Bodansky and Jaffe (1931) found that bone deformities and fractures were most severe in those animals on

a calcium deficient diet. Histological studies on the bones of young guinea pigs given parathormone injections confirmed the observations as resembling osteitis fibrosa (Jaffe et al., 1931). The bones of rats in which experimental hyperparathyroidism was produced by parathyroid extract injections also showed changes diagnosed as osteitis fibrosa (Johnson, 1932). Intermittent injections of parathyroid hormone produced periodic decalcifications and restorations in the bones of young guinea pigs--a feature not seen in chronic experimental hyperparathyroidism (Jaffe et al., 1932).

Hyperfunction of the parathyroids is associated with hypercalcemia and decalcification of the bones (Shelling et al., 1933). However, overdosage of parathyroid hormone does not always lead to bone resorption in rats (Selye, 1932a). The first response to large doses of hormone is bone resorption by osteoclasts, whereas continued doses lead to bone formation, as shown by increased bone density (Selye, 1932b). After repeated injections of parathormone in dogs, Thompson and Collip (1932) noted the presence of calcifications in kidneys, bronchi, and interstitial tissue and cardiac muscle of the heart.

Pugsley and Selye (1933) examined the bones of dogs made hyperparathyroid by parathormone injections during different stages of reaction to the hormone. First there occurred the formation of numerous osteoclasts, followed on the

fourth day after injection by the appearance of many osteoblasts. This is a constant feature of osteitis fibrosa. During the ninth to the twelfth day the osteoclasts disappeared and, if the treatment was continued, osteoblasts increased in number. This led to the typical picture of "marblebone" in which huge amounts of bone tissue are formed.

McJunkin et al. (1932) found that parathyroid hormone injections in rats caused a reduction in mitotic activity in the parathyroid glands over that in the control animals. These authors concluded that there is an inhibition of mitosis in the parathyroid by an amount of hormone insufficient to produce destructive lesions in the soft tissues. In fact, they considered the inhibition of mitosis a more delicate test for excess parathyroid activity than either the production of lesions in parenchymatous organs or the elevation of the serum calcium.

Hyperactivity of the parathyroids is one of the possible causes of renal calculi (Colby, 1934). In acute parathormone poisoning in dogs which led to death in a few days there were calcium deposits in the kidney parenchyma but no chronic renal changes (Albright et al., 1934). These authors also stated that the degree of bone involvement is an index to the duration of the hyperparathyroidism, not to its severity.

In his book on the parathyroids, Shelling (1935) described histologically the different types of enlargement that may be recognized under different physiological conditions. He designated the parathyroid hypertrophy seen in experimental production of faulty bone calcification by dietary means as probably the secondary result of a primary disturbance in the metabolism of lime salts.

Giving parathormone to chickens deprived of vitamin D produced hypertrophy of the parathyroids, i.e., an increase in the amount of cytoplasm of the chief cells with an accompanying hyperplasia (Wilder et al., 1934).

In making parathyroid cell counts Rosof (1934) found the average cell size of the 60-day albino rat comparatively larger than that of the 90-day animal but cautioned against speaking of cellular hypertrophy since there exist great variations in normal cell size. In a study of 25 cases of human hyperparathyroidism, Castleman and Mallory (1935) found evidence for the support of the monophyletic theory of the origin of various parathyroid cell types. Their study showed the chief cell as the only invariable cellular component, obviously the basic fundamental cell, and possibly the only proliferative form.

IV. RENAL INVOLVEMENT

In human cases of hyperparathyroidism, renal disease is a more frequent manifestation than is bone disease (Albright

and Bloomberg, 1935). Chute (1934) found that in fifty per cent of human cases renal calculi were present.

It was found that experimental reduction of renal tissue brought about a significant increase in parathyroid volume in the rat and that the enlargement was a result of an increased volume of both nucleus and cytoplasm of the cells (Jarrett et al., 1935). The degree of hypertrophy was closely correlated with the intensity of the kidney lesion (Pappenheimer, 1936). Examination of the parathyroids and kidneys of 27 human nephritic cases showed enlarged glands and deposition of calcium in the renal tissue (Pappenheimer and Wilens, 1935). In one case of human nephritis, Magnus and Scott (1936) found parathyroid enlargement involving both cell types; no mitoses were noted. They called this "simple hyperplasia" of the chief cells and believed the renal lesions to be the primary change with the parathyroid enlargement as secondary.

Parathyroid hyperplasia in conjunction with renal rickets has been designated as primary hyperparathyroidism (Shelling and Remsen, 1935).

Hyperparathyroidism, if long continued, will lead to renal insufficiency because of calcium deposits in the kidney parenchyma (Castleman and Mallory, 1937). These authors used the term "hyperplasia" to designate both unusually large cells ("primary hyperplasia") and closely-packed, normal-sized cells ("secondary hyperplasia").

Highman and Hamilton (1937) thought that chronic renal insufficiency might cause hyperplasia of parathyroid tissue which could go on to multiple tumor formation. They determined that the hyperplasia was accompanied by a hyperfunction. In such cases, the kidney damage might be the cause and not the result of the tumors (Highman and Hamilton, 1938). Parathyroid hyperplasia seen in partially nephrectomized rats was not prevented by injecting large doses of parathyroid extract (Pappenheimer and Johnson, 1938). Since kidney calcium occurred to only a limited degree in parathyroidectomized rats that were partially nephrectomized, it was shown that the calcium increase in the kidney which follows renal insufficiency is induced by the parathyroid hyperplasia and not by the reduction of kidney substance per se (Donahue et al., 1937). This confirmed the work of Morgan and Samisch (1935) which showed that even small doses of parathormone can induce a significant increase in kidney calcium.

When Cowdry and Scott (1936) gave monkeys concentrated viosterol they found cellular hypertrophy of the parathyroids. Since they observed only one mitotic figure, they concluded that definite signs of hyperplasia were lacking. Kidney damage in these animals was focused in the distal convoluted tubules.

Histological studies of rabbit parathyroids from animals injected with buffered sodium phosphate showed definite

hyperplasia of the chief cells, although measurement of cell size showed practically no increase over the normal and only occasional mitotic figures were found (Drake et al., 1937). Examinations of the bones and kidneys showed no differences from the normal.

Anderson (1939) described cases of hyperparathyroidism in man in which specific interstitial renal damage was present. The microscopic appearance of the kidney in these cases was thus distinguished from that in acute hyperparathyroidism as produced experimentally in animals.

Extensive calcium deposits in the kidney have been reported in cases of parathyroid adenomas in man (Bogdonoff et al., 1956; Schneider, 1957), while one study (Rich et al., 1958) reports two such cases without apparent manifestations of either renal or bone complications.

Renal secondary hyperparathyroidism in dogs produced parathyroids enlarged two and one-half to five times and frequent renal calcinosis (Krook, 1957).

Engfeldt et al. (1958) produced experimental hyperparathyroidism in rats and confirmed results of a previous study (Canterow et al., 1938) in which kidney tubular damage was demonstrated with calcium salts deposited in the proximal convoluted tubules.

V. EFFECTS OF DIET, PREGNANCY, LACTATION

Bodansky et al. (1930) fed guinea pigs a normal diet of oats, hay, carrots, and cabbage, and then fasted them for 60 hours or longer. They found that maximum effects of a single dose of parathormone may be brought out after the fasting period, presumably due to the removal of the normally basic diet. These authors felt, therefore, that the dietary factor may be important in that the effectiveness of parathormone may be defined in terms of changes in the acid-base equilibrium.

Sekiguchi (1930) fed albino rats a vitamin D phosphorus-deficient diet and noted that the parathyroids consisted mainly of "young transparent cells" in contrast to the darker staining cells of the controls. Examination of the "rachitic" tibias showed a widened cartilage zone in comparison to the narrow zone of the normal bone.

Adult rabbits maintained on a carrot and oat diet (Ca:P=0.5) had parathyroids enlarged two or more times their normal size. These glands contained hypertrophied cells and showed an increase in vascularity (Bauman and Sprinson, 1939). Compensatory hypertrophy of the parathyroids was found in rats fed a vegetarian diet (Ca:P=0.75) in which a deficiency of vitamin D was given as the cause of the changes seen (Chang and Chen, 1940). DeRobertis (1941) found that both low calcium (0.05 per cent) and low phosphorus

diets caused parathyroid hypertrophy in rats, but it was more marked in the case of the low calcium group. He also observed that the hypertrophy was caused principally by an increase in the number of cells and to a lesser extent by an increase in individual cell volume. This author stated that the hyperplasia was probably produced during the earlier stages of the diet. In another study (Ham et al., 1940) parathyroid enlargement was noted more often with low calcium and relatively high phosphorus than with high calcium and low phosphorus diets.

Histological examination of the bones of rats fed a low calcium diet showed poorly calcified trabeculae with many osteoclasts near them (Boelter and Greenberg, 1941). Low calcium diets have been associated also with markedly diminished fertility in the rat (Bodansky and Duff, 1941).

Saxton and Ellis (1941) found that the parathyroids of mature female rats fed phosphate compounds were enlarged two to eight times over those of controls. The glands showed both hypertrophy and hyperplasia of the cells. Calcium deposits were present in the kidney tubules and x-rays showed gradual decalcification of the bones. Liegeois and Derivaux (1949) observed hyperplasia of the parathyroids in pigs fed a diet high in phosphorus and low in calcium.

Blumenfield and Rice (1937) confirmed other studies (Morgan 1936, Gilmour and Martin 1937) in which the parathyroids

were shown to be larger in the female than in the male rat. They assumed that the larger glands in the female were associated with the functions of gestation and lactation.

The effects of pregnancy in rats on diets of controlled calcium and phosphorus content were studied by Sinclair (1941). He found that a diet adequate for reproduction (Ca:P=1) produced a "simple hypertrophy" of the parathyroids which was cumulative in repeated pregnancies. Sinclair did nuclear counts and proved that this enlargement was due to cellular hypertrophy and not to hyperplasia. On a diet with a Ca:P of 0.5 this hypertrophy was more pronounced. This author reported that in long-continued stimulation of the gland there is, in addition to the hypertrophy, a hyperplasia which may more than double the cell number. Further study showed that in both low calcium-low phosphorus and low calcium-high phosphorus diets the parathyroids of maternal rats were very large (Sinclair, 1942).

Feeding rats phosphate and calciferol produced hypertrophy of the parathyroids which was verified by cell counts (Duguid, 1942). In a later study the parathyroid cells of 60-day rats maintained on a high phosphate diet showed hypertrophy and increased mitotic activity (Van Dyke, 1959). Enlargement of the parathyroids of rats fed low calcium diets was termed "hyperplasia" by Carnes et al., 1942 and 1943, who found that large doses of viosterol inhibited the

enlargement. A low calcium-high phosphorus vitamin D-free diet produced parathyroid hypertrophy and decalcified bones in rats (Crawford et al., 1957),

Varying the calcium-phosphorus ratio of the diet of rats demonstrated that the relationship between parathyroid volume and the log of the dietary calcium-phosphorus ratio closely approximated a straight line--i.e., the lower the ratio the greater the volume (Stoerk and Carnes, 1945).

