1967

The Effect of Adrenocorticosteroids on Magnesium Metabolism in the Albino Rat

John Harry Fournier
Loyola University Chicago

THE EFFECT OF ADRENOCORTICOSTEROIDS ON MAGNESIUM METABOLISM IN THE ALBINO RAT

by

John Harry Fournier

A Thesis Submitted to the Faculty of the Graduate School

of Loyola University in Partial Fulfillment of

The Requirements for the Degree of

Master of Science

June

1967

BIOGRAPHY

Dr. John Harry Fournier was born in Chicago, Illinois, on August 30, 1942. In June, 1959, he graduated from the Oak Park and River Forest High School, Oak Park, Illinois and then attended the Chicago Undergraduate Division of the University of Illinois, Navy Pier, being enrolled in the pre-medical curriculum until June, 1962.

In September, 1962, the author began his freshman year in medical school at Loyola University Stritch School of Medicine. In July, 1963, he began his studies in the Graduate School of Loyola University, Department of Anatomy, as an M.S. - M.D. candidate. In June, 1966, he was awarded the degree of Doctor of Medicine.

From July, 1966 until the present time he has held the position of Research Associate in Anatomy, Department of Anatomy, at the Loyola University Stritch School of Medicine.

On March 5, 1962, the author married the former Miss Katherine Kassaris of Athens, Greece and on January 22, 1963 became the proud father of a baby daughter.

Dr. Fournier is a member of the National Association of Interns and Residents and the Illinois Society for Medical Research.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to his advisor, Dr. Lincoln V. Domm, Professor of Anatomy, and Chairman, Department of Anatomy, for his guidance and encouragement in this investigation and for providing the rare intellectual climate for serious research. Were it not for his kind understanding nature and his keen interest in his students problems this work would not have been even attempted. This work then bears testimony to the inspirational effect of this man and his supreme ability to guide and direct those around him without seeming to.

The author wishes to express publicly his gratitude for all the consideration and interest shown him by the staff of the Department of Anatomy throughout the years spent at the Graduate School, also, let it be known that Dr. Lincoln V. Domm was a major force in influencing the author's life an unforgettable influence.

My thanks also go to Mrs. Lucia Smelte, Senior Technician in the Department of Anatomy for her help and counsel in preparing the histological material.

Finally, but not least of all, I would like to take this opportunity to express my gratitude for the aid and encouragement which my wife, Kathy so generously provided.

The study was supported in part by USPHS., NIH, Research Grant DE-00694, and a USPHS., NIH, General Research Support Grant. Grants administered by Dr. Lincoln V. Domm.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Forty-eight years have now passed since Osborne and Mendel (1918), discouraged by the lack of knowledge during their time pertaining to the role of inorganic elements in nutrition, investigated the effect of depriving animals of various inorganic elements (K, Mg, Ca, Na, Cl). Theirs was the first attempt to deprive an animal of magnesium but their diet was not sufficiently low in magnesium content (0.012%) to permit the development of the currently well-known and characteristic symptoms of Mg-deficiency. They observed that white mice maintained on their diet, low in magnesium showed good growth, however, they did not conclude that magnesium is a dietary essential.

More diffuculty existed in those days in obtaining a diet low or deficient in magnesium than is the case today. LeRoy (1926) recognized the difficulty in obtaining a diet sufficiently refined to have a low magnesium content and stated that Osborne and Mendel (Loc cit) could not have arrived at any significant conclusions as to the effect of magnesium deprivation since their diet was not sufficiently deficient. He succeeded in obtaining a purified diet containing only 1.03 mg of Mg/100 gm of diet. He was the first to observe the essentiality of magnesium for the growth and development of "la souris blanche". He noted that the growth of Mg-deficient rats was arrested and that within 24 to 35 days following the feeding of such a diet some of his animals died. LeRoy did not describe the manner of death of his experimental animals. However, he concludes his 2-page publication by saying that,

It appears well demonstrated that magnesium plays a very important role and is absolutely necessary for the growth and maintenance of weight of the white rat".

Man's inquisitive mind and nature would not permit him to remain satisfied with only the knowledge that magnesium was an absolute dietary essential. Soon after Kruse, Orent and McCollum (1932) published the results of their pioneer work, describing the signs of Mg-deficiency, such as hyperemia of the ears, scaly dermatitis, and the characteristic tonico-clonic convulsions, which often led to the death of the animal, many other investigators attempted to discover what unknown homeostatic mechanisms were involved in the regulation of magnesium metabolism.

Many experiments were devised and executed and much knowledge was obtained concerning the role of the endocrines in the maintenance of body and plasma magnesium within such narrow limits. In brief, the thyroid, the parathyroid, the adrenal cortex, the pancreas, and the pituitary were all shown to be directly or indirectly involved in the hormonal regulation of magnesium.

An intensive search of the literature reveals few pertinent publications, past or current, relating to the effect of adrenocorticosteroids (cortisone) on the regulation of magnesium. These are at times contradictory and confusing, some investigators noting an increase in urinary and fecal excretion of magnesium following cortisone administration and others observing no change in their external balance studies.

It is now known that in Cushing's syndrome, where endogenous excesses of corticosteroids exist, increased amounts of magnesium may be lost in the urine (Ingbar, et al., 1951). Aikawa, (1960) however, studying the effect of cor-

isone administration in rabbits in a Mg-deficient state, did not observe any hange in the external balance studies for magnesium following cortisone treatent. Other investigators studying the administration of hydrocortisone in ases of Cushing's syndrome or in cases of Addison's primary adrenal insuffiiency did not observe any change in urinary magnesium excretion in their xternal balance studies (Doe, et al., 1960).

This investigation was undertaken with the hope of clarifying and putting nto clearer perspective, the role of cortisone in magnesium metabolism. The xternal balance, as represented by the urinary magnesium and calcium excreion, was chosen to be followed, for as is now well known there exists a defiite inter-relationship between the bivalent calcium and magnesium ions. The ndocrine regulation of magnesium, unlike that of calcium is not yet known.

REVIEW OF LITERATURE

The Discovery of Magnesium as a Dietary Essential

The first attempt to deprive an animal of magnesium was made by Osborne and Mendel (1918) during the course of a general study of the inorganic elements in the nutrition of the rat. Their "magnesium free" diet contained over 100 parts of Mg per million (0.012%) and no untoward effects were apparent. These investigators expressed the view that the animal body could thrive and complete its growth on a diet low in magnesium. They did not however, attach any significance to the failure of weight gain which occurred after the completion of growth and which disappeared with the addition of magnesium to the diet. Their results taken alone could hardly justify the assertion that magnesium is essential to life and growth. We now know that perhaps their diet was not significantly below the critical level in magnesium content and that further reduction would have revealed the necessity of this element.

LeRoy (1926) must be given credit for the first experimental study on the effects of magnesium deficiency. He refined his test diet to the point where it only contained 0.00103% magnesium. When this diet was fed to white mice at 26 days of age their growth was arrested within nine to 13 days and death occurred in 24 to 35 days. His control mice received 0.023% magnesium and exhibited normal growth and development. His mice exhibited a parabolic growth curve. The animals thrived for a time, then ceased to grow, dropping to their initial weight before death. LeRoy made no mention of any of the symptoms now known to be characteristic of magnesium deprivation.

In 1932 Kruse, Orent and McCollum reported experimental results indicating that magnesium is a dietary essential. They fed a diet analyzed as containing 0.18 mg of Mg/100 gm of food. These investigators demonstrated for the first time the following spectacular symptoms as characteristic of severe magnesium deficiency:

"When weaned rats weighing 35 to 45 gm are placed on this low magnesium diet, they pass through a spectacular series of events leading to an early and violent death. Within three to five days, average four days, all the exposed skin areas become vividly red from vasodilatation and hyperemia in the vascular bed. The reddened appearance of the animals becomes intensified until the 11th to 14th day, average 12th day, when it slowly subsides to be followed in turn by marked pallor and finally by slight cyanosis. In animals which are older and heavier when restricted to the diet, the red stage may reappear after it has once faded. During the hyperemic period the animals are extremely irritable and hyper-excitable as is evidenced by the readiness with which they are startled by slight noises or shadows. The hyperexcitability becomes progressively more pronounced until the 18th day, when any sudden disturbance throws the animal into fright that is followed by a convulsive seizure. Although the convulsive attack may appear as early as the 11th day, the more usual time is the 18th day, and by the 23rd day practically all animals have had a first seizure. The sudden onset of convulsions is a striking feature. They defined a convulsion as follows:

"The excitable animal, startled by sound, races at rapid speed in a wide circle until it finally falls on its side. The entire body of the animal is now rigid, with head stretched back, fore limbs extended at three upper joints

and flexed at the metacarpo-phalangeal joint, and hind limbs extended backward. All respiratory movements cease during the attack and return with relaxation of the musculature. This stage of spasticity is succeeded by a period of relaxation lasting only a very short time. While still lying on its side, the animal exhibits twitchings in various regions, or paddles rapidly with all extremities. Then reappears a tonic spasm in which the rigid body assumes a typical position, with fore limbs pressed tightly against the thorax, fore paws clenched, and hind extremities extended. Next the animal may suddenly leap into the air, at the same time spinning laterally several times or it may curl up and do neither. Within a short time the animal rears from the dorsal or lateral recumbent position in an attempt to stand, but its extremities will not support it. The animal buries its head in its out-stretched fore limbs and propels itself forward entirely by its hind limbs. Instead of forward motion, fine tremors may appear over the body. Following the convulsive stage comes a recovery phase".

Exploring the possibility that magnesium as well as calcium could be responsible for producing a condition of tetany Kruse, Orent and McCollum 1933 pursued their study of the above described symptom complex, by analyzing the following blood constituents: sodium, potassium, calcium, magnesium and non-protein nitrogen. They observed an early and progressive decrease of magnesium in the blood and also found that terminally the non-protein nitrogen rises. No other blood constituents underwent any alteration. What is most important, is that tetany was shown to occur with normal calcium values and that their results appeared to support the view that lowered magnesium in the blood is capable in itself of inducing tetany independent of any changes in

calcium level. Lavollay (1931) reported similar effects in rats by keeping them on a diet containing 30 parts of Mg per million.

Factors Affecting the Development of the Characteristic Symptoms of Mg-Deficiency

Tufts and Greenberg, (1937) reported that, with a diet containing 10-20 parts of Mg per million, the onset of convulsions depended on the level of vitamin B complex in the diet, and that with ample supplies of this vitamin there were no trophic changes such as loss of hair and emaciation or edema of the feet. These authors established the fact that the border line magnesium content necessary for good growth was 5 mg/100 gm of food and that females on this diet gave birth to young of normal weight and magnesium content. They also noted that a high dietary calcium intake increased the severity of magnesium deficiency and raised the amount of magnesium necessary to meet minimal requirements. They divided the course of magnesium deficiency in the rat into two fairly definite phases, the first being characterized by vasodilatation, hyperemia and hyperexcitability, and the second by nutritional failure, cachexia and kidney damage. They regarded the second phase of magnesium deficiency as the time elapsing between the onset of hyperexcitability and the death of the animal. They found that in the latter phase of magnesium deficiency the growth rate dropped and symptoms of malnutrition, such as general loss of hair, a rough and sticky coat, diarrhea and finally edema, appeared. (Tufts and Greenburg 1937).

Watchorn and McCance (1937) maintained rats on a diet containing approximately 4 mg/100 gm and observed that they developed diarrhea and melena beginning about the tenth day. This was followed by hyperemia of the skin, loss

of hair and in some cases hyperpnea and nervousness. These symptoms disappeared in seven-ten days and thereafter the rats appeared to be normal until near the end of the experimental period (12 weeks) when they began to lose appetite and started to lose weight.

The magnesium deficiency of Watchorn and McCance is a milder form than that observed by Kruse, Orent and McCollum (1932) and may be characterized as a subacute condition of magnesium deficiency. In this milder form of deficiency the tonicoclonic convulsions and other manifestations of severe magnesium deficiency were delayed.

It is therefore logical to assume that the dietary level of magnesium, considered here as the sole experimental variable, is directly proportional to the degree of magnesium deficiency manifested in laboratory animals. With very low levels of dietary magnesium such as the levels used by Kruse, et al. (1932) the severe aspects of the Mg-deficient syndrome, as manifested by tonicoclonic convulsions, appeared in about 18 days, whereas, with levels such as those employed by Watchorn and McCance (1937), these symptoms did not become manifest until the 12th week. The age of the animal also plays a role in the development of the Mg-deficient symptom, as Kruse et al. (1932) noted that the younger the animal before being placed on a Mg-deficient diet the shorter its life and the more spectacular its death. Probably the increased storage of magnesium in the older animals is a deciding factor in their longer survival. Kruse and coworkers (1932) found that the older animals survived a greater number of seizures than the younger ones. Smith and Field (1963) also studied the role of age in Mg-deficiency in rats. They observed that young rats on their Mg-free diet developed vasodilatation of the ears and

hyperirritability two to three days earlier than did adult rats. They also noted that only young males showed an increased concentration of calcium in the kidneys.

The effect of varying the dietary ratios of calcium, magnesium and phosphorus as concerns the mineral utilization in the rat has been studied by several investigators.

Toothill (1963) observed a significantly reduced absorption of magnesium with increases in the level of either phosphate or calcium in the diet and that absorption was further reduced when both calcium and phosphate were simultaneously increased. Furthermore, an increase in dietary phosphate had no significant effect on the percentage of calcium absorption at either of the two levels of dietary calcium employed, whereas, an increase in dietary calcium decreased significantly the percentage of phosphate absorbed at each level of phosphate. The finding that the absorption of magnesium was reduced with an increase in dietary calcium and that the severity of the signs of Mg-deficiency in rats was increased by an increased calcium content of the diet confirms the findings of Tufts and Greenberg, (1937).

Toothill (1963) observed that the absorption of magnesium was significantly reduced by an increase in the dietary level of calcium from 0.34 to 0.68% or of phosphate from 0.39 to 0.79% and was further reduced when calcium and phosphate were simultaneously increased to levels of 0.65 and 0.78% respectively. The increased dietary phosphate had no significant effect on the percentage absorption of calcium at either level of calcium intake, but the increased dietary calcium significantly reduced the apparent percentage absorption of phosphate at each level of phosphate intake.

Tufts and Greenberg, (1937) found that the severity of Mg-deficiency in rats was increased when the calcium and phosphate levels of a diet containing 5 mg/100 gm, Mg (0.87% Ca and 0.80% P) were changed respectively to 1.16% calcium and 0.75% calcium and 0.75% phosphate. When the levels of calcium and phosphate were 1.66 and 1.0% respectively, the minumum magnesium content of the diet necessary for normal growth was increased to about 13 mg/100 gm.

O'Dell et al. (1957) reported that an increased severity in the signs of Mg-deficiency occurred with an increase in the level of dietary phosphate and that in the absence of adequate magnesium guinea pigs, receiving a diet containing a high level of calcium and phosphate gained less weight and died sooner than animals given diets containing less calcium and phosphate.

Forbes (1963) studying the effect of two levels of dietary calcium and phosphate found that the visible signs of Mg-deficiency in rats were produced most readily on diets containing the higher levels of calcium and phosphate.

Changes Observed Following Return to a Normal Diet in Mg-deficient Rats

Alcock and MacIntyre (1964) and Forbes (1964) investigated the changes occurring in magnesium and calcium metabolism following the restoration of magnesium to rats maintained on a Mg-deficient diet. The former noted, following deprivation of magnesium for 20 days in normal growing female rats previously receiving a diet normal in magnesium content, that during the period of magnesium restriction, the urinary and fecal excretion of calcium were less than normal, the plasma calcium level was elevated (hypercalcemia) and the plasma magnesium level was decreased. Following a normal intake of magnesium in Mg-deficient animals fecal excretion of calcium and absorption of magne-

sium from the gut was greater than in controls. Also, during the first 24 hours and for the remaining three days the urinary calcium excretion was double that of normal animals. They considered this to be highly significant as it represented a tenfold increase over the calcium excretion during the period of deprivation. It was found that the plasma calcium had decreased to normal by the second day and that the plasma magnesium was significantly reduced after four days of normal magnesium intake.

Alcock and MacIntyre (1964) assume that there is a common transport system for both calcium and magnesium and that an increase in the concentration of one ion would depress the absorption of the other. They also believe that this common transport system for magnesium and calcium exists in the gut as well as in the renal tubule. They present impressive data to substantiate this theory describing, in the rat as well as in man, experiments in which the oral administration of magnesium, to subjects already depleted of magnesium, was followed by an increase in urinary calcium excretion.