Enlargement of the parathyroids resulting from both hypertrophy and hyperplasia of the cells was reported by Baker (1945) in rats 50 hours after ureteral ligation or nephrectomy. His observations were verified by noting a reduction in number of nuclei per unit area and increased mitotic activity. No significant changes in the vascularity or in the connective tissue of the gland were noted. He believed that the first response of the cells in one of hypertrophy followed or accompanied by hyperplasia. These results in general were confirmed by later observations (Weymouth, 1957).

METHODS AND PROCEDURES

I. ANIMAL PREPARATION AND MAINTENANCE

The animals used in this study were 90-day old female rats of the Long-Evans hooded strain, weighing approximately 150 grams at the beginning of the study.* In each of the experimental procedures the animals were run in duplicate plus a control.

The control rats were maintained on the nutritionally adequate stock diet developed by Drs. Ullrey and Miller of the Animal Husbandry Department of Michigan State University and were given tap water to drink. A calcium deficient, vitamin D-free diet devised by Crawford et al. (1957) and modified by Buckner (1959) was given to the experimental animals. This diet (C-D diet) contained 0.001 per cent calcium and 2.4 per cent phosphorus, in contrast to the stock diet which contained 0.8 per cent calcium and 0.4 per cent phosphorus. These animals were supplied with distilled drinking water which contained less than 2 parts per million of calcium. Buckner's work showed that rats fed this diet developed hyperparathyroidism, since injected doses of their blood sera produced parathormone-like responses in thyro-parathyroidectomized rats.

*All animals were supplied by Dr. J. E. Nellor, Head of the Endocrine Research Unit, Michigan State University.

The animals were grouped according to physiological states as follows:

Group I. Thirty non-pregnant animals were placed on the C-D diet from 1 to 20 days. Two rats were killed each day for the first 10 days and on alternate days thereafter up to 20 days. A control animal was killed with each pair. These animals have been divided into 3 sub-groups, 1A, 1B, and 1C, to designate animals maintained on the C-D diet for 1-5, 6-10, and 12-20 days, respectively.

Group II. Eight pregnant animals, 4 maintained on the C-D diet for 10 days and 4 on the stock diet, were killed 2 days before parturition was expected.

Group III. Four lactating animals, 2 of which were on the C-D diet for 14 days and 2 on the stock diet, were killed on the day of weaning (21 days post-partum).

Six lactating females, all maintained on the stock diet, were killed at various intervals: 2 at 5 days post-partum, 2 at 15 days post-partum, and 2 at 10 days post-weaning (30 days post-partum).

Table 1 shows the complete design of the experiment.

II. PREPARATION OF MATERIALS

Hoskins and Chandler (1925) stated that accessory parathyroid tissue in the rat was so infrequent as to be negligible, and this work has been cited by most investigators in support of work with parathyroidectomized animals.

TABLE I
DESIGN OF EXPERIMENT

	Group I -- Non-Pregnant Animals										Group II--Pregnant Animals										Group III--Lactating Animals														
	Control Group					Sub-Group A					Sub-Group B					Control Group					Sub-Group A					Sub-Group B					Sub-Group C				
	30	10	10	10	10	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2						
Number of Animals	30	10	10	10	10	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2						
Av. Weight in Grams	182	175	188	180	180	207	190	190	207	190	207	172	240	245	246																				
Type of Diet	Stock	C-D	C-D	C-D	C-D	Stock	C-D	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock	C-D	Stock						
Number of days on diet	1-20	1-5	6-10	12-20	12-20	10	10	10	10	10	14	14	14	5	15	30																			

However, Van Dyke (1959) has found accessory parathyroid tissue in 62 per cent of 73 normal postnatal Wistar rats. Since this study is based on representative changes in the rat parathyroid under conditions which did not necessitate complete parathyroidectomy, the presence or absence of accessory parathyroid tissue was not determined.

The thyroid-parathyroid glands, kidneys, and tibias were removed from each freshly killed animal and placed immediately in 10 per cent neutral formalin buffered with a mixture of mono- and dibasic sodium phosphate.

The bones were treated with Decal* and all tissues were dehydrated and cleared with an ethyl-butyl series of alcohols (Johnson, 1943). They were then embedded in Tissuemat,** blocked, sectioned at 6 microns, and stained as follows: Parathyroids: Hematoxylin (Malewitz and Smith, 1955) and Eosin, Crossman's Modification of Mallory's Triple Stain (Crossman, 1937). Kidneys: Hematoxylin and Eosin, Mallory's Triple Stain, Von Kossa's Method for Calcium (Mallory, 1942), Periodic Acid Schiff-Alcian Blue (Mowry, 1956). Tibias: Hematoxylin and Eosin, Toluidine Blue.

III. HISTOLOGICAL METHODS

Paraffin sections of the tissues from all animals were examined with the light microscope.

*Scientific Products, Evanston, Illinois

**Fisher Scientific Co., Pittsburgh, Pennsylvania

To determine whether or not hypertrophy of individual parathyroid cells occurred, nuclear density counts were made using a 5 sq. mm. net reticule ruled into 0.5 mm. squares. Ten 1 mm. square fields of each section were counted, the average of the total equaling 529 sq. μ of tissue. Hereafter, these measurements will be referred to as the average number of nuclei per unit area. Since the density of a nuclear population, i.e., the number of nuclei per unit volume of tissue, can be calculated by random sampling (Trowell and Westgarth, 1959), an analysis of variance was used as a test for significance. Each section of parathyroid was also examined for mitotic figures to determine whether hyperplasia was present, and these were tabulated as to total number per section.

Kidney sections were examined microscopically for calcium deposits. A visual quantitative estimation was made of the amount present, and each kidney was assigned a value of "-" to "++++", based on the following criteria: "-" denoting an absence of calcium, "+" indicating a scattering of a few deposits, "++" meaning several calcium areas present, "+++" showing extensive calcium distribution along with some tubular damage, and "++++" indicating very extensive deposits as well as widespread damage to the affected tubules.

Epiphyseal disc widths of the tibias were measured with an ocular micrometer using the 10X objective, each division equaling 10.1 microns. Five measurements were made of each tibia and the averages recorded. An analysis of variance was used as a test for significance.

RESULTS AND DISCUSSION

I. PARATHYROIDS

Group I.--Non-pregnant animals on the C-D diet. The parathyroid glands of all non-pregnant control animals on the stock diet presented the typical microscopic structure that has been described for the rat (Figure 1). The nuclear density count was 13.6 nuclei per unit area, and no mitotic figures were seen (Table II).

The glands of the C-D diet animals will be considered in 3 sub-groups of 5 each, according to the number of days on the experimental diet, i.e., 1-5, 6-10, or 12-20. These will be designated as 1A, 1B, and 1C, respectively.

It was evident from the nuclear density count that the parathyroids from the 1A animals showed hypertrophy of the individual cells (Table II). This enlargement occurred 24 hours after the beginning of the C-D diet, and the cells increased in size progressively through the fifth day (Figure 2). The mean of the nuclear density count of this group was significantly different from the mean of the controls ($P < .01$).

The enlargement appeared to involve both cell nuclei and cytoplasm. The former were sharply outlined and appeared hypochromatic, with finely dispersed chromatin and prominent nucleoli. Since the nuclei of normal parathyroid cells vary

somewhat in shape, there did not seem to be any particular changes in this respect in the dietary glands. The cytoplasm stained lightly, and because it was increased in amount the entire gland was lighter in appearance. The cells in general appeared normal, showing no signs of degeneration. In some cases, the cytoplasm appeared to be vacuolated, giving a "water-clear" appearance to the cell. The number of these vacuolated cells appeared to increase in direct proportion to the length of time the animal was on the diet.

The usual cord-like arrangement of the parenchymal cells was interrupted in various areas of the gland, with the cells tending to form acinar-type groups.

Two or three mitotic figures per section were counted in glands from animals on days 2 through 5, but this was not considered a sufficient increase in mitosis over normal controls to justify using the term hyperplasia to describe the enlargement noted.

There appeared to be an increase in vascularity from the second dietary day on. This observation was based on the presence of an increased number of endothelial cells (sinusoidal) and capillaries and was most evident in the glands from animals on the fifth dietary day (compare Figures 3 and 4).

It was difficult to ascertain by visual estimation whether there were any changes in the connective tissue stroma or capsules of these glands. However, the glands from an

TABLE II
PARATHYROID NUCLEAR DENSITY COUNTS AND MITOTIC FIGURES;
EPIPHYSEAL PLATE WIDTHS OF TIBIAS*

	Parathyroids		Tibias
	Nuclear Density**	Mitotic Figures#	Width of Epiphyseal Plate (μ)
GROUP I			
Controls	13.6	0	303
Sub-group A	8.8	2.2	157
Sub-group B	6.9	2.8	145
Sub-group C	8.4	1.0	118
GROUP II			
Stock diet	9.8	0	105
C-D diet	5.9	0	119
GROUP III			
Stock diet	10.1	0.7	134
C-D diet	6.4	0	117

* All values reported as means

** Number of nuclei/529 sq. μ of tissue

Number per section

animal on the third day did show greater amounts of connective tissue stroma than was found in the control glands. This finding was not a consistent one in glands from animals examined on the other 4 days.

The nuclear density count on the group 1B animals showed that the cells continued to hypertrophy; the group mean again was significantly different from that of the control animals ($P < .01$). The greatest amount of cellular hypertrophy was seen on the tenth dietary day (Figure 5). Two to 5 mitotic figures were counted in glands from animals on the sixth, seventh, ninth and tenth days (Figure 6). Although this was a slight increase over group 1A, it does not seem to justify the use of the term hyperplasia to describe the enlargement.

There appeared to be an increased vascularity of the glands in group 1B, especially on days 8, 9, and 10. There were no consistent changes in the connective tissue of the capsule or stroma of the gland.

The cell groups in these glands presented an increasing acinar-like arrangement, with many of them appearing in "nests." There were increased numbers of vacuolated cells, particularly at the periphery (Figure 7). Also present were larger "nests" composed of from 1 to 2 dozen cells surrounded by, or surrounding, a capillary. These were especially prominent in parathyroids from the 9-day C-D diet animals. In

sections from an 8-day-diet animal there were several smaller cell "nests," each consisting of from 5 to 10 cells. The cytoplasm of these cells was very vacuolated, and the nuclei were eccentrically positioned.

As in the two previous groups, the mean nuclear density count of the parenchymal cells in group 1C differed significantly from that of the normal control animals ($P < .01$). Contrary to the pattern observed thus far, there was somewhat less hypertrophy than in the previous group, even though these animals were maintained on the C-D diet for a longer interval (Figure 8). Only one or two mitotic figures were noted in the glands from animals on the twelfth, fourteenth, sixteenth and eighteenth days, while none were seen on the twentieth day. For the first time there appeared what seemed to be several tissue spaces among masses of the parenchymal cells (Figure 9). This could denote that some shrinkage had taken place which might account for the slightly higher nuclear density count found in this group. Although this feature could be the result of artifact, it was present in the majority of the glands from these animals. The acinar-like arrangement of the cells noted in the previous 2 groups was present, but there were fewer cell "nests." Also decreased in number were cells with vacuolated cytoplasm.