Thus, in short, it appears from the above investigations, that rats maintained on a Mg-deficient diet develop hypomagnesemia and hypercalcemia. The increased level of blood calcium can be explained by using Alcock and MacIntyre's (1964) theory as follows: Due to a lack of magnesium in the diet, there is an increased absorption of calcium from the gut. This results in a lowered fecal as well as a lowered urinary excretion of calcium despite the hypercalcemia which exists. Following return to a diet normal in magnesium content, there is an increased fecal calcium excretion, indicating an increased magnesium intake from the gut, at the expense of calcium. The plasma magnesium level starts to rise towards normal and the blood calcium level

ls from its high level towards the normal level. Perhaps a renal conser-
tion mechanism exists so that with the higher levels of magnesium relative-
there is an increased absorption of magnesium and calcium is preferential-
excreted. Thus far, the source of the calcium excreted in the urine, fol-
wing the return in Mg-deficient animals to a diet normal in magnesium, is
till uncertain.

e Role of Endocrines in Mg Homeostasis

The hormonal regulation of magnesium metabolism has been the subject of
ntensive investigations since magnesium was discovered to be a dietary es-
ntial. The belief that the serum magnesium, like calcium, sodium, and
otassium may be actively controlled by hormones is supported by observations
n the fluctuation of this cation in certain clinical diseases. Kashiwa
1961) observed the effects of Mg-deficiency in intact, in adrenalectomized
nd in hypophysectomized rats. He observed that the adrenalectomized rat
eveloped Mg-deficiency symptoms coincident with the intact rats, whereas the
eficiency symptoms were significantly delayed in the hypophysectomized rat.
e believed that this delayed depletion of serum magnesium and the consequent
ppearance of symptoms in the hypophysectomized animals was due to the Mg-
eficiency and he suggested that a relationship must exist between the pitui-
ary and magnesium metabolism..Kashiwa (Loc cit) confirmed, in the intact as
ell as the adrenalectomized rats, the observations of Kruse, Orent and Mc-
ollum (1932) as concerns the characteristic symptoms of Mg-deficiency. He
elieved that the lowered serum magnesium was coincident with the appearance
f the renal lesions in the intact and adrenalectomized Mg-deficient rat.
he delay in the depletion of serum magnesium and the appearance of symptoms

n hypophysectomized rats suggested to him that the pituitary might be in-
olved either directly or indirectly in the regulation of serum magnesium.
e observed that in the absence of the pituitary the normal serum magnesium
evel persisted in spite of the low magnesium consumption. Although he could
ot explain the delay in the depletion of serum magnesium in the hypophysec-
omized rats, Kashiwa inferred, from his data on adrenalectomized rats, that
he absence of ACTH was not the factor responsible since the adrenalectomized
t did not deviate from the time sequence established for intact rats.

Corradino and Parker (1962) studied the effect of the thyroid on magne-
ium in the rat. Their results indicated that the functional status of the
hyroid can be influenced by dietary magnesium levels and that there is an
ntagonism as concerns the regulation of oxidative phosphorylation between
he thyroid hormone and magnesium. These investigators mention the work of
leiber et al. (1941) who observed thyroid enlargement in magnesium deficient
ats. They also discuss the work of Bain (1954) who demonstrated that a func-
ional interrelationship exists between magnesium and thyroxin and that mag-
esium antagonized the action of thyroxin in the in vitro uncoupling of oxi-
ative phosphorylation.

Aikawa (1960) in studying the effect of thyroxin on magnesium metabolism
bserved that the serum magnesium was significantly depressed in rabbits two
ays following the second injection of thyroxin.

Of unsual interest is the work of NeGuib (1963) who reported the effects
f magnesium in disorders of the thyroid. He observed that in myxedema all
he magnesium was ionized while in hyperthyroidism up to half of it was pro-
ein bound. NeGuib reviewed the subject of magnesium and its effect on the

thyroid. He cited Tapley's (1956) results which demonstrated that the administration of triiodothyronine in patients with myxedema caused a rise in the urinary excretion of magnesium. NeGuib treated three patients with toxic goiter. In one patient he observed a 12 cm diminution in the circumference of the neck in a goiter while all three patients became non-toxic, having been toxic prior to treatment. It appeared to NeGuib that, although it is not known how magnesium produces its effect, a high level of magnesium can apparently cause a notable diminution in the size of the thyroid gland in both toxic and non-toxic goiter. From his results in the use of magnesium in the treatment of patients with toxic goiter, and from the above mentioned results of investigations on the effect of magnesium as concerns thyroid function and vice-versa, it seems likely that there is an antagonistic action between magnesium and thyroxin, in vivo and in vitro.

Cheek and Leng (1960) studied the changes in tissue composition of the rat, in parathyroid intoxication produced by large doses of commercial extract, and observed the effect of magnesium loading on animals so treated. In all their rats receiving commercial parathyroid extract an elevation in plasma magnesium was noted.

Selye (1958) reported a striking inhibition of parathyroid hormone intoxication following the administration of magnesium salts in his investigations. He also observed that magnesium salts antagonized the parathyroid hormone intoxication only when the latter was aggravated by the concurrent administration of phosphates. In other words, in order for the magnesium salt to exert its antagonistic effect, the animals must first be sensitized to parathyroid hormone by concurrent treatment with phosphate. He could not

explain why this occurs. MacIntyre, Boss and Troughton, (1963) advanced the hypothesis that the parathyroid is involved in magnesium homeostasis. Using purified parathyroid hormone (2000 units/mg) they studied its effect on the urinary excretion of calcium and magnesium. Their experiments showed conclusively that this hormone brought about a marked conservation in urinary magnesium and calcium. These investigators postulated that this is due to a direct renal effect secondary to a change in the tubular handling of these cations. They suggest that the mechanism whereby plasma magnesium is maintained in the body, within very narrow limits, is directly attributable to a variation in the secretion of parathyroid hormone in response to changes in the plasma magnesium level. This hypothesis implies that a rise in plasma magnesium will inhibit the secretion of parathyroid hormone thus producing an increased urinary excretion of magnesium and a subsequent return of the plasma level towards normal. Similarly, a fall in plasma magnesium level should stimulate production of parathyroid hormone thereby leading to an increased renal conservation.

MacIntyre et al. (1963) concluded that magnesium and calcium homeostasis are interdependent and in the face of marked abnormalities in plasma magnesium the calcium homeostasis cannot be maintained. To support their view they offered the following evidence:

Since in experimental magnesium deficiency in the rat there exists hypercalcemia, hypophosphatemia and an increased phosphate clearance all of which suggests hypersecretion of parathyroid hormone and since following total parathyroidectomy in the rat there is a fall in plasma magnesium which is not so marked as that seen in calcium. These authors concluded that the parathyroid

hormone is essential for magnesium homeostasis through its effect on renal regulation. As concerns calcium homeostasis the renal effect of the parathyroid hormone is not so significant as is the effect on bone.

Hanna and MacIntyre (1960) reported that the administration of aldosterone in both normal and adrenalectomized rats resulted in a decrease in the apparent availability of dietary magnesium. They observed that relatively small doses of aldosterone injected into normal and adrenalectomized animals resulted in an increased urinary and fecal excretion of magnesium.

In a review of magnesium metabolism, MacIntyre (1963) postulates that although his results provide a satisfactory explanation for the loss of magnesium in Conn's syndrome, a direct effect of aldosterone, they provide no evidence as to whether or not aldosterone is concerned in magnesium homeostasis. He maintains that if aldosterone were concerned in magnesium homeostasis, the administration of excess magnesium would be expected to stimulate the secretion of aldosterone and its deprivation to depress it. As yet, however, no conclusive results are available to substantiate this involvement of aldosterone in the homeostatic regulation of magnesium.

Aikawa (1960) investigated the effect of cortisone on magnesium metabolism in the rabbit. He observed that on the eighth day of cortisone administration there was a slight but significant decrease in the mean serum magnesium concentration. He did not observe any significant changes as a consequence of his external balance studies and found that cortisone did not cause a change in the excretion of urinary magnesium.

Care and Ross (1963) reported that the apparent availability of dietary magnesium was decreased in intact sheep following the administration of deox-

ycorticosterone acetate. Since, in most circumstances the diet provides excessive amounts of magnesium, it would seem likely that endocrine factors function to reduce the availability of dietary magnesium rather than to increase it.

It is hoped that the results of the following experiments will shed new light on the problem of magnesium metabolism and its relation to the adrenal corticosteroids.

MATERIALS AND METHODS

This investigation concerns itself primarily with the role of cortisone and its effect upon the metabolism of magnesium in a strain of female Sprague-Dawley albino rats[1]. The results of cortisone administration in three experimental groups, Group A, maintained on a magnesium deficient diet, Group B, on a magnesium supplemented diet[2], and Group C, on the standard Rockland rat-mouse pellets, are reflected in changes in the urinary excretion of magnesium and calcium ions which were the two bivalent cations selected to be followed through routine urine analyses throughout the investigation. These groups were established by selecting at random animals of the same sex and age and each group consisted of the same number of animals. The growth curves of the animals employed, as reflected by the weight changes of their respective, groups, were followed throughout by individual weekly weighings.

In Group A, experiment 1, tap water was supplied whereas in experiments 2 and 3 distilled water was provided. Following experiment 1, tap water was provided only in Groups B and C. The animals were permitted to feed and drink ad libitum, food containers and water bottles attached on outside of doors were provided for each cage.

---

[1]Purchased from Hormone Assay Laboratories, Inc., Chicago, Ill.

[2]The Magnesium Deficient and the Magnesium supplemented diets were prepared by General Biochemicals, Laboratory Park - Chagrin Falls, Ohio.

The diets were analyzed[3] and found to have the following magnesium and calcium content: Experiments 1 and 2, (1963, 1964), the Mg-deficient diet, 10 mg% Mg, the Mg-supplemented diet, 63 mg% Mg. No calcium determinations were requested. For experiment 3, (1966) the deficient diet contained less than 2 mg% Mg and 0.899% Ca, and the Mg-supplemented diet 75 mg% Mg and 0.850 % Ca. The Rockland pellets employed in all three experiments were found to contain 330 mg% Mg and 1.86% Ca.

The urine samples were collected in special stainless steel metabolism cages[4] which were designed so as to prevent fecal contamination of urine specimens. These cages were coated with a special silicone preparation (siliclad^{R}) so as to avoid metal ion contamination of urine samples.

The investigation consisted of three experiments each of which was divided into two parts. In Part I, the duration of experiment 1 was ten weeks of experiment 2 five weeks and of experiment 3 seven weeks. During this period Groups A and B of experiment 1, and Groups A, B, and C of experiments 2 and 3 were maintained on their respective diets without cortisone administration. In Part II, the duration of experiment 1 was nine weeks of experiment 2 four weeks and of experiment 3 three weeks. Part II differed from Part I by the introduction of cortisone therapy (3 mg/Kg in experiment 1 and 6 mg/Kg in experiments 2 and 3)[5]. In other words, each of the experiments

---

[3]The Wisconsin Alumni Research Foundation, Madison, Wisc.

[4]Manufactured by the Acme Metal Products Inc., Chicago, Ill.

[5]Generously supplied to Dr. L. V. Domm by Sharp and Dohme, Division of Merck and Company, Inc., Philadelphia, Pa.

consisted of two parts, the first without and the second with cortisone therapy.

Experiment 1 began with 16 rats 120 days of age. The animals were divided into two Groups, A and B, each consisting of eight rats. The diets of Groups A and B differed only with respect to the quantity of magnesium contained in each. Experiment 2 employed 36 rats 40 days of age. The animals were divided into three groups, A, B, and C, each consisting of 12 rats. Experiment 3 employed 60 rats aged 50 days. These were divided into three groups as above each consisting of 20 rats.

The same animal quarters were employed throughout. The animal room was air-conditioned and maintained within a controlled temperature range of 68-72 degrees. The animals were permitted to feed and drink ad libitum. The only difference with respect to water supply, between experiment 1 and experiments 2 and 3 was that in the former tap water was employed and in the latter distilled water for Group A, the Mg-deficient group. The tap water contained 0.0113 mg of Mg / cc[6] which constituted the introduction of an unintentional experimental variable in experiment 1. This was corrected in experiment 2 and 3 by the use of distilled water.

Rats of the respective groups were selected at random and placed into metabolism cages for urine collections between 5 P.M. to 7 A.M. for experiments 1 and 2, and from 10 P.M. to 7:30 A.M. for experiment 3. In experiments 1 and 2, only six urine specimens per day could be analyzed for magne-

[6]Information obtained from North Side Water Filtration and Purification Plant, Chicago, Ill.

sium and calcium due to the time consumming nature of the method[7] employed, however, in experiment 3, by using a flame photometer[8] it was possible to make determination on as many as twenty per day.

The analytical method employed for determinations of urinary magnesium and calcium concentrations in experiments 1 and 2 was that of Kovacs et al. (1959). It was as follows:

Reagents Employed

2% NH_4 oxalate solution

2% NH_4 OH

25% HC1

Concentrated NH_4 OH

Buffer Solution, 0.83 gm NH_4 C1 was dissolved in a small amount of double distilled water after 12 ml concentrated NH_4 OH was added to it and made up to 100 ml with double distilled water.

Indicator Solution, 10 mg Plasmocorinth B dissolved in 100 ml of double distilled water.

0.001 M (EDTA) Ethylene-diamine tetraacetate dinatrium Complexion.

Procedure

The procedure used in our determinations of magnesium was as follows: Two mls of 4-5 times diluted urine was put in a centrifuge tube. To this 1 ml of 2% NH_4 oxalate was added and after standing at room temperature for one hour the mixture was centrifuged. Two ml of the supernatant fluid was put

---

[7]Kovac's Complexometric Method (1959)

[8]In accord with the Coleman Operating Instructions for Model 21 Flame Photometer, Coleman Instruments Corporation, Maywood, Ill.

into a beaker and to this 1.5 ml of buffer solution and 1 ml of indicator solution was added. With continuous shaking it was titrated from a 5.0 ml graduated burette with 0.001 M EDTA. The end point of the titration was reached when the red indicator color suddenly turned blue.

The procedure employed in our determinations of calcium was as follows:

The urine remaining after centrifugation was poured off after having added 8 ml of 2% NH_4OH, the precipitate being dissolved with three drops of 25% HCl. It was then washed into a beaker with 4.5 ml of fistilled water. To the dissolved precipitate four drops of concentrated NH_4OH, 1.5 ml buffer solution, and 1 ml ind9cator solution was added. The precipitate was then titrated with 0.0001 M EDTA until the red color changed to blue.

Calculations

One ml (0.0001 M EDTA) was equal to 0.04008 mg Ca or 2 micro Eq Ca or 0.02432 mg Mg or 2 micro Eq Mg. On the basis of this, the concentration of magnesium and calcium in the urine specimens could be calculated as follows:

ml X H = mEq/L Ca

ml X H X 2/3 = mEq/L Mg

ml X H X 2 = mg% Ca

ml X H X 1.82 = mg% Mg

In the above formulas the ml is equivalent to the 0.001 M EDTA used for the titration of either calcium or magnesium and the H represents the dilution of the urine.

Kovacs et al. (1959), in order to test the reliability of the Complexometric method compared it with the calssical calcium and magnesium determinations of Tisdall, method (Ca) Winis procedure (Mg) and found his technique to

be accurate and his results to correspond with those obtained with standard techniques.

Each urine sample was diluted five times and a minimum of four two ml samples were obtained and placed in four centrifuge tubes and analyzed for magnesium and calcium concentrations by the above mentioned technique. The results of the four determinations of each sample were averaged and the average used in the statistical analyses as the value of the magnesium and calcium excretion for that particular sample.

Throughout our investigation, special care was taken to follow precisely the various steps of Kovacs' Complexometric method in each determination in order to avoid the introduction of errors in technique.

The analytical method employed in determining the urinary Mg and Ca concentrations in experiment 3 is with some minor modifications, taken mainly from the "Operating Directions for the Model 21 Coleman Flame Photometer". The method is as follows:

Reagents Employed:

1% Sterox

0.02% Sterox

8 mEq/L Magnesium flame standard

5 mEq/L Calcium flame standard

3% 8-Hydroxyquinoline solution

1 N Hydrochloric Acid

2 N Ammonium Chloride

2% Ammonium Oxalate solution

Procedure

Magnesium

A 2 ml aliquot of urine is delivered into each 15 ml graduated centrifuge tube. One ml 2% Ammonium Chloride and 2 ml concentrated NH_4OH are added. The test tube is warmed to 70°C in a waterbath followed by the addition of 2 ml 3% 8-Hydroxyquinoline solution. The tubes are maintained at 70°C for 15 minutes, then centrifuged at 2500 rpm for an equal period. The supernatant is then poured off, 2 ml 1 N HCl is added in order to dissolve precipitate, and the tubes are mechanically shaken on a vortex mixer to insure that all the precipitate goes into solution. One tenth ml 1% Sterox is added to each tube and with de-ionized single distilled water the dissolved precipitate is diluted to the 5 ml mark.

The unknown samples are delivered into 5 ml capacity beakers[9] and labelled one through twenty. The flame photometer is now turned on and the zero (blank scale) is set using 0.02% Sterox in the blank beaker. The standard setting (black scale) is set using 8 mEq/L flame standard. The system is flushed between each sample with 0.02% Sterox (blank solution) at the same time checking to see that the zero mark has not shifted. Between every four determinations, the standard mark is checked and if found to have changed it is corrected. For every twenty determinations, five checks are made to insure a constant standard setting. Determinations are made in the above manner on unknown samples one through twenty and the values obtained recorded.

---

[9]Disposo Beakers, Scientific Products, Inc., Chicago, Ill.

Calcium

A 2 ml aliquot of urine is delivered into each 5 ml graduated centrifuge tube and 1 ml 2% Ammonium oxalate solution is added. The tubes are permitted to stand for 15 minutes at room temperature, then centrifuged at 2500 rpm for an equal period of time. One ml of 1 N HC 1 is added to each tube and the tubes are mechanically shaken on a vortex mixer to insure all of the precipitate going into solution. To each tube is added 0.1 ml 1% Sterox and with de-ionized single distilled water the dissolved precipitate is diluted to the five ml mark.

The unknown samples are delivered into the five ml capacity beakers and labelled one through twenty. The zero (blank scale) is set using 0.02% Sterox and the standard mark (black scale) is set using 5 mEq/L Ca flame standard solution. The procedure is essentially the same as for the magnesium determinations. The zero point is set first, then the standard mark, followed by flushing of the flame photometer with 0.02% Sterox between successive readings. Between every four determinations the standard setting is checked, and adjusted if it is found to have shifted. The zero setting is checked between every determination in the process of flushing the atomizer and adjusted as needed. Determinations are made in the above manner on unknown samples one through twenty and the values obtained recorded.

In order to avoid certain commonly encountered variables in flame photometry the following measures were taken:

All chemical reagents were purchased ready for use[10]. This saved time

[10] Trade name Harleco[R] Scientific Products, Inc., Chicago, Ill.

and eliminated variations in concentrations of standards and other reagents.

A solid state voltage regulator eliminated day to day fluctuations by maintaining a constant voltage delivery to the photometer at all times. An oxygen valve regulator[11] was used to insure a constant pressure during the operation of the photometer.

Thus, the major variables of different concentrations of chemical reagents, fluctuations in voltage, and fluctuating oxygen pressures were minimized or eliminated.

Calculations

In order to determine the number of mEq/L for magnesium and calcium the following formula was employed:

$$\frac{\text{critical dilution}}{\text{aliquot urine}} \times \frac{\text{Scale Reading}}{\text{Scale Setting of Standard}} \times \text{Concentration of Standard Solution}$$

For Magnesium

$$\frac{5}{2} \times \frac{\text{Scale Reading}}{80} \times 8 \text{ mEq/L Mg} = \text{mEq/L of Mg in sample}$$

For Calcium

$$\frac{5}{2} \times \frac{\text{Scale Reading}}{50} \times 5 \text{ mEq/L Ca} = \text{mEq/L of Ca in sample}$$

The data obtained from this investigation was subjected to statistical analysis[12] and, in accordance with the degrees of freedom the probability was

---

[11] Harris Regulator Valve. It maintained a 13 lbs/in^2 pressure.

[12] The t-test significant at 5% or less.

determined, and the results reported as significant or not significant as the case may be. A table of the statistical analyses is included under the results section.

In experiments 3 tissue specimens of adrenal, parathyroid, heart, eye, kidney, skeletal muscle (hamstring) and bone (femur), were taken and were fixed in Ca acetate formalin and Bouin's solutions. A histological study of the kidneys and adrenal were selected for inclusion in the thesis. Sections were stained with hematoxylin and eosin, Von Kossa (Mallory, 1942) and Alizarin red (Dahl's Method, 1952).

EXPERIMENTAL RESULTS

Observations Part I (Pre-Cortisone)

Experiment 1

Group A, fed the Mg-deficient diet, started off at approximately the same weight as Group B, fed the Mg-supplemented diet, and toward the end of the experiment both groups revealed approximately the same overall weight. This is in sharp contrast to the results observed in experiment 2. It was surprising to find that the Mg-deficient group throughout Part I was consistently slightly heavier than the Mg-supplemented group. This is in contrast to experiment 2 in which Group A failed to gain weight as compared with Group B, which thrived and progressively gained weight.

The animals employed in experiment 1 were older and weighed more initially than those of experiment 2. Groups A and B averaged 240-250 gms whereas the animals in experiment 2 (all groups) initially averaged 130-140 gms and only Group C reached an average of 240 gm by the end of the experiment, (graph 1). Perhaps the discrepancy in the overall weights of these animals can be explained by the fact that they were older to begin with in experiment 1, (approximately 120 days) than in experiment 2, (50 days) thus allowing for greater maturity in the former prior to the establishment of experimental conditions.

The urinary excretion of magnesium in Group A, fed the Mg-deficient diet, differed significantly when compared with that of Group B, fed the Mg-supple-

mented diet. The former consistently excreted lower levels of magnesium than the latter. Perhaps this represented an attempt at renal conservation of magnesium as suggested by Smith et al. (1962). The level of urinary magnesium excretion in Group A did not fluctuate significantly and remained essentially within a narrow range whereas that of Group B exhibited fluctuations but was always significantly greater than that of Group A, (graph 4). This difference between Group A and Group B was statistically significant throughout the experiment.

Group A consistently exhibited a lower level of urinary calcium excretion than Group B. The difference between the two groups was statistically significant and prevailed throughout the experiment. Both groups revealed a progressive increase in the level of urinary calcium by 59 days reaching a peak (2.9 mEq/L Group A; 6.5 mEq/L Group B) followed by a gradual decrease to a level (3.2) below the initial level of Group B. (5.2) and to a level, (2.0 mEq/L), slightly above the initial level, (1.7 mEq/L of Group A, graph 4).

An interesting inverse relationship between urinary magnesium and calcium excretion was noted for Groups A and B. In these groups an increased magnesium excretion was accompanied by a decreased calcium excretion and an increased calcium excretion by a decreased magnesium excretion. Group B exhibited this relationship more so than Group A (graph 4). This interrelationship between these two bivalent ions was also observed in experiment 2.

Perhaps this inverse relationship can be best explained by the hypothesis of MacIntyre (1963) which is considered in the discussion that follows.

Experiment 2

In this experiment Groups A, B, and C differed significantly as concerns weight. Group C, fed Rockland rat pellets revealed a progressive and consistent gain in weight from an initial group average of 140 gm to a maximum of 240 gm at the conclusion of the experiment.

In sharp contrast to Group C, Group A, fed the Mg-deficient diet, failed to thrive and did not exhibit a progressive increase in weight. Instead this group showed noticeable fluctuations consisting of small gains and losses. From an initial group average of 130 gm, Group A did not increase significantly and at the end of the experiment this average was the same. This failure of the Mg-deficient animals to thrive and exhibit optimum growth curves has also been noted by others since the original investigations of Kruse, Orent and McCollum (1932).

Group B, fed the Mg-supplemented diet, weighed approximately the same as Group A at the beginning of the experiment and after seven days showed a steady increase in weight. The group averaged 126 gm at the beginning of the experiment and 150 at its conclusion.

Our observations seem to indicate that the nutritional differences in mineral (content) of each group are reflected in the growth curves. Group A, deficient in the dietary essential magnesium did not thrive and its members remained dwarfed in size. Group B, receiving a Mg-supplemented diet showed a progressive weight gain which tapered off toward the end of the experiment. The members of Group B were significantly larger at this time and appeared to be healthier than those of Group A.

Group C, receiving the Rockland rat pellet diet, manifested optimal development and growth and all the animals were healthy and well nourished

throughout the experiment. This group exceeded all others in weight gain. At the end of the experiment this group averaged 234 gm, Group B 149 gm and Group A 130 gm, (graph 2).

Groups A, B, and C each revealed different levels of urinary magnesium excretion, Group A showed the lowest level (0.54 mEq/L Mg), while B, exhibsignificantly higher one (1.6 mEq/L Mg), and C the highest, higher than A and B combined (4.7 mEq/L Mg).

Thus, as with the growth curves, the respective nutritional differences between Groups A, B, and C are reflected in the urinary magnesium output of each group. Group A, fed the Mg-deficient diet, revealed the lowest urinary magnesium while B, fed the Mg-supplemented diet showed a significantly higher output, and C, fed the Rockland Rat pellets exhibited the highest level. The differences in urinary magnesium excretion between these groups were statistically significant throughout the first half of experiment 2.

The urinary calcium excretion for Groups A, B, and C, in this experiment as in experiment 1, showed that the Mg-deficient group excreted the lowest level (1.6 mEq/L). Group B excreted a higher level than A (2.5 mEq/L) and C the highest level (10.7 mEq/L). These differences in urinary calcium excretion between Groups A, B, and C were statistically significant.

Experiment 3

Groups A, B, and C as in experiment 2, differed significantly as concerns weight changes. Group C, fed the Rockland rat pellet diet, showing an initial group average weight of 160 gm, progressed to a maximum group average of 270 gm toward the end of the experiment. Group B, maintained on the Rock-

land rat pellet diet during the first four weeks of the acclimatization period increased in weight from an initial 160 gm group average to 205 gm at four weeks just prior to being transfered to the Mg-supplemented diet. In sharp contrast to the observations in experiment 2, Group B, after being maintained on the Mg-supplemented diet, failed to thrive and from an initial group average of 205 gm it dropped to a group average of 143 gm at seven weeks, a level far below that of Group A, (225 gm) or Group C, (270) at the end of Part I. Thus Group B whose weight was between Group C. (optimum) and Group A (poorest) in experiment 2, in experiment 3 exhibited a failure to thrive, reaching a weight level significantly below Group A, the Mg-deficient group.

Except for a transient vasodilatation of the ears and scaly dermatitis, in some of the animals in Group A maintained on the deficient diet, none of the more severe signs of Mg-deficiency were noted.

In Group A the analysis revealed an overall urinary magnesium level of 2.1 mEq/L with a range of 1.3-3.20. In Group B the magnesium level was 10.3 mEq/L, with a range of 6.8-18.4 and in Group C it was 22.90 mEq/L with a range of 16.50-45.0. The average magnesium excretion of Group B was approximately five times that of Group A while that of Group C was approximately twice that of Group B and ten times that of Group A.

The average calcium excretion in Group A was found to be 3.7 mEq/L with a range of 0.40-8.7. In Group B the average was 2.9 mEq/L with a range of 0.8-4.50 while in Group C it was 4.4 with a range of 2.50-8.00. The rate of calcium excretion was highest in Group C followed by Group A and Group B in order of decreasing amounts.

The rats in Group C of experiments 1, 2 and 3 received Rockland rat pel-

lets. However, in experiment 3, certain unexpected difficulties appeared to result from their use. It was noted that the animals on these pellets progressively excreted a more milky white urine during the course of the experiment with fine white particles held in suspension which gradually settled to the bottom of an undisturbed test tube as a whitish precipitate. Upon shaking, the urine developed a foamy, sudsy appearance. The urine was in the alkaline pH range (7.8) and an analysis of the white particles[13] revealed them to be triple magnesium phosphate and amorphous phosphate crystals. Further analysis in our laboratory revealed excessive quantities of magnesium in the range of 50 mEq/L as well as an elevated calcium content of 5 mEq/L as compared to Group A (0.65 mEq/L and Group B 0.85 mEq/L). Since these particles interfered with our urine analyses due to their unequal distribution in the aliquots Group C was transferred to Purina rat chow pellets. The day following after this transfer the urine, had cleared noticeably in the majority of the animals and, although the cloudy, milky condition disappered, the urine continued to show, to a lesser degree, whitish particles in suspension for the remainder of experiment 3. On casual examination the urine seemed to be clear, but when held up to the light a fine granular suspension was immediately visible. Analysis of the Rockland pellets[14] revealed the magnesium content to be 330 mg% and the Ca 1.86%.

---

[13] The Rosner-Hixson Laboratories

[14] The Wisconsin Alumni Foundation, Madison Wisc.

A new group of rats (25 female Spraque-Dawleys, aged 40 days) was obtained in order to determine the presumed dietary etiology of the above observations. These were randomly divided into three groups, one to receive Rockland, another Purina and the third Wayne Lab-Blox rat pellets. Prior to putting these animals on their respective diets their urine was collected and examined. All of these had white particles in suspension but to a lesser degree than in the older animals of Group C, experiment 3. Upon inquiry we discovered that these rats had been fed Rockland pellets from the time they were weaned. An at random selection of other rats maintained on Rockland pellets in our central animal quarters revealed that they all excreted an alkaline urine containing white particles in suspension. The phenomenon was more pronounced in pregnant rats and the urine of females in general demonstrated a greater opaqueness and an increased number of particles than that of males. Since the solubility constant of magnesium was exceeded, resulting in the precipitation of Mg-triple phosphate in the urine, one may also assume that the (Ca X PO_4) solubility product, of necessity, was exceeded, resulting in the deposition of calcium salts in the kidneys (renal calcinosis). The diet of these rats contained 1.86% Ca, which is three times the recommended value of 0.6%, and 0.8% phosphate, which is well within the normal recommended range of 0.6-0.8%. Thus an excessive and elevated magnesium and calcium content of the diet resulted in exceeding the solubility constant for both, although once one of their solubility constants is exceeded the other is carried along with it, resulting in the deposition of their respective salts in the kidneys. The severity of the renal damage appeared to be directly proportional to the length of exposure to the diet (see histological

servations) as indicated by different degrees of urine turbidity and opaqness, quantitatively associated with different degrees of renal damage. ter considering the varying compositions of standard diets, Wayne Lab-Blox s selected for experiment 4 since it was found to be the least nephrotoxic those used in this investigation.

bservations Part II (Cortisone)

Experiment 1

There were not enough observations to warrant a statistical study of the ta collected for the cortisone-treated and non-treated groups in experiment , Part II. However, even though a statistical analysis of the data was not ossible, it is interesting to note that a study of the data revealed certain rends.

The urinary magnesium excretion of the rats in Group A receiving cortisone increased steadily for 23 days after the initiation of treatment, from n initial level of 0.50 to 1.2 mEq/L, a level well above the non-treated roup which was 0.23. For the next 31 days, however, the members of Group A receiving cortisone excreted less magnesium gradually reaching a level of .40 mEq/L which was lower than that of the non-treated deficients the level f which was 0.90.

The cortisone-treated members of Group B, after 54 days of treatment, id not excrete relatively, much more urinary magnesium than had been found rior to the initiation of treatment. From an initial level of 2.1 mEq/L, the evel was found to be 2.4 after 24 days, and 2.4 after 55 days of treatment,

ereas in the non-treated group it was 5.0 after 24 and 1.5 after 55 days spectively. Perhaps the decrease in urinary magnesium excretion in the reated, as compared to the non-treated group at 24 days, represented a cor- isone effect. It is also conceivable that the relative increase in magnesium 55 days in the treated as compared to the non-treated group, was the result cortisone administration.

An inverse relationship between the excretion of urinary calcium and mag- sium was noted in the rats of Group A receiving cortisone. An increase in e excretion of calcium over the 55 day period of treatment, from a level of 2 mEq/L at 23 days to 0.60 at 55 days was associated with a decrease in mag- sium excretion from a level of 1.2 to 0.40 mEq/L respectively.

In the cortisone-treated Group B, an initial decrease in calcium excre- on from 3.2 to 1.3 mEq/L at 23 days was followed by an increase to 6.0 mEq/L 55 days. The non-treated rats of Group B excreted progressively less cal- ium, from a level of 8.8 mEq/L at 23 days to 3.3 at 55 days.

It is interesting to note that the magnesium deficient rats of Group A, rt I, weighed slightly more at the end of four weeks than the magnesium pplemented, (Group B) however, thereafter and throughout Part II, Group B ogressively gained more weight than the Group A deficients so that they timately differed significantly in this respect. The treated deficients roup A) and the treated supplemented (Group B, Controls weighed less (255.6 d 271.5 gm) than the non-treated rats of these two groups (268.7 and 288.7) respectively, (graph 2).

Experiment 2

During the first week of treatment, the cortisone-treated animals of ups A and B, revealed significant increases in levels of urinary magnesium. se values were 0.700 mEq/L for Group A and 4.3 for Group B, compared to and 2.4 respectively for the non-treated rats of these two groups. In trast to Groups A and B, the cortisone-treated animals of Group C excreted mEq/L compared to 2.0 for the non-treated. This value was found not to significant.