Vascularity was increased, i.e., large numbers of endothelial cells were present. There appeared to be no change in the amount of connective tissue present.

Group II.--Pregnant animals. Nuclear density counts on the parathyroids of these animals indicated cellular hypertrophy in both the stock diet rats and those on the C-D diet. The means were significantly different in each case, but only the C-D diet animals differed significantly from the normal control mean ($P < .01$). Of all the animals used in this study, the C-D diet pregnant rats showed the greatest cellular hypertrophy. No mitotic figures were noted in any of these glands.

There was increased vascularity of the glands in both stock diet and C-D diet animals, but it was more marked in the latter. No changes in connective tissue were noted.

The cells tended to lie in the acinar-type arrangement noted in previous groups, but the pattern was very irregular. There were many vacuolated cells present, with cell "nests" of the smaller type (8 to 10 cells) observed in all glands. (Figure 10).

The nuclei of the cells from animals on both diets were similar to those seen in the non-pregnant C-D diet animals--sharply defined, with finely dispersed chromatin and prominent nucleoli. Shrinkage spaces between cell masses were observed in both but seemed more prominent in the stock diet animals.

Group III.--Lactating animals. There was a significant difference between the means of the nuclear density counts

of tissues from lactating animals on the stock diet and those on the C-D diet. In addition, the mean of the latter was also significantly different from that of the non-lactating animals ($P < .01$). Only two mitotic figures were found, one each in the 5-day and the 30-day post-partum animals on the stock diet.

The glands from the control lactating rats appeared to show increased vascularity over those from the non-lactating controls. The acinar-type arrangement of the parenchymal cells was prominent in all glands. In some areas the cell groups were surrounded by capillaries, while in others a capillary was the center of the "acinus" (Figure 11). The nuclei showed varying degrees of chromatin density ranging from moderate hypochromasia to the pattern seen in the normal non-lactating controls.

There was increased vascularity in the glands of the C-D diet lactating animals as compared with those on the stock diet. Also, there were many cell "nests" consisting of clusters of vacuolated cells with the nuclei eccentrically placed. These cells were especially prominent at the periphery of the gland (Figure 12). The cell nuclei showed the pattern already described, which seems typical in animals on the C-D diet.

Nuclear density counts of glands from lactating control animals did not vary significantly from one another, although as has been pointed out they were significantly different from the means of both the C-D diet lactating group and the non-lactating controls.

Parenchymal cells of all these glands resembled the regular arrangement of cord-like rows which characterized the non-lactating control glands. However, there was a scattering of vacuolated cells, especially in those glands from the 5-day post-partum animals. Although there were some "nest" type cell arrangements, more often these "water-clear" cells were scattered at random throughout the more normal-appearing cells. With the exception of these, most of the cell nuclei showed normal chromatin pattern and density.

Glands from the 5- and 15-day post-partum animals appeared to have increased blood supply as compared to the controls. The 30-day post-partum group appeared to have a vascular supply similar to that of the control animals.

The terms "hypertrophy" and "hyperplasia" have been used in the literature to describe the type of parathyroid enlargement seen in various conditions of experimental hyperparathyroidism. Confusion in the use of these two terms has occurred because (1) some investigators apparently make no cytological distinction between them, using either or both terms to denote increase in cell size or in cell number, and (2) disagreement exists as to whether the parathyroid enlargement seen in different types of parathyroid stress (diet, pregnancy, lactation, etc.) is a result of hypertrophy or hyperplasia.

An attempt at clarification was made by Wilder et al. (1934) who stated that "hypertrophy" should be used to describe

enlargement due to an increase in size of the individual epithelial cells of the gland. Shelling (1935) noted that there was a lack of unanimity as to what constituted either hypertrophy or hyperplasia. He recognized the former as an increase in cell size rather than in numbers and the latter as enlargement due to an increase in cell numbers without an increase in their size or in the supporting tissue of the gland.

The data from this study indicates that under the stress of a calcium-deficient, vitamin D-free diet, the parenchymal cells of the parathyroid glands of Long-Evans hooded rats become hypertrophied. That is to say, there is an increase in volume of individual cells without an increase in cell numbers. Furthermore, in non-pregnant stressed animals, the degree of hypertrophy is significantly different from the normal and increases with the length of time on the experimental diet. This observation is based chiefly on nuclear density and mitotic figure counts (Table II). The lack of increase in the latter appears to rule out the possibility that enlargement of the glands under these dietary conditions is a result of cellular hyperplasia.

The parathyroids of pregnant and lactating animals showed this hypertrophy to an even greater degree. In addition, it can be seen that the pregnant dietary group had the most significant increase of all dietary groups. Apparently the added stresses of pregnancy and lactation are responsible for this difference.

In general, these results agree with those of Ham et al. (1940) and Sinclair (1941). Ham showed quantitatively that low calcium rickets caused marked hypertrophy of the parathyroids of rats, and Sinclair found simple hypertrophy in parathyroids from pregnant and low calcium diet rats. On the other hand, DeRobertis (1941) used the term hypertrophy in a confusing sense when he described the enlarged parathyroids of rats on low calcium diets as "hypertrophy due principally to an increase in the number of cells (hyperplasia) and to a less extent to an increase in volume of the cells (hypertrophy) . . ." The observations made in this study disagree with those of Luce (1923)--who stated that the marked enlargement found in the parathyroids of rats on a calcium deficient diet was due to hyperplasia, even though she observed no increase in mitotic divisions or other cytological evidence. In addition, Kurokawa (1925) described "nodular hyperplasia" in parathyroids from pregnancy cases in man without presenting any quantitative data.

Vacuolated cells and acinar-like arrangement of the parenchymal cells were rather consistent findings in glands from all three dietary groups. The appearance of "Wasserhelle" (water-clear) cells in enlarged parathyroids was first described by Getzowa (1907), and later studies confirmed the presence of these cells in various types of hyperparathyroidism (Castleman and Mallory, 1935; Duguid, 1942; Van Dyke, 1959).

The significance of these vacuolated cells may indicate that in attempting to respond to the stresses of diet, pregnancy, and lactation, they increase in cytoplasmic volume until a state of exhaustion is reached. This compensatory hypertrophy is evident in all three groups of dietary animals in this study and is not considered to be indicative of true degeneration. This observation assumes that the secretion made by the parenchymal cells is produced by cytoplasmic constituents, the nature of which has not been clearly defined.

In this study the acinar-like arrangement of the cells, resulting in cell "nests," parallels rather closely the degree of vacuolization. That is to say, the "nests" are composed of these vacuolated cells and their appearance in the gland increases with the degree of hypertrophy. Acinar-like configuration was noted by Krook (1957) in cases of van Recklinghausen's disease. A tendency toward acinar arrangement was also noted by Castleman and Mallory (1937) in more advanced cases of parathyroid enlargement.

The mechanism involved in the simple hypertrophy seen in the animals in this study would seem to be one of a direct stimulus of the parathyroid cells, causing them to increase their cellular volume in an attempt to compensate for lack of adequate calcium and vitamin D in the diet. This hypertrophy appeared to follow a general pattern which was dependent on the length of the dietary period. The maximum response occurred

about the tenth day, after which the gland appeared to stabilize for a time. The tissue spaces noted between the parenchymal cell masses near the end of the 20-day dietary period could indicate that some of the cells were shrinking from their enlarged state, i.e., that a form of "regression" similar to that described by Nonidez and Goodale (1927) was occurring. It would be of interest to determine by further study whether this regression would continue if the dietary interval was prolonged beyond 20 days.

The differences in vascularity and connective tissue content found in this study were too inconsistent to analyze with any degree of certainty. One might assume that increased cellular activity in an endocrine gland would call for an increased blood supply, but this is only speculation in this case.

II. KIDNEYS

Gross appearance. Grossly there were two main differences between the kidneys from animals on the stock diet and those from animals maintained on the C-D diet. In contrast to the dark purple color of the normal kidney, these organs from the C-D diet animals were a dull tan. In addition, the cut surfaces of coronal sections of kidneys from these animals presented a grainy appearance in the cortico-medullary region.

Group I.--Lesions of metastatic calcifications were noted in all non-pregnant animals maintained on the C-D diet, and in some animals there was also degeneration of the tubular epithelium. The amount of calcium present ranged from "+" to "++++" and was roughly proportional to the number of days the animals were fed the C-D diet. The calcifications appeared either as dense, homegenous masses or as mixtures of calcium and cellular material (Figure 13). In the more severely damaged kidneys these deposits were large and occupied the lumina of many tubules in the cortico-medullary region.

Calcium deposits were not noted in the cortical area, and the glomeruli appeared normal. In general, the masses were found in the lumina of the thick limb of Henle, and as the dietary interval lengthened the collecting tubules were similarly involved (Figure 14).

In animals from the fourteenth dietary day on, i.e., those animals in which the calcium deposits were heaviest, the tubular epithelium of the thick limbs of Henle and the collecting ducts showed distinct signs of degeneration. The cells showed pyknotic nuclei and disintegration of the cytoplasm. It was not determined whether the tubular cells themselves were calcified. Many of the affected tubules appeared dilated and their lumina were completely obstructed by the calcium masses (Figure 15).

On the basis of the PAS reaction, Bowman's capsule appeared to gradually thicken in an irregular fashion as the time interval on the C-D diet increased (Compare Figures 16 and 17). PAS-positive homogenous masses were noted in some of the collecting ducts of all animals, including the normal controls, but again the amount of this material seemed to increase with the length of time the animals remained on the C-D diet.

Vacuolated tubular cells were noted in the medullary area, appearing on the tenth day of the C-D diet. This type of cell also increased in frequency in proportion to the length of the dietary period. Syncytial giant cell formation inside the tubules was noted, probably as a result of the loss of some of the tubular epithelium (Figure 18).

Group II. The kidneys of the pregnant control animals showed no differences from those of the non-pregnant

controls. Their counterparts on the C-D diet for 10 days exhibited the same type and extent of damage seen in the 10-day dietary animals in Group I. Calcium deposits were heavy (++++) in the loops of Henle and in the collecting system, and Bowman's capsule appeared thickened. In addition, the damage to the tubular epithelium seemed more extensive in the pregnant dietary animals than in the non-pregnant group (Figure 19).

Group III. Kidneys from lactating animals maintained on the stock diet presented no evidence of calcium deposition or tubular epithelial damage.

Periodic acid Schiff-positive casts of varying sizes were present in the collecting ducts of all animals except those killed at 30-days post-partum. The tubules containing these casts seemed dilated in many cases, causing a flattening of the epithelial cells (Figure 20).

Lactating animals on C-D diet for 15 days showed slightly less calcium deposition (+++) and cellular damage than the 15-day C-D diet animals in Group I. PAS-positive casts were also present in the collecting ducts, and the glomerular capsules appeared slightly thickened.

In the area of the collecting ducts in the kidneys of all animals in this group many of the epithelial cells displayed a distinct vacuolation, giving a "spongy" appearance to the section when viewed under lower power. This

manifestation was particularly noticeable in the 5-day post-partum animals (Figure 21). It seems probable that this vacuolation was caused by hydropic swelling.

Since it is generally conceded that the parathyroid hormone is involved in regulating the excretion of phosphate by the kidney, it is to be expected that any upset in the calcium-phosphorus metabolism would be reflected in this organ. The kidney findings of this study agree in general with those of other investigators who have described morphological changes in the kidneys of animals with hyperparathyroidism produced by different methods (Hueper, 1927; McJunkin et al., 1932; Pappenheimer and Wilens, 1935; Shelling and Remsen, 1935; Magnus and Scott, 1936; Pappenheimer, 1936; Cowdry and Scott, 1936; Donahue et al., 1937; Highman and Hamilton, 1937; McFarlane, 1941; Duguid, 1942; Bogdonoff et al., 1956; Rich et al., 1958).

In contrast to the interstitial involvement found by Anderson (1939) in cases of chronic hyperparathyroidism, it can be seen that the renal lesions found in the C-D diet animals in this study involved the kidney tubules themselves. This finding agrees with that of Engfeldt et al. (1958). Noting the lack of agreement with respect to localization of renal lesions in hyperparathyroidism, Carone et al. (1960) used microdissection to determine the exact location of kidney damage produced by one dose of parathyroid extract in

dogs. They found tubular lesions in the ascending limb of Henle, the distal convoluted tubules, and the collecting system.

In view of the comparatively gradual appearance of changes that occurred in the morphology of the parathyroids and tibias of the dietary animals in this study, it was surprising to note the appearance of metastatic calcifications as early as 24 hours after feeding the C-D diet. According to serum calcium determinations by Buckner (1959), animals on the diet up to 10 days maintained normal levels in the 9-11 mg. % range except on the fifth day. On the other hand, serum inorganic phosphorus increased from the second day and continued rising. Since serum inorganic phosphorus usually decreases in hyperparathyroidism, the high phosphate content of the diet used in this study apparently caused a heavy renal phosphate load which resulted in precipitation of calcium phosphate in the kidney as early as the first dietary day. This finding agrees with the work of Saxton and Ellis (1941) who noted calcium deposits in the kidney tubules of rats on high phosphate diets.

As might be expected, the amount of calcium deposition roughly paralleled the length of the dietary period in all three groups. Tubular cell degeneration became evident when this deposition was judged to be "+++" by visual estimation.

The presence of PAS-positive material in the collecting ducts of lactating animals up to 15-days post-partum on the stock diet is apparently a normal finding. The nature of this material was not determined qualitatively, but it is suggested that it is of a mucoprotein nature. Apparently it is not of functional significance, since these animals remained as healthy as the non-lactating rats of stock diet.

Thickening of Bowman's capsule in the kidneys of the dietary animals, first noticed on the tenth dietary day, was the only glomerular change observed. This manifestation was also noted by Anderson (1939) in cases of chronic hyperparathyroidism. The cause of this change was not determined, but it was due apparently to a form of hyalinization rather than calcium deposition, since there was no positive test for calcium in this area.

The vacuolated tubular cells also made their appearance on the tenth dietary day in the Group I animals and could be an indication of growing tubular cell degeneration. This explanation, however, does not account for the presence of these cells in large numbers in the kidneys of the 5-day post-partum animals on stock diet. Further study is needed to determine the significance, if any, of this manifestation.

III. TIBIAS

Group I. In general, bone changes occurred more slowly than parathyroid and kidney changes in the C-D diet animals. One of the characteristic features of the normal control rat tibia are the regularly arranged columns of cartilage cells in the epiphyseal plate, with a prominent zone of hypertrophied cells. The average width of the entire plate was 303 μ , with the margins fairly regular on both the metaphyseal and epiphyseal sides. Metachromasia was most apparent in the area composed of younger cartilage cells. In addition, the bony spicules formed numerous trabeculae with marrow spaces between (Figure 22).

During the first 5 days on the C-D diet the most noticeable change was a progressive narrowing of the epiphyseal plate with irregularity of the margins. Hypertrophied cartilage cells were still present, although the normal columnar arrangement was disturbed. The bony spicules were fewer in number and showed an increasing degree of metachromasia. Calcification occurred during this dietary period, although it proceeded at a reduced rate from normal.

In the 1B animals, the epiphyseal plate width remained reduced and the margins were irregular. On days 6 through 9 the hypertrophied cartilage zone was much larger relative to the total cartilage area than in the normal controls. On day 10, however, these hypertrophied cells

were greatly reduced in number, the younger cells being more numerous. Bony spicules were present in tibias from all animals, but the definite trabecular formation found in the control group was lacking. Marrow was present in increasing amounts between the reduced number of spicules. Calcification appeared to be proceeding at a progressively slower rate as the dietary interval increased.

By the sixteenth dietary day the bony spicules had all but disappeared and the shaft was filled with marrow containing many fat globules. The epiphyseal plate, which continued to narrow, still presented some hypertrophied cells. They were greatly reduced in number, however, and most of them were no longer arranged in orderly columns. On the eighteenth day there were only a half dozen spicules present and only a small number of cartilage cells making up the epiphyseal plate were hypertrophied. Calcification appeared to have almost ceased.

The tibias from the 20-day C-D diet animals showed the most extensive changes from the normal (Figure 23). The epiphyseal plate was reduced to its narrowest width. There were only two or three small bony spicules present, and the marrow contained much fat. Very few cartilage cells were in the hypertrophied stage, resulting in a highly meta-chromatic epiphyseal plate.

Group II. The pregnant animals on the stock diet showed a different bone picture from that of the non-pregnant

controls. The epiphyseal plate width was reduced by more than half and was very irregular in its margins; there were few hypertrophied cartilage cells present. The metachromasia of the plate resembled the intensity seen in the Group 1C animals. There was only a scattering of bony spicules, with no trabecular formation evident. There was a large amount of marrow present, but it contained little fat.

The tibias from pregnant animals on the C-D diet for 10 days did not vary from the stock diet animals as much as might be expected. The main difference was that these bones showed a greater number of hypertrophied cartilage cells in the epiphyseal plate. The marrow was relatively free from fat. There was more spicule formation in these tibias than in the 10-day C-D diet animals in Group I, but the plate width was approximately the same (Figure 24).

Group III. In the lactating animals on the stock diet the epiphyseal plate width was slightly more than half that of the non-pregnant, non-lactating control group, but greater than that of the pregnant control animals. The hypertrophied cartilage zone of the plate was larger and more regularly arranged than in the pregnant group. There were only a few bony spicules present and almost no trabecular formation. The large amount of marrow contained heavy deposits of fat.

The epiphyseal plate of the animals maintained on the C-D diet for the last 15 days of lactation was very

irregular and reduced in width by half over that of the control group (Figure 25). Hypertrophied cartilage cells were greatly reduced in number and very irregularly arranged. There were very few bony spicules with no trabecular formation evident. The marrow contained much fat and fibrous connective tissue.

On the fifth post-partum day the epiphyseal plate showed an increased width over that of the animals observed at weaning, with many hypertrophied cartilage cells in fairly regular columns. There were many spicules of bone forming small trabeculae particularly prominent adjacent to the metaphyseal side of the plate. Osteoblasts were present in fairly large numbers, giving evidence that calcification was proceeding at a fairly rapid rate. The marrow contained some fat but little fibrous connective tissue.

By the fifteenth post-partum day the epiphyseal plate had increased in width by some 20 μ . The zone of hypertrophied cartilage was larger, with the cells in regularly arranged columns. The large number of bony spicules formed many trabeculae and osteoblasts were present in large numbers.

The increasingly wider epiphyseal plate on the thirtieth post-partum day abounded in hypertrophied cartilage cells. There was a definite trabecular formation of ossifying spicules, and many osteoblasts were present. The marrow contained some fat and a small amount of fibrous connective tissue.

Ingalls (1941) made a detailed study of the normal epiphyseal growth in the bones of albino rats. He described the series of integrated processes of proliferation, degeneration, and calcification of cartilage and its subsequent removal and replacement by calcified osteoid matrix.

Although the exact relationship has not been clearly defined, there is an interrelationship between the parathyroids and resorption of bone. Since the parathyroid glands regulate the plasma calcium ion concentration, they respond to a diminution in this concentration by increased activity, which leads to resorption of bone with subsequent release of dissolved calcium into the blood. Selye (1942) believes that the primary site of hormone action is in the bone itself, i.e., that it stimulates osteoclast formation and bone absorption.

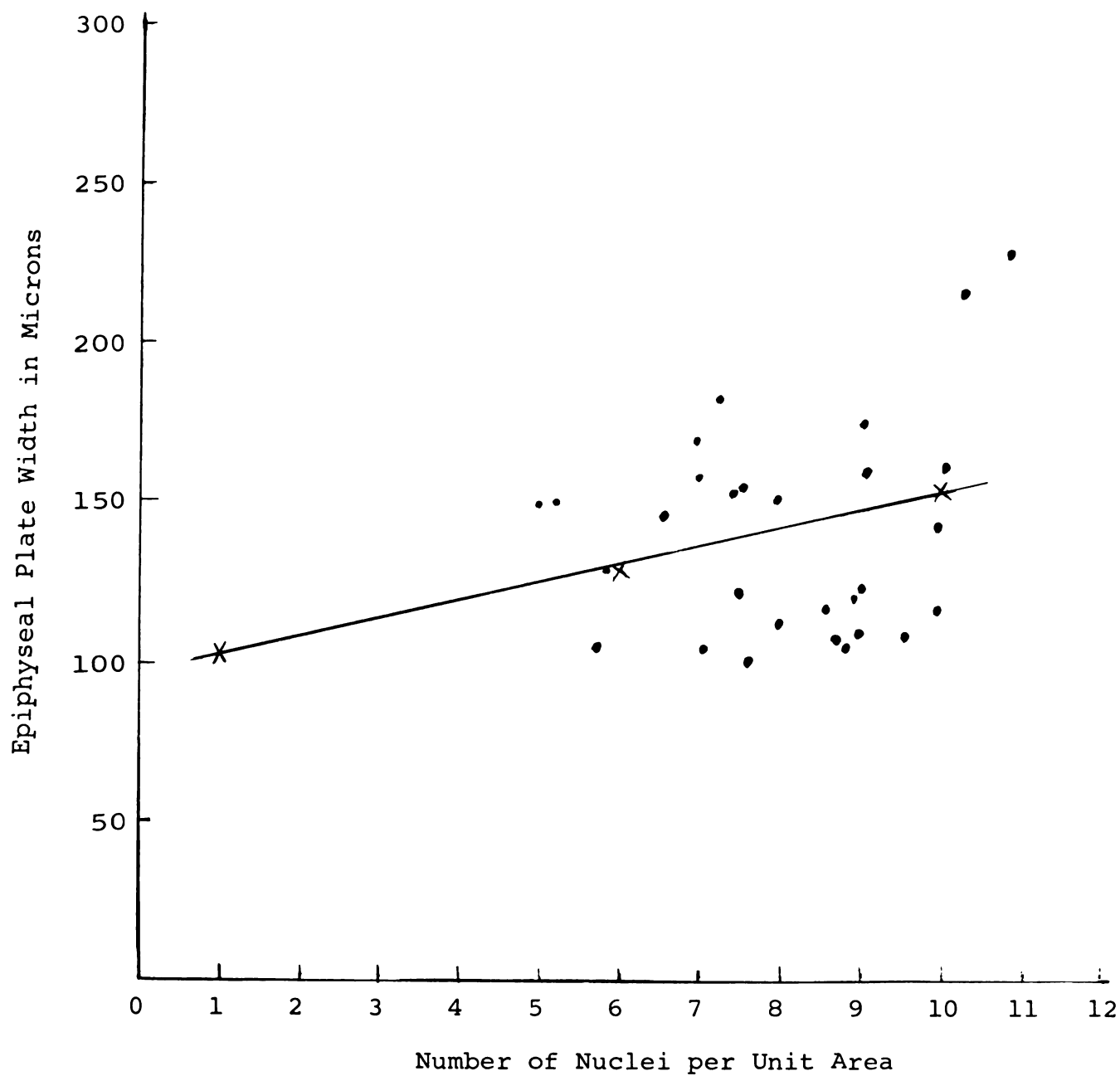
The results of this study show that the bones of the animals on the C-D diet reflected the fact that the serum calcium was being maintained by dissolution of bone salts. The width of the epiphyseal plate correlated rather closely with the parathyroid nuclear density count (Figure 26), becoming narrower as the gland size increased. This correlation was not statistically significant, but there is at least a general relationship between the degree of parathyroid stress and the tibia response.