Only the cortisone-treated rats of Group B, excreted an overall signifi- t increase in level of magnesium after the first week. The average magne- level of the cortisone-treated animals of this group was 2.7 mEq/L com- ed to 1.7 for the non-treated controls. Although the cortisone-treated s of Group A revealed an overall increase in the level of magnesium (0.60 /L) when compared with the non-treated controls (0.60 mEq/L) this differ- e was not significant.

The overall level of urinary magnesium for the cortisone-treated rats of up C was 1.9 and that of the non-treated controls 1.6 mEq/L. This increase ever, was not statistically significant.

The cortisone-treated rats of Groups A and C revealed an overall in- ase in levels of urinary calcium when compared with the non-treated con- ls. The cortisone-treated animals of Groups A and C, excreted 3.6 and 3 mEq/L respectively, throughout the entire period of Part II, compared to t of the non-treated controls 2.8 and 12.0 mEq/L. This increase in cal- m was not significant in either group.

In Group B, in contrast to Groups A and C, the cortisone-treated rats raged less urinary calcium (6.7 mEq/L) for the entire period of Part II,

n the non-treated controls whose average was 10.6. However, this decrease calcium excretion was not significant.

As was noted in experiment 1, by the end of experiment 2, the cortisone-ated animals of Groups A, B and C differed significantly in weight from non-treated in that the cortisone-treated animals in all three groups ighed less than the non-treated controls, thus demonstrating the well known abolic effect of cortisone resulting in a negative nitrogen balance. The rtisone-treated rats of Groups A, B and C, at the end of the experiment, ighed 108, 113 and 219 and the non-treated 116, 158 and 250 gm respectively raph 2).

As in Part I, Group C exhibited the greatest weight gain and Group A the ast while Group B was intermediate between Groups A and C.

Convulsions as described by Kruse, Orent and McCollum, as well as the chexia of severe magnesium deficiency were first observed in Group A of periment 2.

Experiment 3

The cortisone-treated animals of Group A revealed a continuous, gradual crease in urinary magnesium for the entire period of Part II, when compared th the non-treated controls. The values for the treated deficients averag- 0.97 and those of the controls 2.92 mEq/L. This decrease in urinary mag-sium was significant.

Due to the unexpected loss of many of the rats of Group B during the irst week of cortisone treatment an insufficient number of observations were de to justify any evaluation of the results.

The cortisone-treated Group C rats excreted less urinary magnesium than the non-treated controls. The values for the treated averaged 14.0 and those of the non-treated controls 15.8 mEq/L. However, this decrease was not significant.

The cortisone-treated rats of Group A excreted less urinary calcium than the non-treated controls, the average values being 2.6 and 7.0 mEq/L respectively. This decrease in calcium was not significant. The urinary calcium excretion in the cortisone-treated rats of Group C averaged 4.7 and that of the non-treated controls 4.2 mEq/L. This difference was not significant.

As in experiments 1 and 2 by the end of experiment 3, the cortisone-treated rats of Groups A and C differed significantly in weight from the non-treated controls. The former of both groups weighed less than the latter. The cortisone-treated animals of Groups A and C, at the end of the experiments, weighed 204 and 272 gm and the non-treated controls 208 and 283 gm respectively.

During the first week of cortisone treatment, after six out of eight non-treated animals of Group B had died, it was decided to sacrifice the entire group. The non-treated controls of this group, in contrast to Groups A and C above weighed less than the treated ones. They appeared to be severely undernourished and averaged 105 gm in weight as compared to 138 gm for the cortisone-treated group.

Histological Observations Parts I and II

Experiment 3

Kidney

In general, careful examination of kidney sections from Groups A, B, and C, stained with hematoxylin and eosin, revealed essentially normal renal histology, with the exception of scattered, amorphous, gray staining material noted in the lumina of the proximal and distal convoluted tubules, and very rarely an occasional hyalinized glomerulus. The normal renal morphology in all sections was grossly disrupted by numerous artificial spaces and tears presumably created by the excessive renal calcium during sectioning of the paraffin blocks. Difficulty in sectioning was encountered in all three groups, but mainly in Groups B and C.

Sections of kidneys treated with histochemical procedures specific for calcium salts and stained with Von Kossa and alizarin red dyes revealed calcium salt deposition in Groups A, B, and C. However, the degree of calcium deposition differed quantitatively and in location between members of the same group as well as between the respective Groups A, B, and C as a whole. There was considerable overlap in the extent and distribution of the calcium deposits and although it is difficult to quantitate these differences, it was evident that Group A showed the least calcium, with minimal cortical deposits, Group B a somewhat greater degree of deposition, with extensive cortical and cortico-medullary deposits, and Group C, the greatest degree of renal calcinosis with cortical, cortico-medullary and papillary deposits.

No differences in degree or location of calcium deposition was observed between the cortisone-treated and non-treated members of Groups A, B, and C.

Adrenal

Careful examination of sections of adrenal stained with hematoxylin and

eosin did not show any significant difference between the cortisone-treated and non-treated members of Groups A, B, and C. The histological structure appeared to be normal in all the sections studied. No decrease in size of the zona glomerulosa in the adrenals of treated animals was observed when compared with the non-treated ones.

DISCUSSION

Part I (Pre-Cortisone)

Throughout experiments 1 and 2, the magnesium deficient groups revealed statistically significant decreases in urinary magnesium and calcium. This decrease in magnesium excretion we believe should not be interpreted solely as a reflection of the decreased magnesium content of the diet, which it probably is in part, but it should also be considered as an attempt at renal conservation of magnesium. Smith, Baxter, Linder and Guiles (1962), observed that a pronounced urinary conservation of magnesium occurred in depleted rats within one week following initiation of the deficient diet. These authors believed that the conservation of body magnesium was due to a renal conservation brought about, either, by an increased reabsorption of magnesium by the renal tubule, or, by a decrease in tubular secretion of magnesium. Martin and Wilson, (1960) observed the virtual absence of magnesium in the urine and stools of rats on a magnesium deficient diet indicating to them a conservation of body magnesium.

Samiy, et al. (1960) found that clearance studies demonstrated that calcium and magnesium each reciprocally affected the excretion of the other and that they probably competed for a common reabsorptive mechanism. Their finding that both ions are reabsorbed against a concentration gradient at the same distal site and that the isotopes of these ions entered the nephron at a common site, are consistent with the hypothesis of a common reabsorptive mechanism.

It is interesting to note that the urinary levels of magnesium excretion were in the same range for the deficients in experiments 1 and 2 (Group A, experimental, 0.53 mEq/L, Group A, experiment 2, 0.54 mEq/L). As the deficients in both experiments were maintained on the same GBI Mg-deficient diet, this is not an unexpected occurrence. The animals in experiment 1 were significantly older from the start (120 days) and did not demonstrate the severe signs of magnesium deficiency such as tonico-clonic convulsions and cachexia. This could conceivably be explained by the fact that older animals have greater body stores of magnesium than younger animals which take a longer time to deplete. Also, in experiment 1, the animals were maintained on tap water. The Chicago water supply is obtained from Lake Michigan and is considered notoriously hard due to its excessive mineral content and is especially high in magnesium and calcium salts. Therefore, the greater body stores, as well as excessive magnesium in the tap water, greatly delayed or prevented the more severe signs of magnesium deficiency from appearing in Group A, experiment 1. On the other hand, in experiment 2, using the same diet, but younger animals, (40 days) which had received distilled water, the tonico-clonic convulsions and cachexia of severe magnesium deficiency were observed.

In both experiments 1 and 2, the level of urinary calcium excretion of Group A was significantly below that of Group B and C. This finding of a decrease in urinary calcium excretion in the magnesium deficient rats confirms the observations of MacIntyre, (1963) who found that, despite the hypercalcemia and hyperabsorption from the gut, much less calcium was excreted in the urine in magnesium deficiency. This investigator maintained, that although one cannot be quite sure that changes in calcium excretion were not

due to a change in the glomerular filtration rate, it seemed more likely that it represented an increase in tubular reabsorption of calcium. He believed that his results suggested the existence of a common reabsorptive path for calcium and magnesium, not only in the gut, but also in the renal tubule, and that this "renal mechanism" would be of such a nature that a deficit or an excess of one ion would lead to an over or under absorption of the other.

In contrast to the results for experiments 1 and 2, in experiment 3, the deficients, which were sexually mature at the onset of the experiment (75 days) and who received only distilled water, did not manifest any of the severe signs of magnesium deficiency. Except for the mild signs of deficiency such as vasodilatation of the ears, hyperemia of the footpads, and diarrhea, no hyperirritability or convulsions were noted. Group A, although maintained on the standard GBI Mg-deficient diet, excreted less magnesium (2.1 mEq/L) than Group B (10.3 mEq/L) or Group C (22.9 mEq/L), however,the level in the Mg-deficient rats was approximately four times that of the deficients in experiments 1 and 2. This increase in urinary magnesium, when compared to similar aged animals in experiment 2 which demonstrated the severest signs of deficiency, is hard to explain. Several possible and plausible explanations are as follows: The first impression one receives when attempting to explain the above differences is that the diets employed in experiments 1 and 2 and in experiment 3 must have differed with respect to magnesium content. Since in all three experiments, the standard GBI Mg-deficient diet was supposedly manufactured from the same formula, one would assume that the diets employed in these experiments were similar ones. In experiments 1 and 2, a quantity of diet was ordered which was sufficient for

both of these experiments. However, in experiment 3, another lot of the standard GBI Mg-deficient diet was ordered which, although similar in formula to the diet employed in the previous two experiments, was however manufactured from different stock components. An analysis by the Wisconsin Alumni Research Foundation (WARF), indicated that the deficient diet employed in experiments 1 and 2 contained 10 mg% / 100 gm and that employed in experiment 2 less than 2 mg% Mg. This analysis indicates that the diet employed in experiments 1 and 2 is exactly double the minimum requirement established by Tufts and Greenberg (1937) of 5 mg% Mg for normal growth and reproduction. The severe manifestations of magnesium deficiency observed in experiment 2 cannot be explained, unless one assumes that the samples analyzed by the WARF were in some way contaminated by the magnesium supplemented diet of the controls. The value reported by the WARF for the diet employed in experiment 3 is within the correct range for a magnesium deficient diet. A conclusion which can be arrived at from our results in experiments 1 and 2, is that the diet employed was below the minimum magnesium requirement for the rat, that is magnesium deficient. Although no indications of severe magnesium deficiency were noted in experiment 3, the result of an analysis of this diet, and the milder transient signs of deficiency observed, indicated that this diet was also deficient. Thus, we are confident that in all three experiments the deficients received diets below the minimum requirements for magnesium.

An explanation for the observation that the Mg-deficient rats of experiments 2 excreted only one-fourth the amount of urinary magnesium observed in those of experiment 3, age 40 and 50 days respectively, may be due to the

fact that the former were 40 days old at the beginning of the experiment and on arrival were immediately placed on the Mg-deficient diet and distilled water, whereas, the Mg-deficient rats of the latter were permitted to acclimate for a period of four weeks during which time they were maintained on the standard Rockland rat pellets and tap water. These pellets were found to contain the highest magnesium content of any of the standard rat diets tested. Furthermore, the rats were 50 days old at the onset of the experiment and after four weeks of acclimatization on the Rockland pellets, with their excessive magnesium content, they were 78 days old, sexually mature, and by this time must have accumulated great stores of magnesium. This would seem to explain the fourfold level of urinary magnesium excretion when comparing this group of Mg-deficient rats to the same group in experiment 2. It is also of interest to note that the Mg-deficient rats in experiment 3, did not reveal a decrease in urinary calcium as did the Mg-deficient rats of experiments 1 and 2. This we believe is due to an accumulated store of magnesium so that even the very low magnesium diet which they received required many more weeks than the course of the experiment, for the severe signs and symptoms of deficiency to appear. Thus, a certified deficient diet had an effect comparable to a borderline deficient one, presumably due to an increase in body stores of magnesium acquired during the acclimatization period.

Of considerable significance is the unexpected discovery of renal damage due to excessive calcium salt deposition occurring in normal rats having received a well known and widely employed laboratory rat pellet diet. The members of Group C, during Part I, experiment 3, excreted a milky, turbid urine excessive in magnesium content (40-50 mEq/L) which exceeded the (Mg) X (PO_4)

solubility constant thus precipitating magnesium which was grossly visible as whitish particles of magnesium triple phosphate. Group C had received Rockland rat pellets which on analysis were found to contain 330 mg% magnesium and 1.86% calcium. When the members of Group C were transferred to Purina rat chow pellets after noting the above phenomenon, the urine cleared significantly within two days and on casual inspection appeared normal. However, for the remainder of experiment 3, when the urine was transilluminated, very fine particles of magnesium triple phosphate were still found to be present. Purina Rat chow pellets contained 260 mg% magnesium and 1.3% calcium.

In order to investigate the presumed dietary etiology of the above phenomenon, 27 female Sprague-Dawley rats 40 day of age were purchased and shortly after their receipt urine specimens were collected and assayed. These were all found to contain magnesium triple phosphate particles. It was later learned that the suppliers of our experimental animals feed all their rats Rockland pellets from the time they are weaned. Three well known varieties of rat pellets were tested by dividing the new animals into three groups of nine rats each. One group received Rockland, the second Purina and the third Wayne Lab-Blox pellets. The Wayne Lab-Blox pellets contained 200 mg% magnesium and 1.2% calcium. The animals which received Rockland and Purina pellets all excreted urine which contained particles of magnesium triple phosphate, whereas, those which use Wayne Lab-Blox excreted a clear, normal appearing urine after three days. The animals maintained on Rockland Pellets excreted an opaque, turbid urine containing more particles than those which received Purina rat chow pellets. The animals that received the Mg-deficient and Mg-supplemented diets that contained respectively, (less than 2 mg% and 75 mg%

magnesium, and 859 mg% and 899 mg% calcium), excreted a clear urine. Since the urine of animals which received diets of less that 2 mg%, 75 mg% and 200 mg% magnesium content with calcium contents of 859 mg%, 899 mg% and 1200 mg% calcium respectively, did not excrete magnesium triple phosphate particles, whereas, those maintained on diets containing 260 mg% and 330 mg% magnesium and 1300 mg% calcium and 1860 mg% Ca excreted a turbid, cloudy urine containing particles of magnesium triple phosphate, we assume that this phenomenon must be directly related to the magnesium content of the diet, and not the calcium, occurring somewhere above the 200 mg% magnesium level. If it were due to the calcium, then the animals maintained on the Wayne pellets, which have a calcium content similar to that of the Purina pellets, 1200mg% and 1300 mg% respectively, would have revealed the above phenomenon. However, this was not the case since only the animals maintained on Rockland and Purina pellets excreted the magnesium triple phosphate particles,indicating that the elevated magnesium content (260 mg%) and 330 mg% was the responsible factor, whereas, the animals maintained on Wayne pellets (200 mg%) excreted a clear urine. The animals maintained on Rockland pellets (330 mg%) excreted the most turbid urine containing the greatest number of particles. Also, the calcium content of the Rockland pellets was the highest, (1.86%) which probably contributed to the opaqueness by the addition of calcium phosphate precipitates. It is of interst to note that the Rockland, Purina and Wayne pellets all contained the recommended phosphorus content of 0.9%. Since all three diets contained the same phosphorus content, excessive phosphorus can be eliminated as being responsible for exceeding the (Mg) X (PO_4) solubility constant resulting in the precipitation of magnesium triple phosphate part-

ales.

The kidney is an organ which concentrates urine mainly at the proximal d distal convoluted tubules during the process of reabsorbing filtered Na, and Ca and, according to the osmolarity of the blood, at the collecting ubules which are the target organs for the hypophyseal anti-diurectic hor-one. Therefore, it is not surprising, since the solubility constants of g and Ca) X (PO_4) are closely related, that in the process of reabsorbing lectrolytes in the proximal and distal convoluted tubules, and water in the ollecting tubules, that magnesium and calcium phosphate salt deposition hould occur at these anatomical locations, the chances being increased due o the renal concentration which lessens the ratio of solute to solvent Eland, 1963).

Histological sections of kidneys treated with histochemical methods pecific for calcium salts and stained with Von Kossa and Alizarin Red dyes emonstrated calcium salt deposition in Groups A, B, and C. However, the egree of deposition differed quantitatively and in location, in direct pro-ortion to the dietary magnesium concentration of the above groups. The kid-eys of Groups A and B revealed renal calcinosis, presumably because they had een maintained on Rockland pellets during a four week acclimatization period rior to being transferred to their respective Mg-deficient and Mg- supple-ented diets. Group A showed the lowest degree of calcium deposition with inimal cortical deposits, whereas, Group B, exhibited a greater degree of ortical deposition, as well as excessive cortico-medullary deposits, and roup C the greatest with cortical, cortico-medullary and papillary deposits.

In order to explain the above, the hypothesis is advanced that the renal

lcium deposits, most probably calcium phosphate, are primarily a secondary ffect of the excessive dietary magnesium content which resulted in exceed-ng the (Mg) X (PO_4) solubility constant as well as secondarily the (Ca) X PO_4) constant resulting in the deposition of both calcium phosphate and mag-esium phosphate salts in the kidney. The positive results obtained with the istochemical methods for calcium show that calcium salts were deposited in he cortex, cortico-medullary junction and renal papilla. However, the histo-hemical methods for magnesium are not so reliable as those for calcium and e are not yet able to prove the presence of kidney magnesium salt deposition, lthough this possibility seems very likely. Thus far, sections stained with istochemical methods specific for magnesium, are only weakly positive at the ites found to contain calcium salts. We hope that before the final copy is ritten magnesium salt deposition will be demonstrated.

Thus, our results seem to indicate that dietary levels of magnesium bove 200 mg% are nephrotoxic resulting in varying degrees of renal calcinos-s. It has been known that rats maintained on a diet of 400 mg% magnesium evelop diarrhea, loss of appetite and die (Cunningham, 1933). The effect of igh levels of magnesium in our diets (330 mg%) resulted in a disturbance of calcium metabolism with a resultant precipitation of calcium salts in the idneys. It is hoped that the presence of magnesium salts will also be emonstrated in these kidneys.

At various times throughout experiments 1, 2, and 3, in both Parts I and II, an inverse relationship was observed to exist as concerns the urinary xcretion between magnesium and calcium. An increased excretion of magnesium as associated with a decreased calcium excretion and an increased calcium

cretion with a decreased magnesium excretion. This observation may be compatible with the existence of a common renal reabsorptive mechanism whereby the renal tubule, in order to reabsorb magnesium, must lose calcium and that the active reabsorptive enzyme system must deal with either magnesium or calcium individually, reabsorbing one at the expense of the other.

art II (Cortisone)

During experiments 2 and 3, significant differences in levels of magnesium excretion between the cortisone-treated and non-treated members of roups A and B were observed. In experiment 2, the cortisone-treated members of Group B excreted significantly increased levels of magnesium as compared to the non-treated animals. In contrast to the results of experiment 2, the cortisone-treated members of Group A, experiment 3, excreted decreased levels of magnesium for the entire period of Part II in comparison with the non-treated members.

Thus in experiments 2 and 3, significant data were obtained indicating that cortisone administration may result in either an increase or a decrease in urinary magnesium excretion. In an attempt to explain this observation the following hypothesis is advanced: Perhaps the effect of cortisone on magnesium excretion is dependent on the relative (Minimal or Maximal) quantity of available magnesium supplied in the diet and water, as well as the total reserve of stored magnesium in the animal. In the rats of experiment 2, Group B, which received a Mg-supplemented diet and tap water, cortisone administration resulted in a significant increase in magnesium excretion, whereas, in experiment 3, Group A, which received a Mg-deficient diet and

distilled water, the injection of cortisone resulted in a significant decrease in magnesium excretion for the entire period of Part II. Perhaps, with normal or excessive amounts of magnesium in the diet, the effect of cortisone on the renal reabsorptive sites or mechanisms, is to eliminate excesses and with diets deficient in magnesium it functions to conserve this electrolyte, thereby maintaining homeostasis.

A review of the literature unfortunately was not very helpful due to the dearth of experimental data dealing with the effect of cortisone on magnesium metabolism and the conflicting clinical reports on the urinary excretion of magnesium following cortisone administration. Some investigators report no change, others either a decrease or an increase in magnesium excretion.

Ingbar, et al. (1951) found that in cases of Cushing's syndrome, where endogenous excesses of corticosteroids exist, increased amounts of magnesium were lost in the urine. On the other hand, Doe, et al. (1960) observed that, in normal subjects treated for adrenal insufficiency (Addison's disease), and in Cushing's syndrome, the administration of hydrocortisone hemisuccinate did not influence magnesium excretion and that the nocturnal excretion of magnesium was normal in five cases of Cushing's syndrome due to bilateral adrenal hyperplasia.

The adreno-cortical hormones are C_{21}-steroids and are therefore closely related chemically to the other steroid hormones. This close relationship is also shown biologically by the fact that in non-functioning of the adrenal cortex, the sex hormones will prolong life, and that certain cortical substances and metabolites of the adreno-cortical hormones show androgenic, progestational and estrogenic activity. The biological action of the individual

steroids show quantitative differences and considerable overlap, (Diem, 1962). Therefore, the effects of a progestational-estrogen compound such as Enovid may in some ways parallel that of cortisone with respect to magnesium regulation. Aikawa, et al. (1960) observed that during a 14 day series of cortisone injections in adult male rabbits the external magnesium balance was not altered, but a significant reduction in serum magnesium concentration was observed, indicating the occurrence of subtle internal changes in the dynamics and dis tribution of magnesium. Goldsmith and Goldsmith (1966) observed that, in women who were taking progestational drugs (Enovid) to suppress ovulation, the serum and urine magnesium was significantly lower than in younger ovulating women (controls). Thus a parallel in the biological action of cortisone and Enovid (norethynodrel with mestranol) was observed by both Aikawa in the rabbit and Goldsmith in the human, to result in a decreased serum magnesium level, however, Aikawa observed no change in the external balance, (urine) whereas, Goldsmith noted a significant decrease in magnesium excretion.

Care, et al. (1963) observed that the absorption of dietary magnesium was decreased in intact sheep, following the administration of deoxycorticosterone acetate, resulting in increased fecal magnesium excretion. If one accepts MacIntyre's (1963) hypothesis of the existence of "common reabsorptive sites" for magnesium and calcium in the gut and renal tubule, it may be possible that the effect of cortisone in the gut is paralleled by the reabsorptive sites in the renal tubule resulting in increased magnesium excretion.

The results of this investigation indicate that the administration of cortisone may cause either an increase or a decrease in magnesium excretion in treated rats. What variables mediate this effect is not known, however, the

dietary concentration of magnesium as well as the total store of body magnesium may be involved in the response to treatment.

SUMMARY AND CONCLUSIONS

The effect of cortisone on urinary magnesium and calcium excretion was studied in three successive experiments. The experiments were each divided into two Parts, designated Part I and Part II. During Part I, Groups A and B of experiment 1 and Groups A, B, and C of experiments 2 and 3 were maintained on their respective diets, Group A, a Mg-deficient Diet, Group B, a Mg-deficient diet, Group B, a Mg-supplemented diet and Group C, a standard rat pellet diet. Part II differed from Part I by the administration of cortison in half of the rats of each group.

Urine analyses for magnesium and calcium were performed in experiments 1 and 2 according to Kovac's Complexometric method (1959) and in experiment 3 employing a Model 21 Coleman Flame Photometer. All data were statistically analyzed.

In all three experiments (Part I) the Mg-deficient rats (Group A) excreted the lowest level of magnesium and in experiments 1 and 2 they also excreted the lowest level of calcium while in experiment 3, this group excreted a higher level of calcium than B but a lower one than C. These differences were statistically significant.

In experiment 1 (Part I) differences in weight gains were observed between groups A and B and in experiments 2 and 3 between groups A, B, and C. In experiments 1 and 2, Group A showed the poorest weight gain whereas, in experiment 3, Group B exhibited a failure to thrive with a weight below.A. In all three experiments, Group C exhibited optimum growth and development with maximum weight gain. In Part II, the cortisone-treated rats of Groups

A, B, and C in all three experiments weighed less than non-treated ones.

In all three experiments during Parts I and II, an inverse relationship was observed between the urinary excretion of magnesium and calcium. An increased excretion of magnesium was associated with a decreased calcium excretion and vice-versa.

In experiment 3, renal calcinosis was unexpectedly discovered in normal rats which had been fed Rockland and Purina rat pellets excessive in Mg content (330 and 260 mg%). It is assumed that this resulted in exceeding the (Mg) X (PO_4) solubility constant precipitating Mg in the urine grossly visible as particles of Mg-triplephosphate. This phenomenon occurred above the 200 mg% Mg level.

In experiment 1, Part II, there were not a sufficient number of observations to warrant a statistical study, however, it appeared that cortisone administration resulted in an increased magnesium excretion in Group A and a decreased excretion in B.

In experiment 2, Part II, cortisone administration resulted in a significant increase in the excretion of magnesium in Group B and in experiment 3 in a significant decrease in excretion in Group A. There were no significant differences with respect to calcium excretion between the cortisone-treated and non-treated members of Groups A, B, and C in experiments 2 and 3.

It may be concluded that rats maintained on a Mg-deficient diet conserve body magnesium through renal mechanisms which decrease magnesium excretion and that cortisone administration may result in either a decreased or an increased excretion of magnesium depending on the level of magnesium in the diet and the store of magnesium in the animal. With normal or excess magne-

sium content in the diet the effect of cortisone is to eliminate the excess by excretion whereas with diets deficient in magnesium cortisone functions to decrease magnesium excretion thus preserving body homeostasis.

LITERATURE CITED

Aikawa, J. K. 1960 Effect of thyroxine and propylthiouracil on magnesium metabolism in the rabbit. Study with Mg^{28}. Proc. Soc. Exp. Biol. Med., 104: 594-597.

Aikawa, J. K., D. R. Harms and J. Z. Reardon 1960 Effect of cortisone on magnesium metabolism in the rabbit. Am. J. Physiol., 199 2: 229-230.

Alcock, N. and I. MacIntyre 1964 Some effects of magnesium repletion on calcium metabolism in the rat. Clin. Sci., 26: 219-225.

Bain, J. A. 1954 The effect of magnesium upon thyroxin inhibition of phosphorylation. J. Pharmacol., 110: 2-3.

Bland, J. H. 1963 Clinical metabolism of body water and electrolytes, Chap, 7, The Kidney: 137-139.

Care, A. D. and D. B. Ross 1963 Factors which affect the availability of magnesium. Res. Vet. Sci., 4: 24-28.

Cheek, D. B. and H. C. Teng 1960 Changes in tissue composition of the rat in parathyroid intoxication (as produced by large doses of commercial extract) and the effect of magnesium loading. Clin. Sci., 19: 195-208.

Corradino, R. A. and H. E. Parker 1962 Magnesium and thyroid function in rat. J. Nutr., 77: 455-458.

Cunningham, I. J. 1933 Magnesium in animal diets. The influence of the level of dietary magnesium on the magnesium and calcium contents of the bones, the bodies and the blood serum of rats. New Zealand J. Sci. Tech. 15: 191-198.

Dahl, L. K. 1952 A simple and sensitive method for calcium. Proc. Soc. Exp. Biol. Med., 80: 474-479.

Diem, K. 1962 Adrenocortical Hormones (corticosteroids) Documenta Geigy. VIth Edition: 487-491.

Doe, R. P., J. A. Vennes and E. B. Flink 1960 Diurnal variation of 17-hydroxycorticosteroids, sodium, potassium, magnesium creatinine in normal subjects and in cases of treated adrenal insufficiency and Cushing's syndrome. J. Clin. Endocr., 20 (2): 253-265.

Forbes, R. M. 1963 Mineral utilization in the rat: I Effects of varying dietary ratios of calcium, magnesium and phosphorus. J. Nutr., 80: 321-326.

_____ 1964 Mineral utilization in the rat: II Restoration of normal tissue levels of magnesium and calcium following magnesium deficiency. J. Nutr., 83: 44-48.

Goldsmith, N. F. and J. R. Goldsmith 1966 Epidemiological aspects of magnesium and calcium metabolism. Arch. Envir. Health, 12: 607-619.

Greenberg, D. M. and E. V. Tufts 1938 The nature of magnesium tetany. Am. J. Physiol., 121: 416-423.

Hanna, S. and I. MacIntyre 1960 The influence of aldosterone on magnesium metabolism. Lancet, 2: 348-350.

Ingbar, S. H., E. H. Kass, C. H. Burnett, A. S. Relman, A. B. Burrows, and J. H. Sisson. 1961 The effects of ACTH and cortisone on the renal tubular transport of uric acid, phosphorus and electrolytes in patients with normal renal and adrenal function. J. Lab. Clin. Med., 38: 533-541.

Kashiwa, H. K. 1961 Magnesium deficiency in intact, in adrenalectomized and in hypophysectomized. Endocrinology, 68: 80-91.

Kovac, G. and K. Tarnoky 1959 Complexometric determination of calcium and magnesium in urine. Kiserl Orvostud, 11: 440-443. (Hun., Kiserletes Orvostudomany, Budapest).

Kleiber, M., M. D. Boelter and D. M. Greenberg 1941 Fasting catabolism and food utilization of magnesium deficient rats. J. Nutr., 21: 363-372.

Kruse, H. D., E. R. Orent and E. V. McCollum 1932 Studies on magnesium deficiency in animals: I Symptomatology resulting from magnesium deprivation. J. Biol. Chem., 96: 519-539.

_____ 1933 Studies on magnesium deficiency in animals; III. Chemical changes in the blood following magnesium deprivation. J. Biol. Chem., 100: 603-643.

LaVollay, J. 1931 Magnesium et croissance du rat. Soc. Chim. Biol., 13: 1205-1209.

LeRoy, J. 1926 Necessite du magnesium pour la croissance de la souris. Compt. Rend. Soc. Biol., 94: 431-433.

MacIntyre, I. 1963 Magnesium metabolism. Sci. Basis Med. Ann. Rev., pp. 217-234.

MacIntyre, I., S. Boss and V. A. Troughton 1963 Parathyroid hormone and magnesium homeostasis. Nature (London), 198: 1058-1060.

Mallory, F. B. 1942 Pathological Technique, W. B. Saunders Co., Phila.,

Chap. 11, p. 144, (A.F.I.P. modification).

Martin, H. E. and M. L. Wilson 1960 Effect of magnesium deficiency on serum and carcass electrolyte levels in the rat. Metabolism. 9: 484-491.

NeGuib, M. A. 1963 Effect of magnesium on the thyroid. Lancet, 1: 1405.

O'Dell, B. L., E. R. Morris, E. E. Pickett and A. G. Hogan 1957 Diet composition and mineral balance in guinea pigs. J. Nutr., 63: 65-77.

Osborne, T. B. and L. B. Mendel 1918 The inorganic elements in nutrition. J. Biol. Chem., 34: 131-139.

Samiy, A. H., J. L. Brown, D. L. Globus, R. H. Kessler and D. D. Thompson 1960 Interrelation between renal transport systems of magnesium and calcium. Am. J. Physiol., 198: 599-602.

Selye, H. 1958 Prevention of experimental hyperarathyroidism by magnesium and potassium salts. Endocrinology, 63: 216-225.

Smith, W. O., D. J. Baxter, A. Linder, H. E. Ginn, and H. Guiles 1962 Effect of magnesium depletion on renal function in the rat. J. Lab. Clin. Med., 59: 211-219.

Smith, B. S. W. and A. C. Field 1963 Effect of age on magnesium deficiency in rats. Brit. J. Nutr., 17: 591-600.

Tapley, D. F. and C. Cooper 1956 The effect of thyroxine and related compounds on oxidative phosphorylation. J. Biol. Chem., 222: 341-349.

Toothill, J. 1963 The effect of certain dietary factors on the apparent absorption of magnesium by the rat. Brit. J. Nutr., 17: 125-134.

Tufts, E. V. and D. M. Greenberg 1937 The biochemistry of magnesium deficiency: I. Chemical changes resulting from magnesium deprivation. J. Biol. Chem., 122: 693-714.

_____ 1938 II. The minimum magnesium requirements for growth, gestation, and lactation, and the effect of the dietary calcium level thereon. J. Biol. Chem., 122: 715-726.

Watchorn, E. and R. A. McCance 1937 Subacute magnesium deficiency in rats. Biochem. J., 31: 1379-1390.