Evidence is also present, by reason of increasing metachromasia of the plate, that as the dietary interval increases there is a growing decline in osteogenesis. This observation is reinforced by the continued decrease in the number of bony trabeculae which are being formed in the bones of the C-D diet animals contrasted to the normal controls. This picture agrees with the findings of Boelter and Greenberg (1941) and Jaffe and Bodansky (1930b), the latter characterizing these changes as osteoporosis. Crawford (1957) also noted that vitamin D-free rats showed marked decalcification at the epiphyses.

Lactation appeared to increase the bone changes resulting from the C-D diet. In these animals calcification proceeded at its slowest rate, apparently as a result of the greatly increased dissolution of bone salts. Pregnancy also produced an increased breakdown in bony trabecular formation, but the C-D diet pregnant animals did not show significant differences from their non-pregnant counterparts in this respect. Apparently the process of dissolution in response to the increase in demand for calcium reaches a stabilized level and no greater dissolution is forthcoming.

The events described here appear to bear out the theories of others that in primary hyperparathyroidism the parathyroid hormone has a direct action on the bone (Thompson and Collip, 1932; Jaffe, 1933; Jahan and Pitts, 1948). It

FIGURE 26
Correlation of Epiphyseal Plate Widths and
Parathyroid Nuclear Density Counts for
Group I C-D Diet Animals



appears, however, that the bone picture depends to a large degree on what method is used to induce the parathyroid stress. The most rapid and dramatic changes are those observed in animals given large doses of injected hormone, while in the case of dietary stress the observable differences from the normal appear more gradually. Also, in the latter case, disruption of the normal calcification process is the most dominant feature and is correlated with the length of the dietary interval.

SUMMARY AND CONCLUSIONS

Thirty-six Long-Evans hooded female rats, divided into non-pregnant, pregnant, and lactating groups were placed on a low calcium, vitamin D-free (C-D diet) for periods ranging from 1 to 20 days; 36 control animals were maintained on a stock diet for the same length of time. Parathyroids, kidneys and tibias from both experimental and control animals were studied for evidence of morphological changes.

Parathyroid nuclear density counts were significantly decreased in all animals on the experimental diet ($P < .01$). This increase in cell size was directly correlated with the length of the dietary interval through the first 10 days, the greatest increase appearing in the glands of the pregnant group. From 12 to 20 days the glands showed a slight regression in cell size, but they were still significantly larger than the controls. There was no significant difference in mitotic activity between any of the C-D diet groups and the controls.

Renal changes consisting of metastatic calcifications, degenerating tubular epithelial cells, and thickening of the glomerular capsules were noted in all C-D diet animals as early as 24 hours after the onset of the diet. The degree of damage was directly correlated with the length of the diet period. In lactating animals on stock diet, periodic acid-Schiff positive material was observed in the collecting ducts.

Bone changes were noticeable on about the third dietary day and were characterized chiefly by a decrease in the width of the epiphyseal plate. This narrowing of the plate was roughly correlated with the time interval of the experimental diet. The pregnant animals on both diets showed the greatest decrease in plate width. Other changes noted were a diminution of hypertrophied cartilage cells in the plate region and a reduction in the number of bony spicules and trabeculae.

The results of this study indicate that in hyperparathyroidism induced in rats by a low calcium, vitamin D-free diet, the parathyroid glands undergo cellular hypertrophy in an attempt to meet the demand for increased calcium metabolism. In view of the insignificant degree of mitotic activity, hyperplasia was not a contributing cause to the enlargement seen. The glands appear to reach the limit of their ability to increase in size on the tenth dietary day, after which, although still enlarged, they tend to regress. The kidneys show the effects of the increased renal phosphate load almost immediately, the damage progressing with the number of days on the experimental diet. The tibial changes, mainly involving disruption of the normal calcification process, likewise reflect the length of the dietary interval.

Since the greatest degree of change in all organs appeared in the pregnant animals on the C-D diet, it would appear that pregnancy places a greater demand on the calcium regulatory mechanism than does either dietary stress or lactation.

Whether or not the patterns of change seen in this study would continue over a longer dietary interval can be determined only by further investigation.

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Figure 1
Section from the parathyroid of a non-pregnant
control rat.
H & E; X710

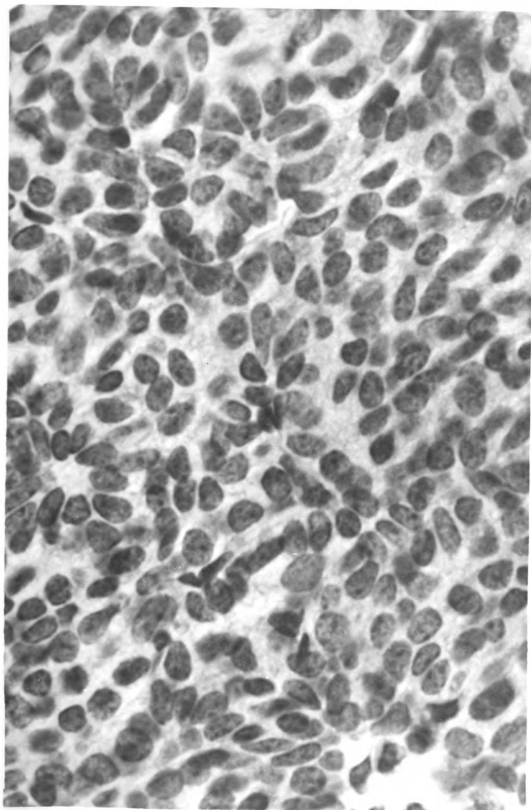


Figure 2

Section from the parathyroid of a non-pregnant rat on
the fifth dietary day, showing cellular hypertrophy.

H & E; X710

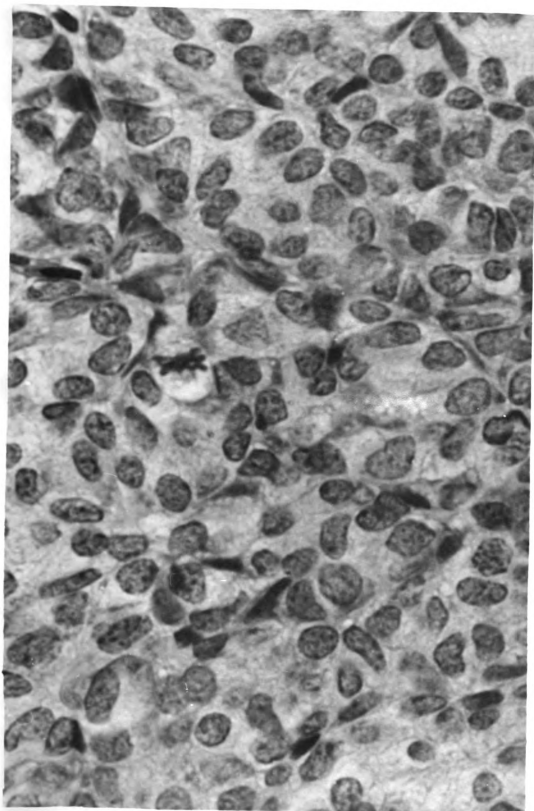


Figure 3
Parathyroid from a non-pregnant control rat, showing
normal vascularity.
Trichrome; X120

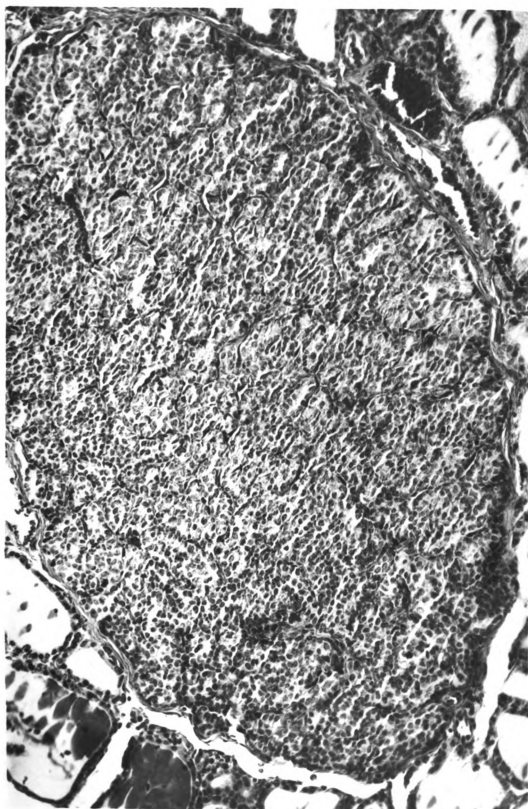


Figure 4

Parathyroid from a non-pregnant rat on the fifth
dietary day, showing increased vascularity.

Trichrome; X110

1. Endothelial nuclei

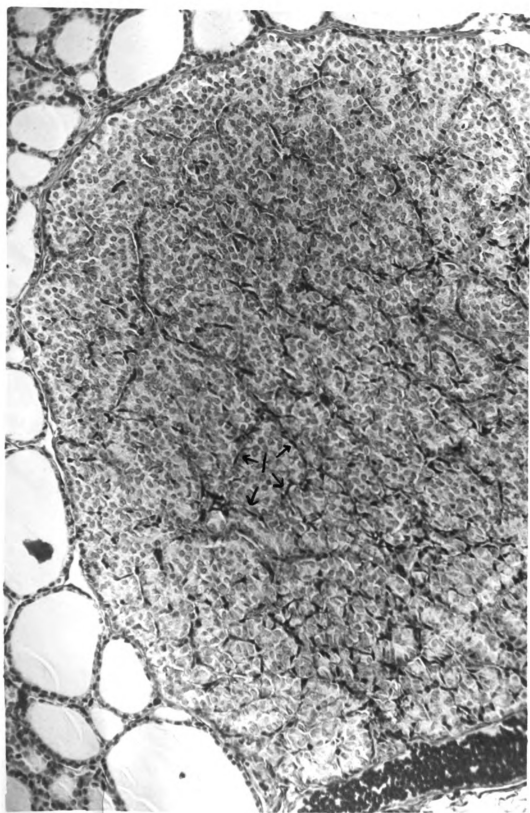


Figure 5

Section from the parathyroid of a non-pregnant rat
on the tenth dietary day, showing the greatest degree
of hypertrophy. Compare with Figure 1.