TABLE 1

AN ANALYSIS OF THE SIGNIFICANCE BETWEEN LEVELS OF URINARY MAGNESIUM AND CALCIUM EXCRETION

Experiment 1, Part I

MAGNESIUM

Groups	No. Rats	No. Obs.	Mean, Urine Mg mEq/L	D.F.[1]	P[2]	Dates Analyzed
A	8	25	0.742	40	0.0005	1-8 and 9-16 all wks.
B	8	17	2.066			
A	8	14	0.700	20	0.0005	All Aug.
B	7	8	2.868			
A	7	10	0.710	17	0.0025	Aug. 15-31
B	7	9	2.346			
A	7	11	0.366	19	0.0025	All Sept.
B	8	10	1.152			
A	6	8	0.416	12	0.010	Sept. 4-11
B	6	6	1.386			
A	8	8	0.502	12	0.0005	Oct 31-Nov 1
B	6	6	2.148			
A	8	8	0.564	13	0.005	Dec. 24-27
B	7	7	1.996			
			CALCIUM			
A	5	5	2.010	8	0.05	Oct 31-Nov 1
B	5	5	3.250			

TABLE 1 (cont'd)

Groups	No. Rats	No. Obs.	Mean, Urine Mg mEq/L	D.F.[1]	P[2]	Dates Analyzed
A	12	12	1.680	18	0.05	All Aug.
B	8	8	5.230			
A	10	10	2.930	16	0.01	All Sept.
B	8	8	6.540			

TABLE 2

AN ANALYSIS OF THE SIGNIFICANCE BETWEEN LEVELS OF URINARY MAGNESIUM AND CALCIUM EXCRETION, GROUPS A, B, AND C

Experiment 2, Part I

MAGNESIUM

roups	No. Rats	No. Obs.	Mean, Urine Mg mEq/L	D.F.[1]	P[2]	Dates Analyzed
A	12	12	0.510	22	0.20[5]	1st run
B	12	12	1.482			
A	12	12	0.433	21	0.0005	2nd run
B	11	11	1.632			
A	10	10	0.430	18	0.10[5]	3rd run
B	10	10	0.946			
A	9	9	0.783	16	0.025	4th run
B	9	9	2.208			
A	43	43	0.539	83	0.00025	All run
B	42	42	1.567			
A	12	12	0.510	22	0.0005	1st run
C	12	12	1.728			
A	12	12	0.433	19	0.10[5]	2nd run
C	9	9	1.812			
A	10	10	0.430	18	0.0015	3rd run
C	10	10	4.595			
A	9	9	0.783	14	0.0015	4th run
C	7	7	10.486			

TABLE 2 (cont'd)

CALCIUM

Groups	No. Rats	No. Obs.	Mean, Urine Mg mEq/L	D.F.[1]	P[2]	Dates Analyzed
A	12	12	1.080	22	0.01	1st run
B	12	12	2.125			
A	12	12	1.470	21	0.20[5]	2nd run
B	11	11	1.865			
A	10	10	2.355	18	0.20[5]	3rd run
B	10	10	3.320			
A	9	9	1.560	16	0.005	4th run
B	9	9	2.520			
A	43	43	1.616	83	0.01	All runs
B	42	42	2.456			
A	12	12	1.080	22	0.00025	1st run
C	12	12	8.010			
A	12	12	1.470	19	0.0005	2nd run
C	9	9	13.905			
A	10	10	2.355	18	0.00025	3rd run
C	10	10	14.145			
A	9	9	1.560	14	0.0015	4th run
C	7	7	6.920			

[5]Not significant

TABLE 3

AN ANALYSIS OF THE SIGNIFICANCE BETWEEN LEVELS OF URINARY MAGNESIUM AND CALCIUM EXCRETION GROUPS A, B, AND C

Experiment 2, Part II

Groups		No. Rats	No. Obs.	Mean, Urine Mg mEq/L	D.F.[1]	P[2]	Periods day
A	T[3]	6	6	0.699	9	0.010	1-9
	C[4]	5	5	0.556			
A	T	6	16	0.606	26	0.450[5]	1-27
	C	5	12	0.587			
B	T	5	5	4.286	8	0.025	1-9
	C	5	5	2.409			
B	T	4	4	2.531	6	0.10[5]	9-18
	C	4	4	1.212			
B	T	5	14	2.684	25	0.050	1-27
	C	5	13	1.665			
C	T	6	16	1.891	26	0.15[5]	1-27
	C	5	12	1.628			
A	T	6	17	3.630	30	0.450[5]	1-27
	C	5	15	2.813			
B	T	5	14	6.715	26	0.10[5]	1-27
	C	5	14	10.650			
C	T	6	15	15.315	25	0.07[5]	1-27
	C	5	12	12.040			

[1]Degrees of Freedom
[2]Probability
[3]Cortisone-treated
[4]Non-treated
[5]Not significant

TABLE 4

A COMPARISON OF URINARY LEVELS OF MAGNESIUM EXCRETION BETWEEN GROUPS A, B, AND C

Experiment 2, Parts I and II

Part I

	Group A Mg-deficient diet	Group B Mg-supplemented diet	Group C Rockland Rat Pellets
1st Run	0.510 mEq/L	1.482 mEq/L	1.728 mEq/L
2nd Run	0.433 "	1.632 "	1.812 "
3rd Run	0.430 "	0.946 "	4.595 "
4th Run	0.783 "	2.208 "	10.486 "
Total Mg Avg.	0.539 mEq/L	1.567 mEq/L	4.655 mEq/L

Part II

	Non-treated	Cortisone treated	Non-treated	Cortisone treated	Non-treated	Cortisone treated
1st Run	0.556 mEq/L	0.699 mEq/L	2.409 mEq/L	4.286 mEq/L	2.035 mEq/L	1.678 mEq/L
2nd Run	0.819 "	0.726 "	1.212 "	2.531 "	1.465 "	2.128 "
3rd Run	0.403 "	0.393 "	1.352 "	1.235 "	1.385 "	1.865 "
Total Mg Avg.	0.587 "	0.606 "	1.665 "	2.684 "	1.628 "	1.891 "

TABLE 5

A COMPARISON OF URINARY LEVELS OF CALCIUM EXCRETION BETWEEN GROUPS A, B, AND C

Experiment 2, Parts I and II

Part I

Periods days	Group A Mg-deficient	Group B Mg-supplemented	Group C Rockland Rat Pellets
1-9	1.080 mEq/L	2.125 mEq/L	8.010 mEq/L
9-18	1.470 "	1.865 "	13.905 "
18-27	2.355 "	3.320 "	14.145 "
27-36	1.560 "	2.520 "	6.920 "
Avg.	1.616 mEq/L	2.458 mEq/L	10.745 mEq/L

Part II

	Non-Tr'd	Cort-Tr'd	Non-Tr'd	Cort-Tr'd	Non-Tr'd	Cort-Tr'd
1-9	2.680 mEq/L	3.200 mEq/L	8.600 mEq/L	3.200 mEq/L	11.915 mEq/L	17.360 mEq/L
9-18	3.835 "	3.420 "	9.795 "	4.325 "	13.350 "	12.165 "
18-27	1.925 "	4.270 "	13.640 "	12.615 "	10.860 "	16.420 "
Avg.	2.813 mEq/L	3.630 mEq/L	10.650 mEq/L	6.715 mEq/L	12.040 mEq/L	15.315 mEq/L

TABLE 6

AN ANALYSIS OF THE SIGNIFICANCE BETWEEN LEVELS OF URINARY MAGNESIUM AND CALCIUM EXCRETION OF CORTISONE-TREATED AND NON-TREATED MEMBERS OF GROUPS A AND C

Experiment 3, Part II

Groups		No. Rats	No. Obs.	Mean Urine Mg mEq/L	D. F.[1]	P[2]	Periods days
A	T[3]	10	10	2.00	18	0.43[5]	Dec. 8
	C[4]	10	10	2.08			
A	T	10	10	1.28	17	0.05	Dec. 12
	C	9	9	2.04			
A	T	7	7	0.46	10	0.0025	Dec. 14
	C	5	5	2.72			
A	T	8	8	1.00	13	0.0125	Dec. 16
	C	7	7	2.86			
A	T	5	5	0.58	8	0.025	Dec. 18
	C	5	5	2.20			
A	T	6	6	1.00	11	0.488[5]	Dec. 23
	C	7	7	0.97			
A	T	35	35	4.74	64	0.0005	Dec. 8-16
	C	31	31	9.74			
A	T	46	46	6.32	87	0.0005	Dec. 8-23
	C	43	43	12.91			
C	T	10	10	14.92	18	0.20[5]	Dec. 10
	C	10	10	19.52			

TABLE 6 (cont'd)

Groups		No. Rats	No. Obs.	Mean Urine Mg mEq/L	D.F.[1]	P[2]	Periods days
C	T[3]	8	8	12.40	14	0.10^{5}	Dec. 15
	C[4]	8	8	17.40			
C	T	8	8	13.76	13	0.25^{5}	Dec. 17
	C	7	7	16.16			
C	T	6	6	15.56	8	0.45^{5}	Dec. 21
	C	4	4	16.05			
A	T	10	10	0.375	17	0.49^{5}	Dec. 8
	C	9	9	0.435			
A	T	9	9	2.48	13	0.10^{5}	Dec. 12
	C	6	6	8.45			
A	T	5	5	3.37	7	0.27^{5}	Dec. 14
	C	4	4	9.01			
A	T	5	5	1.25	13	0.30^{5}	Dec. 16
	C	8	8	5.38			
A	T	5	5	1.79	8	0.10^{5}	Dec. 23
	C	5	5	7.65			
C	T	9	9	6.00	16	0.0025	Dec. 10
	C	9	9	10.82			
C	T	8	8	2.91	13	0.10^{5}	Dec. 15
	C	7	7	4.89			

TABLE 6 (cont'd)

oups		No. Rats	No. Obs.	Mean Urine Mg mEq/L	D.F.[1]	P[2]	Periods days
C	T[3]	8	8	4.56	14	0.35[5]	Dec. 17
	C[4]	8	8	4.88			

Degrees of Freedom

Probability

Cortisone-treated

Non-treated

Not significant

TABLE 7

A COMPARISON OF URINARY LEVELS OF MAGNESIUM AND CALCIUM EXCRETION BETWEEN GROUPS A, B, AND C

Experiment 3, Part I

MAGNESIUM

e	Group A	Date	Group B	Date	Group C
t. 21	2.16 mEq/L	Oct. 20	18.4 mEq/L	Oct. 19	45.0 mEq/L
26	1.28	30	15.2	Nov. 13	18.4
31	1.32	Nov. 2	9.6	16	16.0
v. 3	3.20	6	12.8	21	19.6
10	1.32	11	6.8	25	20.0
12	1.40	14	6.8	28	22.8
18	3.20	17	6.5	Dec. 2	23.2
23	2.40	19	8.0		
27	2.80	22	11.2		
31	2.60	26	8.0		
c. 5	1.96	30	10.4		
g.	2.15		10.34		22.90

CALCIUM

t. 21	0.65 mEq/L	Oct. 20	0.85 mEq/L	Oct. 19	5.20 mEq/L
26	1.27	30	3.25	Nov. 13	2.50
31	1.17	Nov. 2	2.50	16	2.82
v. 3	0.82	6	1.80	21	3.40
10	0.92	11	2.70	25	4.25
12	0.40	14	2.15	28	4.40
18	8.75	17	3.50	Dec. 2	8.00
23	6.40	19	3.10		
27	6.40	22	4.10		
31	6.80	26	3.10		
c. 5	7.00	30	4.50		
g.	3.70		2.87		4.37

ach Value for Mg or Ca represents the average of 20 observes

TABLE 8

A COMPARISON OF URINARY LEVELS OF MAGNESIUM AND CALCIUM EXCRETION BETWEEN CORTISONE-TREATED AND NON-TREATED RATS OF GROUPS A AND C

Experiment 3, Part II

MAGNESIUM

ate	Treated	Non-Treated	Date	Treated	Non-Treated
ec. 8	2.00 mEq/L	2.08 mEq/L	Dec. 10	14.92 mEq/L	19.52 mEq/L
12	1.28	2.04	15	12.40	17.40
14	0.46	2.72	17	13.76	16.16
16	1.00	2.86	21	15.56	16.05
18	0.58	2.20			
23	1.00	0.97			
vg.	1.05	2.14		14.16	17.28

CALCIUM

ec. 8	3.75 mEq/L	4.35 mEq/L	Dec. 10	6.00 mEq/L	10.82 mEq/L
12	2.47	8.50	15	2.91	4.89
14	3.37	9.00	17	4.56	4.88
16	1.60	5.35	21		
23	1.79	7.65			
vg.	2.40	6.97		4.49	6.86