H & E; X710

1. Endothelial nuclei
2. Parathyroid nuclei

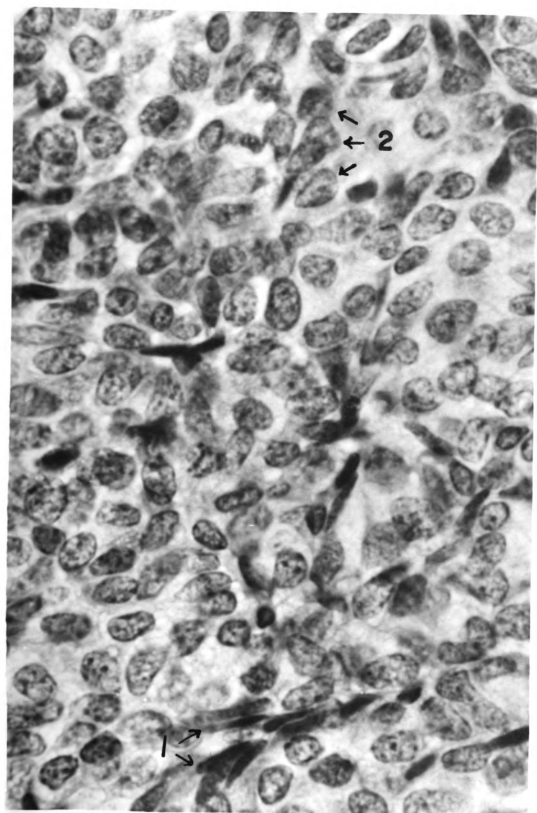


Figure 6

Section from the parathyroid of a non-pregnant rat on the ninth dietary day. Arrows indicate mitotic figures.

H & E; X710

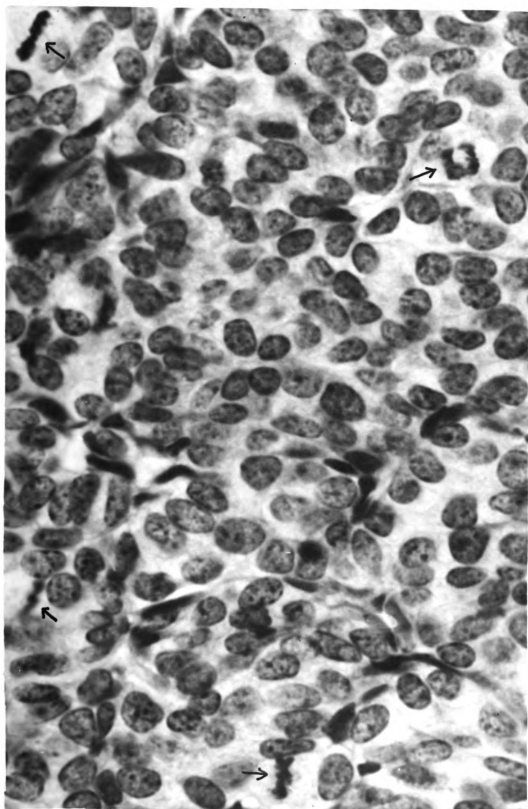


Figure 7

Section from the parathyroid of a non-pregnant rat
on the eighth dietary day. Dotted line indicates
"nest" of vacuolated cells.

H & E; X710

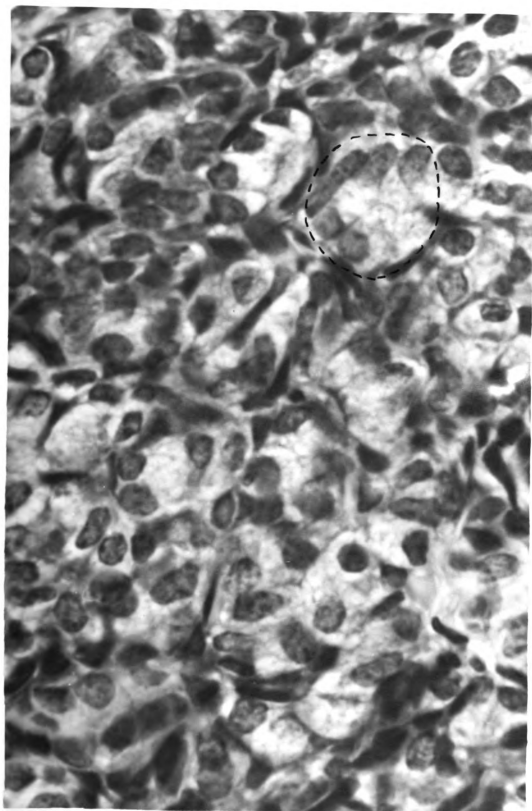


Figure 8

Section from the parathyroid of a non-pregnant rat on the sixteenth dietary day, showing slight regression in cell size. Compare with Figures 2 and 5.

H & E; X710

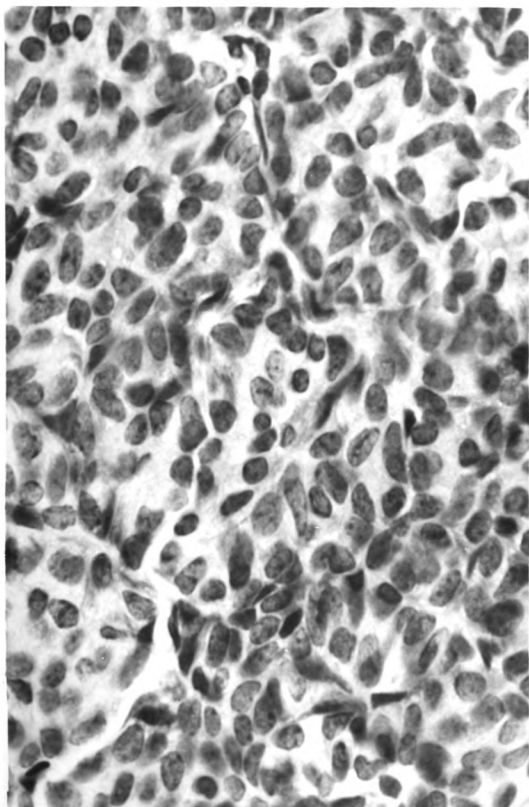


Figure 9

Portion of the parathyroid from a non-pregnant rat on the fourteenth dietary day, showing tissue spaces (arrows).

Trichrome; X210

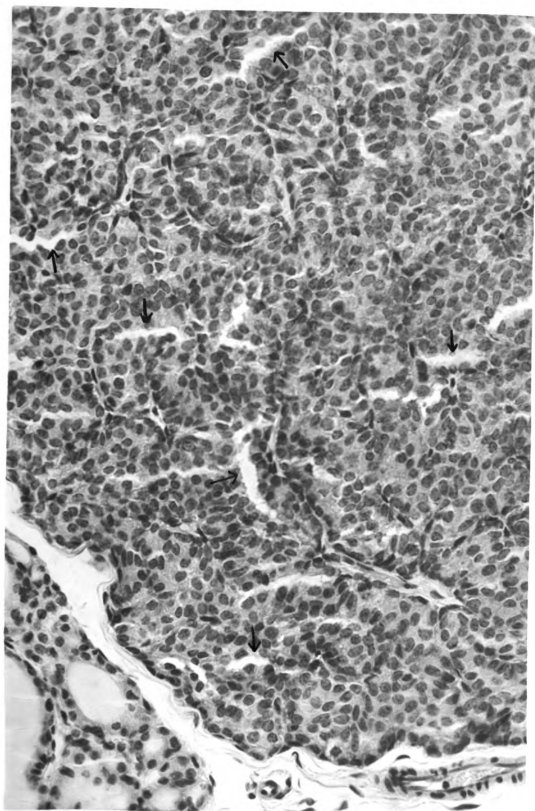


Figure 10

Section from the parathyroid of a pregnant rat on the C-D diet for 10 days. Dotted line indicates "nest" of vacuolated cells.

H & E/ X710

the
" of

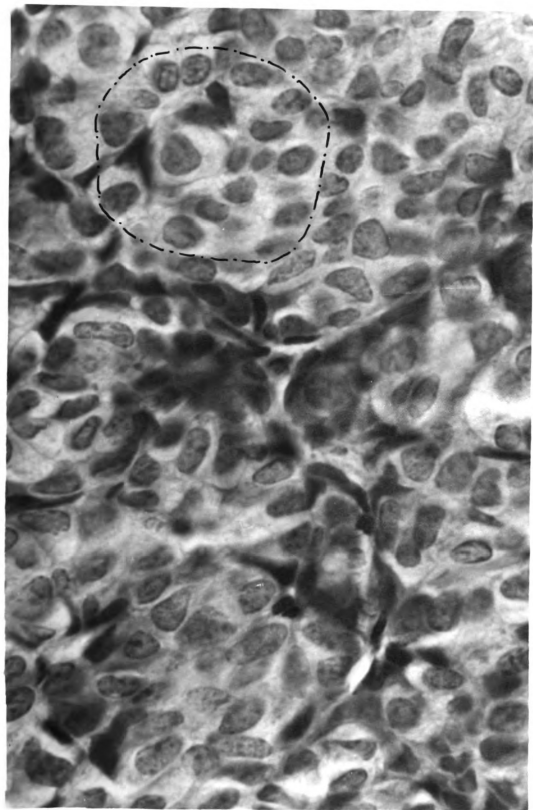


Figure 11

Section from the parathyroid of a lactating rat on
the stock diet.

H & E; X710

1. Capillary in center of cell "acinus"
2. Cells arranged in acinar configuration

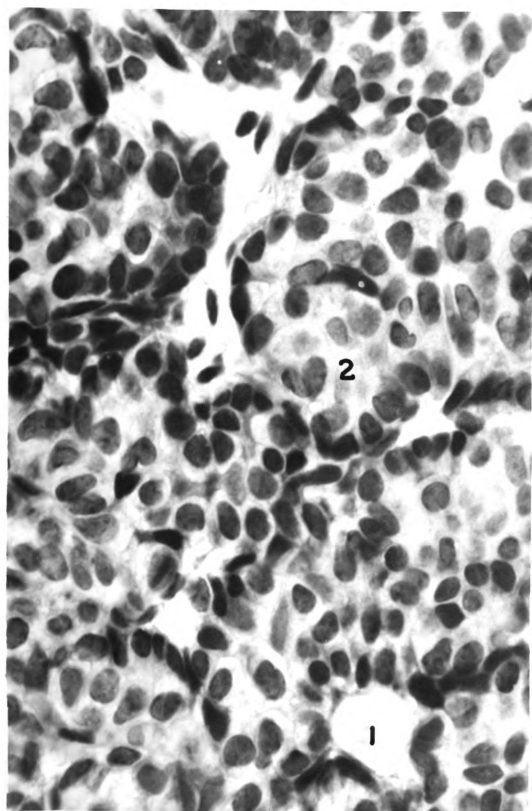


Figure 12

Section from the parathyroid of a lactating rat on
the C-D diet for 14 days, showing vacuolated cells
(X) at the periphery.

H & E; X710

1. Shrinkage space

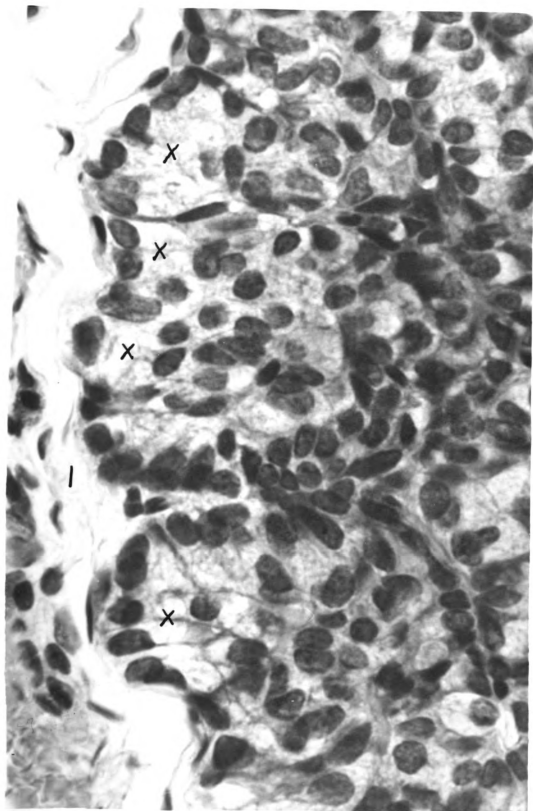


Figure 13

Section from kidney of a non-pregnant rat on the fifth dietary day, showing areas of metastatic calcification of the tubules.

Von Kossa; X135

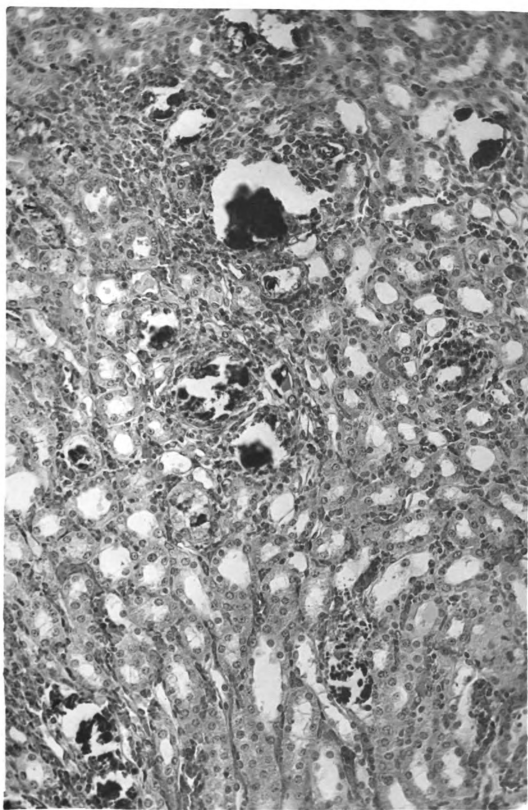


Figure 14

Section of kidney from a non-pregnant rat on the ninth dietary day, showing calcium deposits in the collecting tubules.

Von Kossa; X135

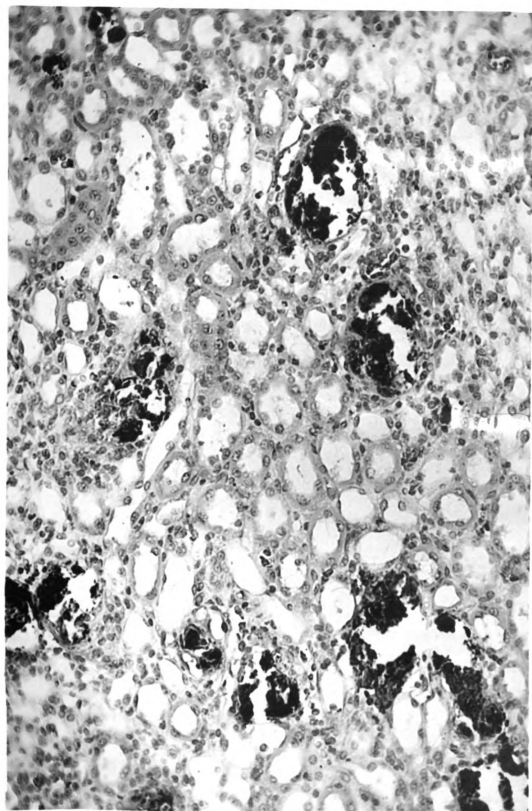


Figure 15

Section of kidney from a non-pregnant rat on the sixteenth dietary day, showing heavy calcium deposits in the collecting tubules.

Von Kossa; X135

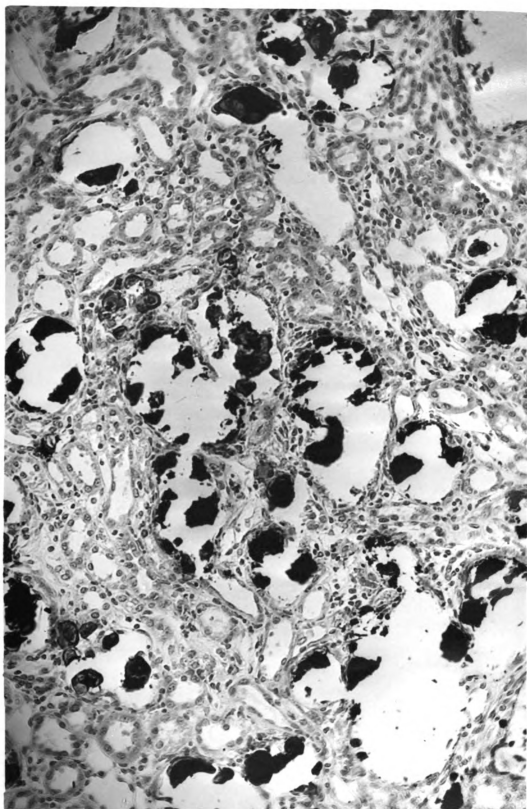


Figure 16

Glomerulus from the kidney of a non-pregnant control
rat showing a normal Bowman's capsule.

PAS; X640

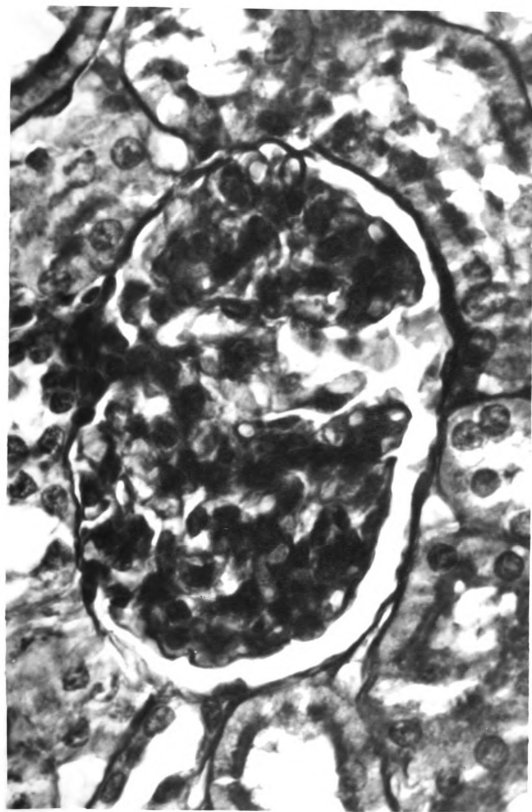


Figure 17

Glomerulus from the kidney of a non-pregnant rat on the twelfth dietary day, showing thickened Bowman's capsule.

PAS; X640

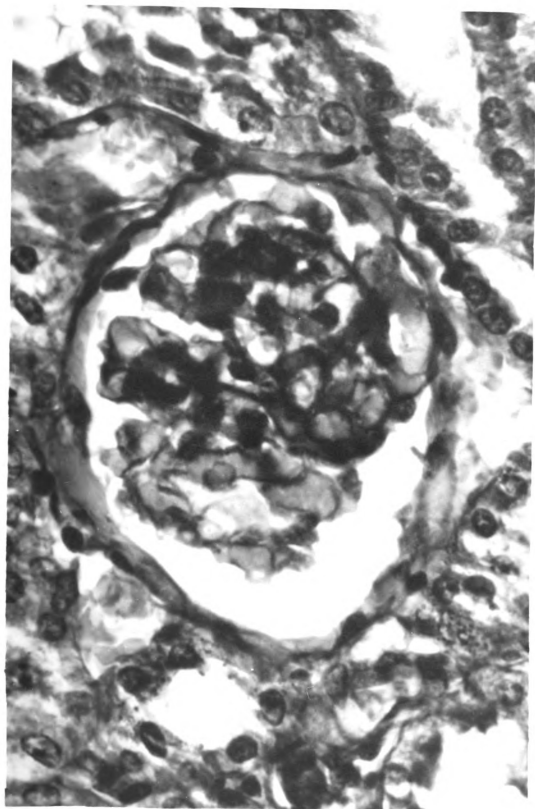


Figure 18

Section of a kidney from a non-pregnant rat on the twentieth dietary day, showing heavy calcium deposits in collecting tubules. Compare with Figures 13, 14, and 15.

H & E; X265

1. Syncytial giant cells

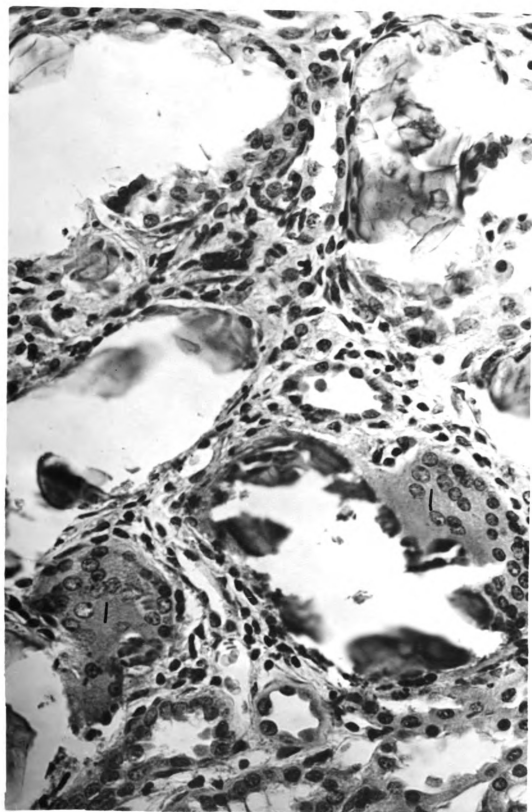


Figure 19

Section from the kidney of a pregnant rat on the
C-D diet for 10 days, showing calcium deposits and
tubular epithelial damage.