PLATE I

EXPLANATION OF FIGURES

1 A comparison of weights between Groups A and B, Experiment 1, Parts I and II.

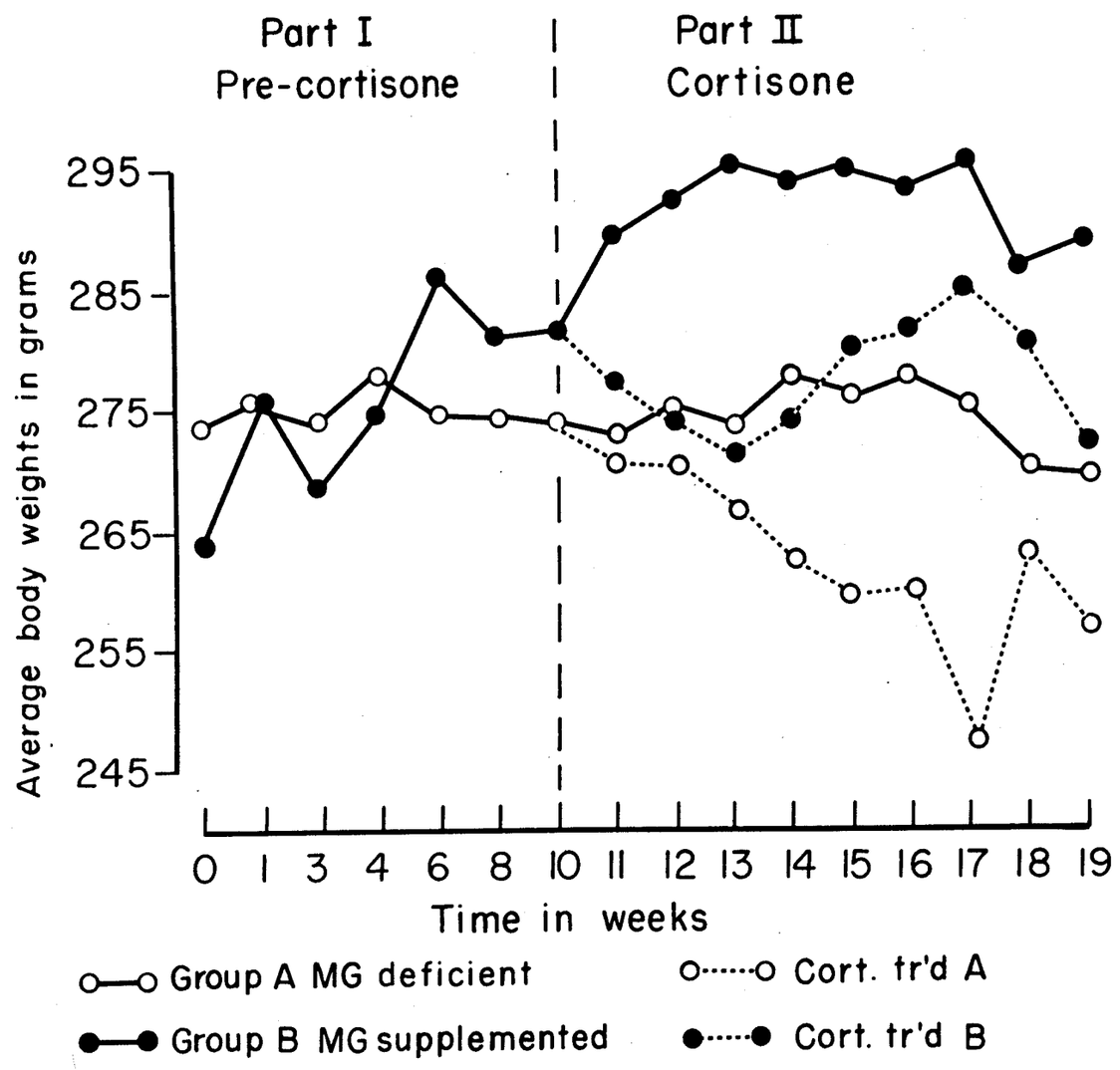

PLATE II

EXPLANATION OF FIGURE

2 A comparison of weights between Groups A, B, and C, Experiment 2.

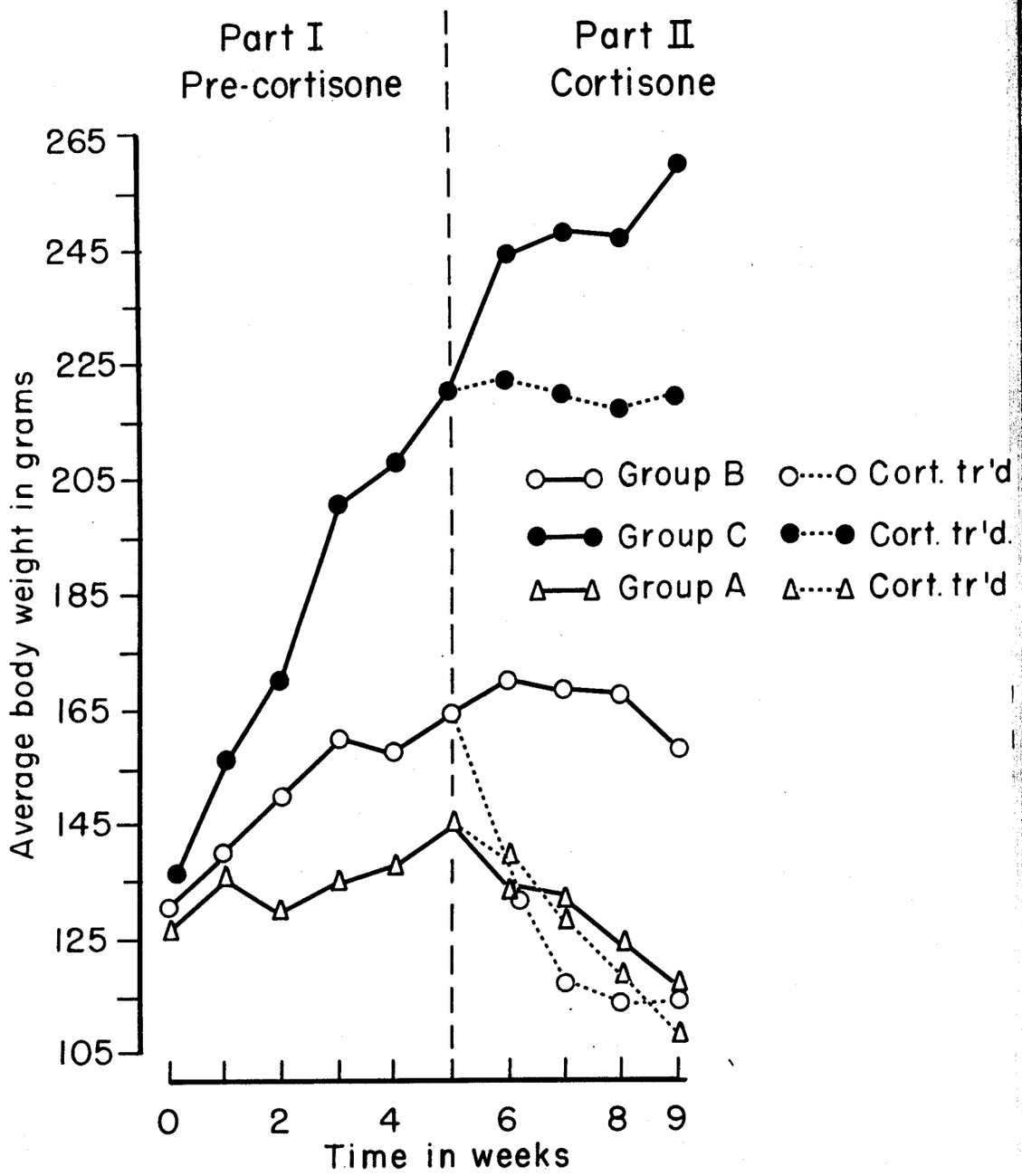

PLATE III

EXPLANATION OF FIGURE

3 A comparison of weights between Groups A, B, and C, Experiment 3. Parts I and II.

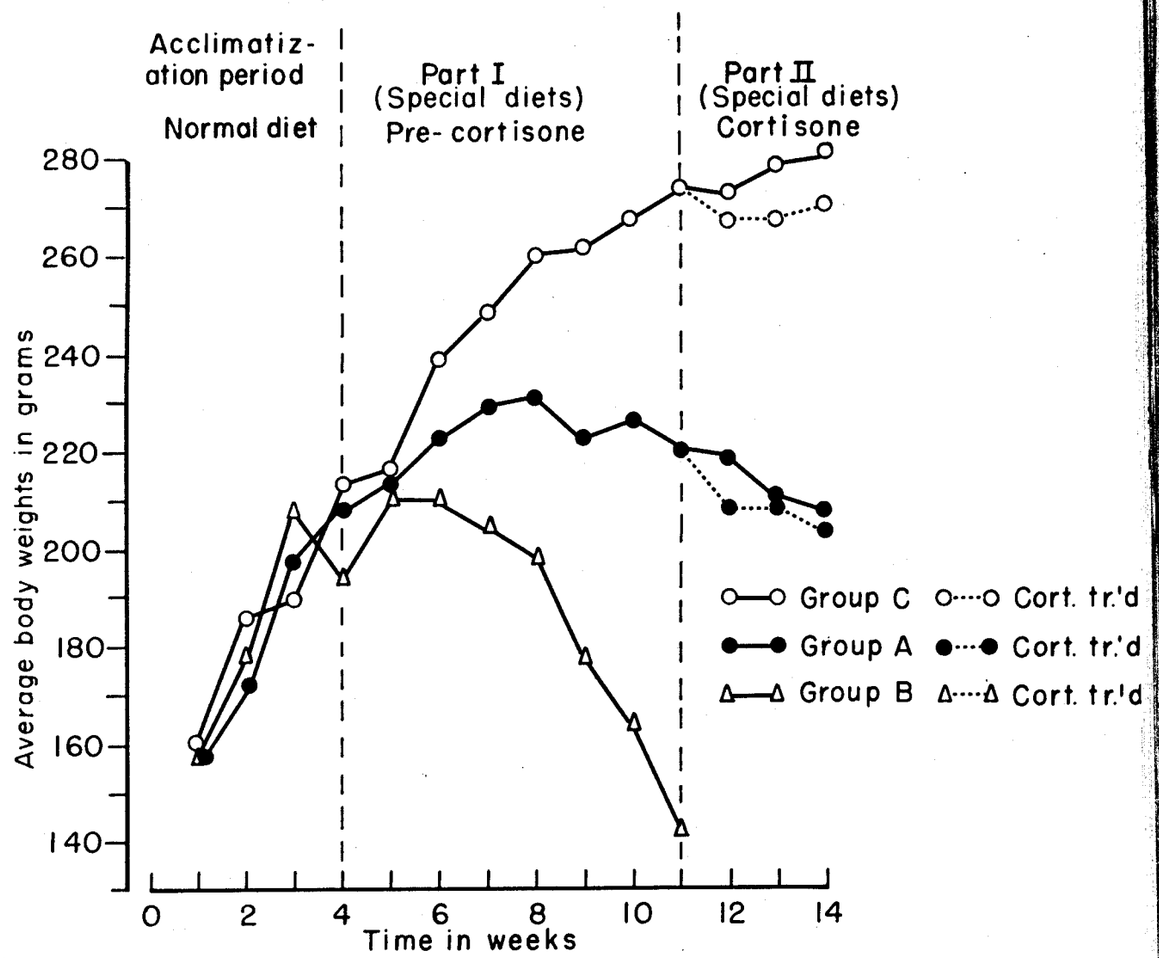

PLATE IV

EXPLANATION OF FIGURE

4 Levels of urinary magnesium and calcium excretion Groups A and B, Experiment 1, Parts I and II.

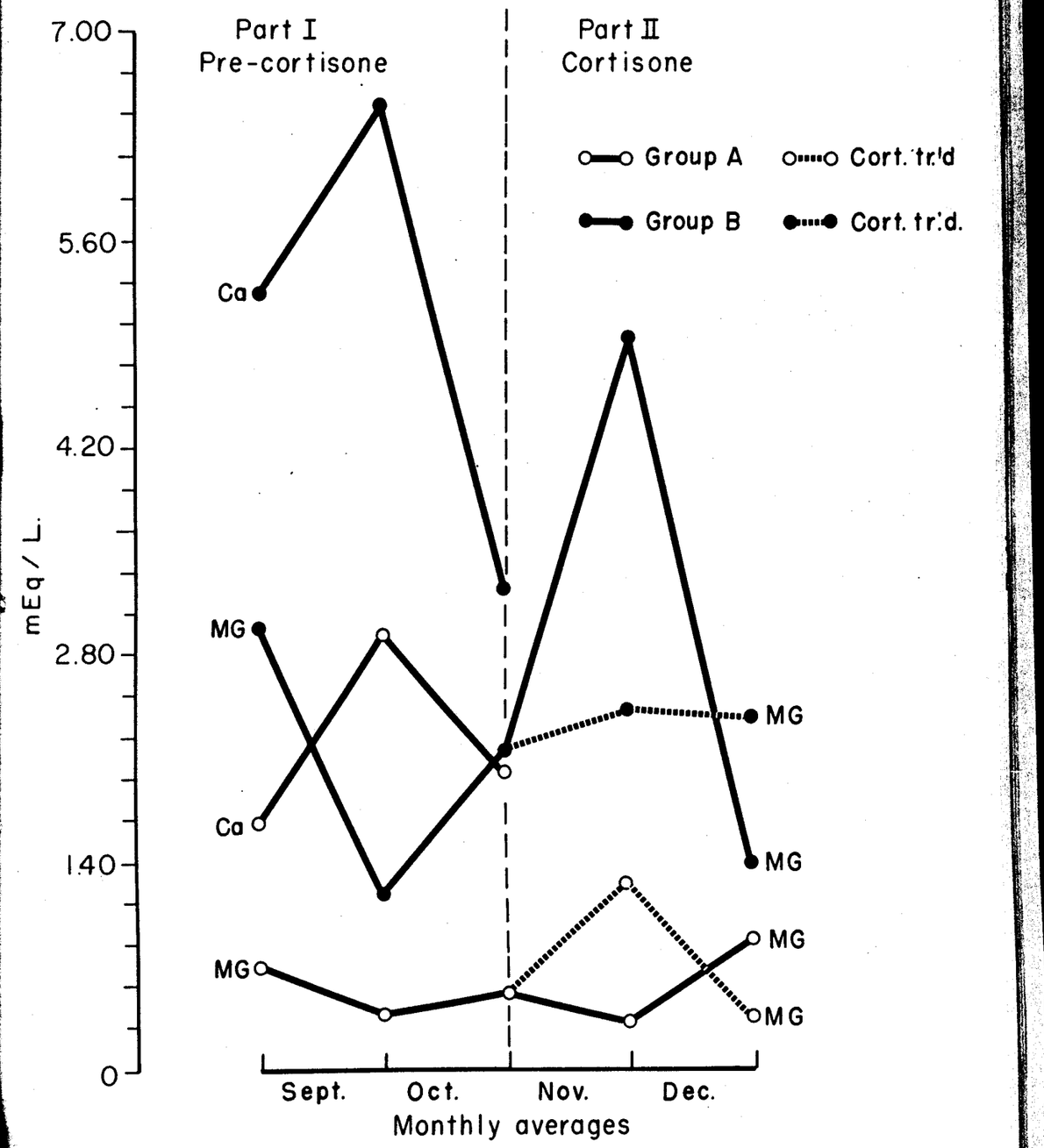

PLATE V

EXPLANATION OF FIGURE

5 Levels of urinary magnesium excretion Groups A, B, and C, Experiment 2, Parts I and II.

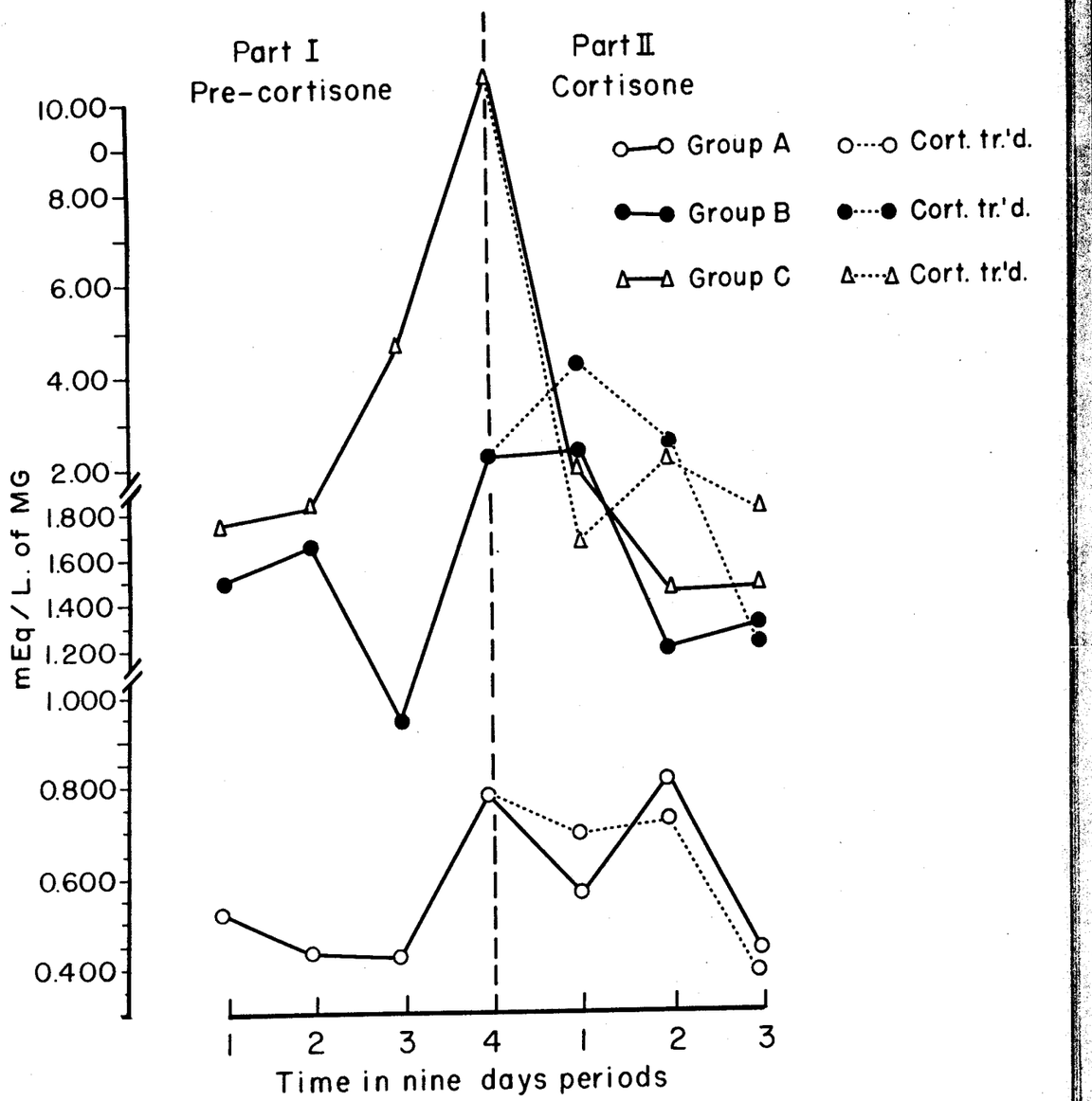

PLATE VI

EXPLANATION OF FIGURE

6 Levels of urinary calcium excretion Groups A, B, and C, Experiment 2, Parts I and II.

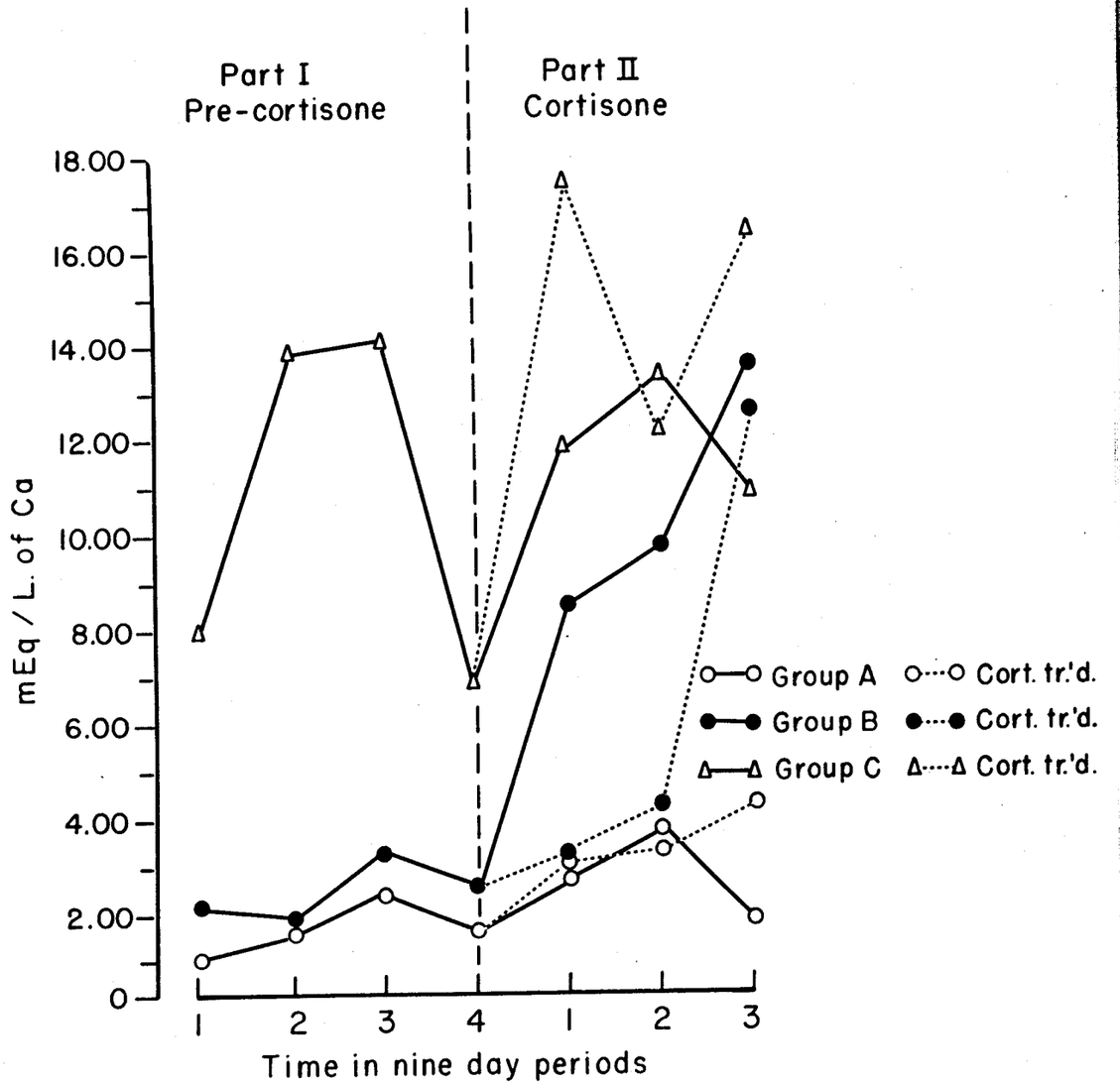