H & E; X265

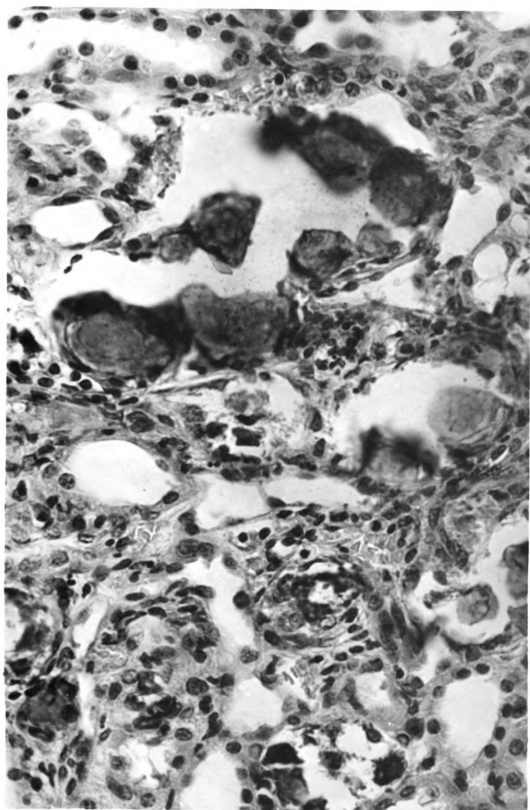


Figure 20

Section of the collecting system from the kidney
of a 5-day post-partum rat on the stock diet, showing
PAS-positive casts.

PAS; X265

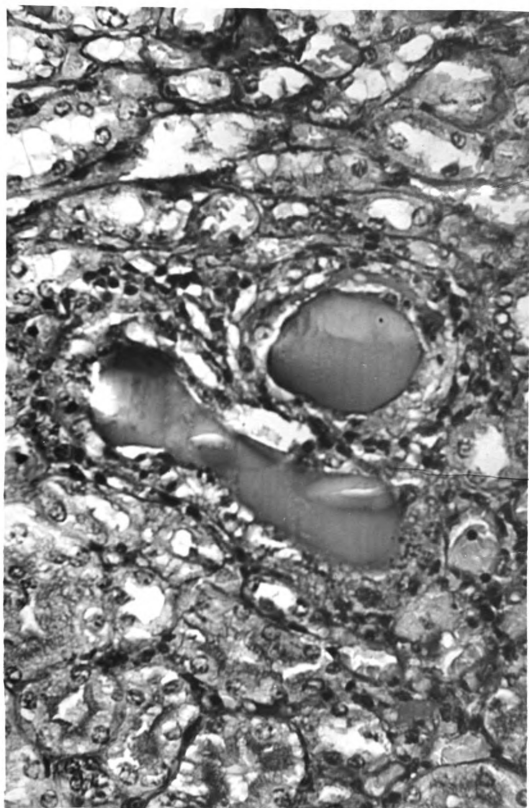


Figure 21

Section of collecting ducts from the kidney of
a lactating rat on the stock diet, showing vac-
uolated cells.

PAS; X265

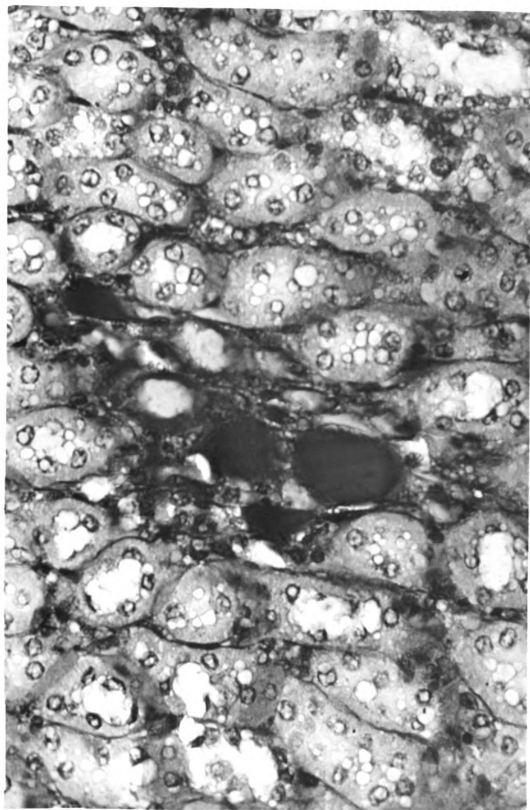


Figure 22

Section from the epiphyseal plate of a tibia from
a non-pregnant control rat, showing normal meta-
chromasia and cartilage proliferation.

Toluidine Blue; X135

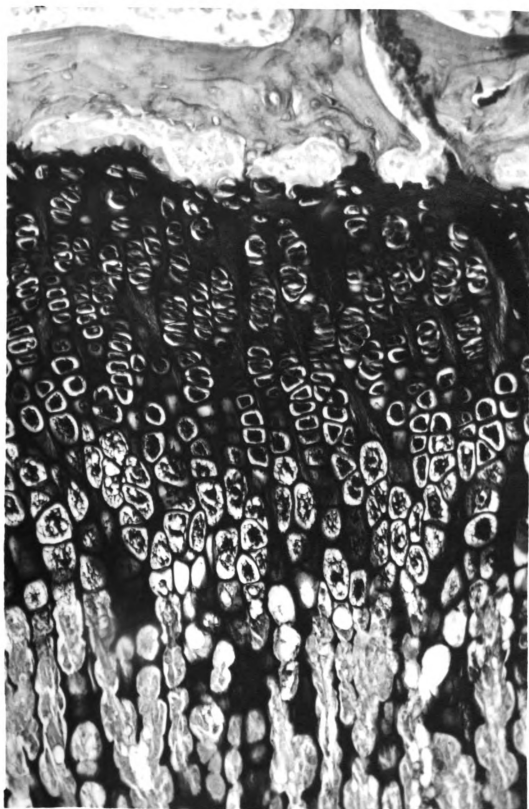


Figure 23

Section from a tibia of a non-pregnant rat on the twentieth dietary day. Note narrowed epiphyseal plate and lack of normal cartilage proliferation.

Toluidine Blue; X135

1. Fat in the diaphyseal marrow
2. Bony spicule

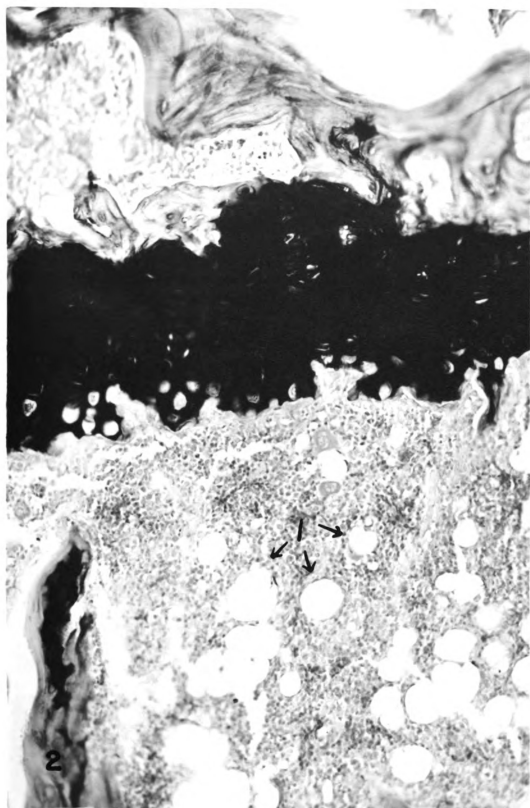


Figure 24

Section from a tibia of a pregnant rat on the tenth dietary day. Note the irregular epiphyseal plate.

Toluidine Blue; X135

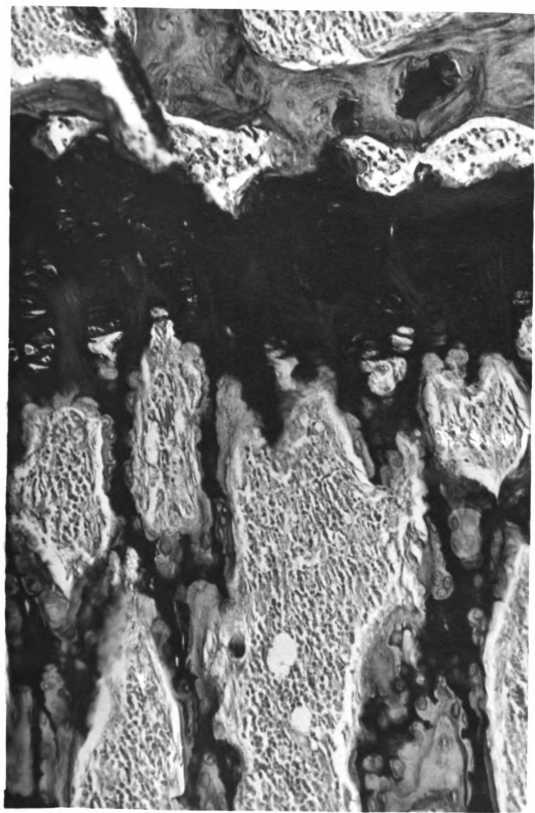
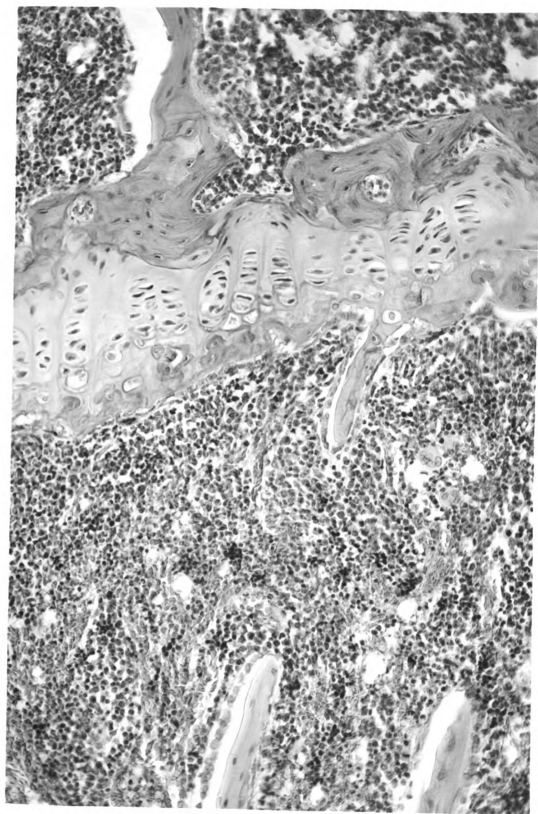


Figure 25

Section from a tibia of a lactating rat on the
C-D diet. Note irregular epiphyseal margins
and lack of cartilage cell hypertrophy.

H & E; X135



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