PLATE VII

EXPLANATION OF FIGURE

7 Levels of urinary magnesium excretion Groups A, B, and C, Experiment 3, Part I.

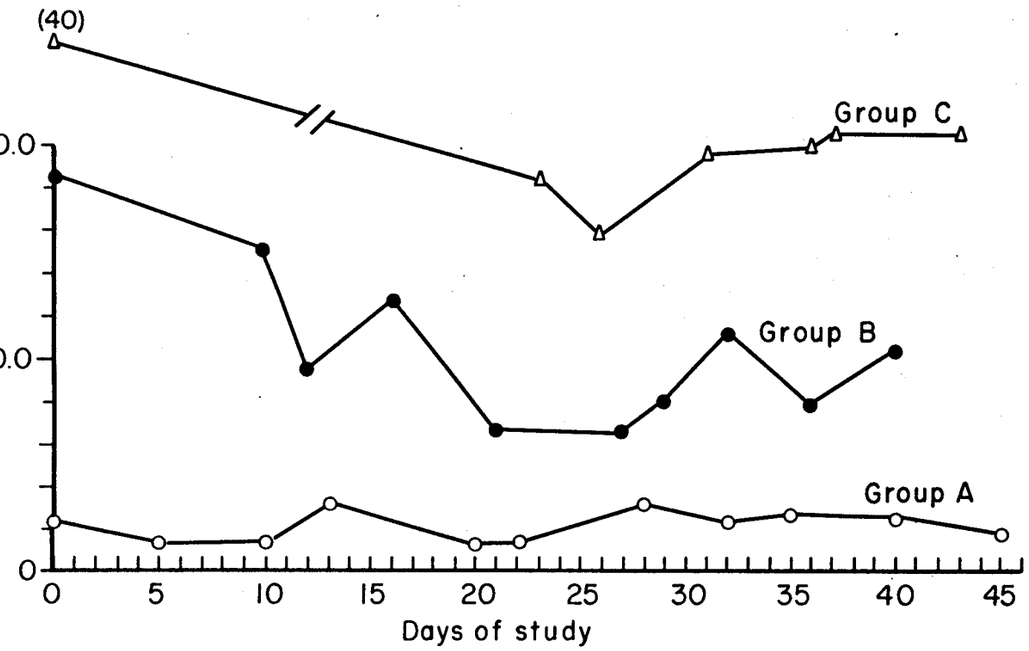

PLATE VIII

EXPLANATION OF FIGURE

8 Levels of urinary magnesium and calcium excretion Group B, Experiment 2, Parts I and II.

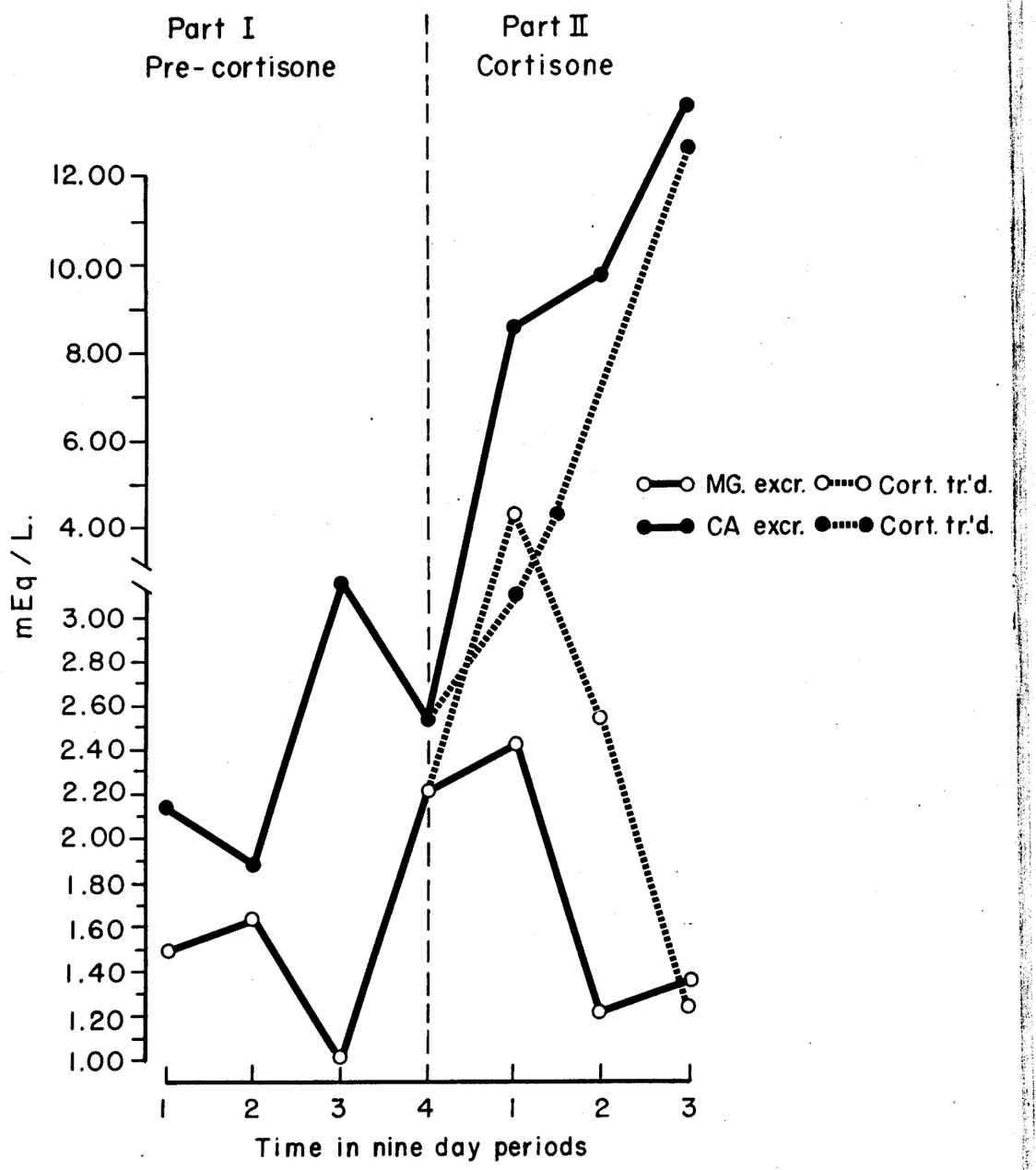

PLATE IX

EXPLANATION OF FIGURE

9 Levels of urinary magnesium and calcium excretion Group A, Experiment 3, Part II.

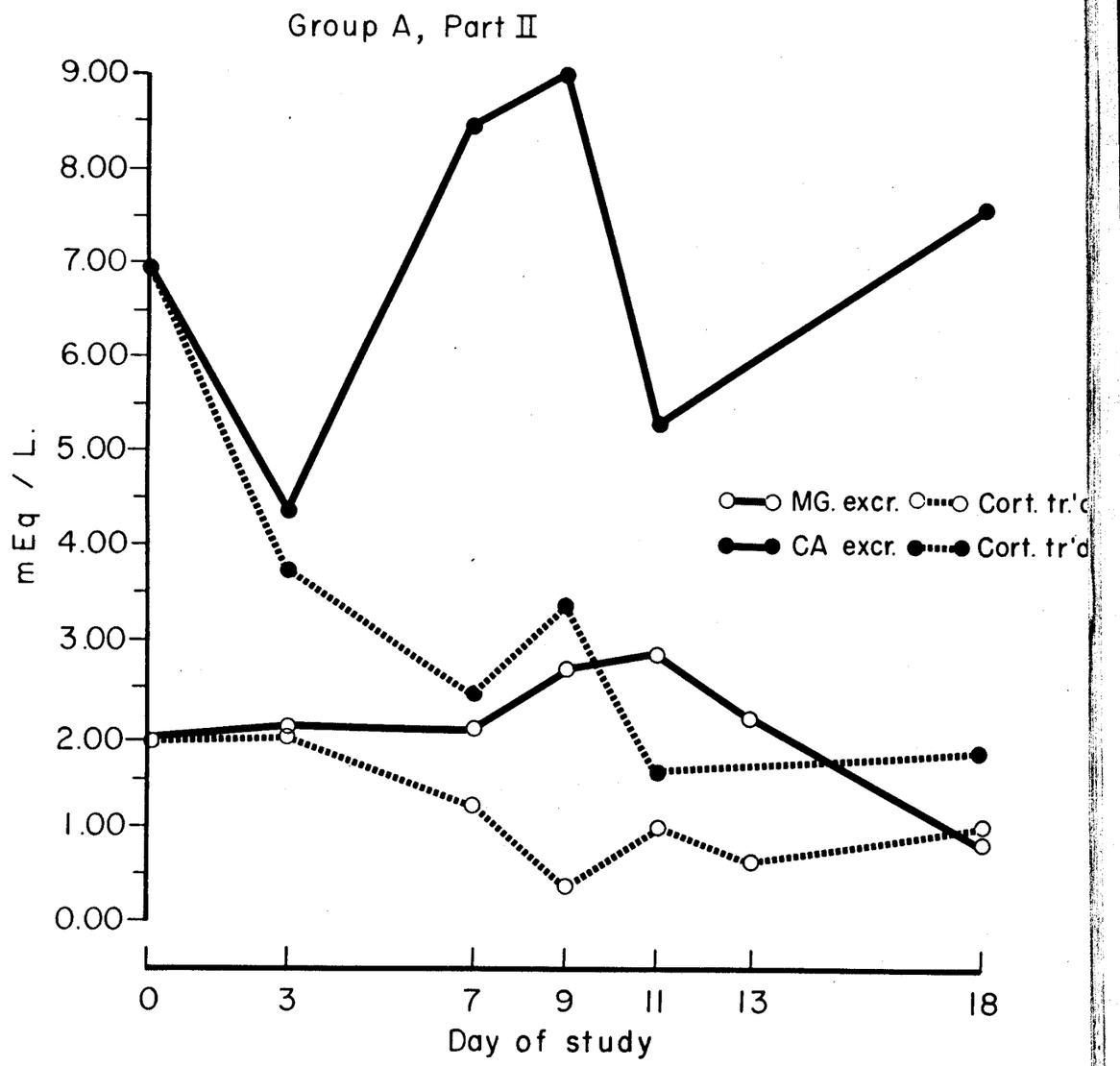

PLATE X

EXPLANATION OF FIGURES

10 A low power view of a longitudinal section of a kidney in a rat of group A showing the cortical calcium salt deposition. X 10.

11 A low power view of a longitudinal section of kidney in a rat of Group B showing the cortical and extensive cortico-medullary calcium salt deposits. X 10.

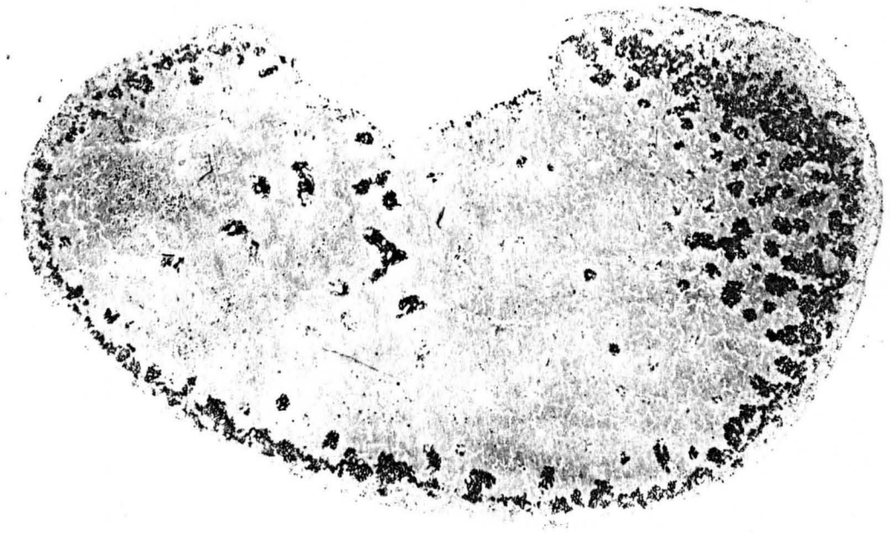

FIGURE 10

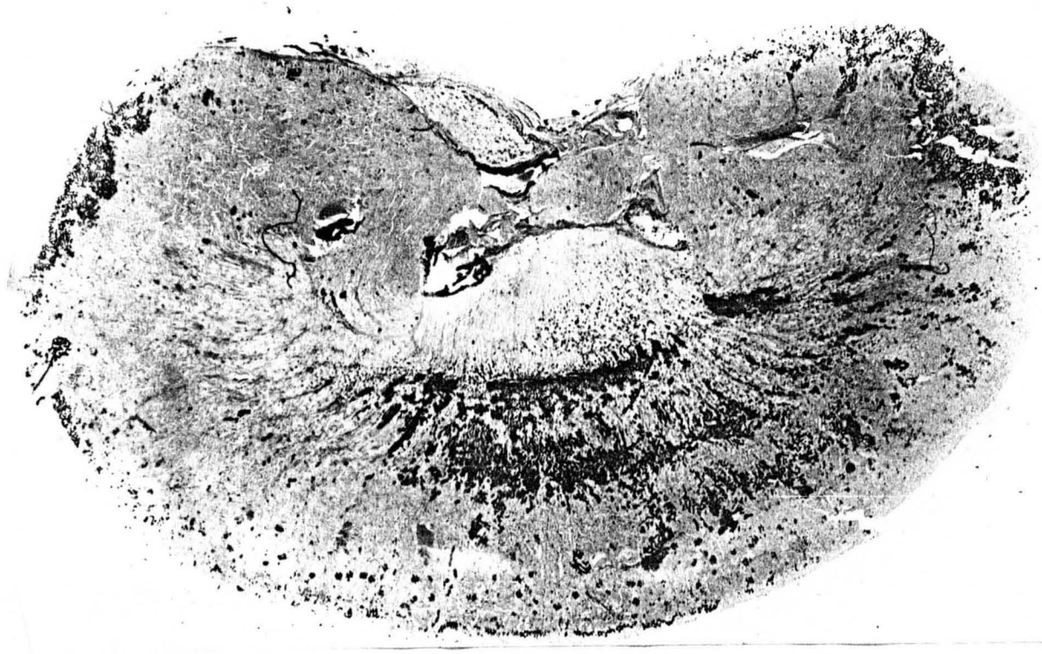

FIGURE 11

PLATE XI

EXPLANATION OF FIGURES

12 A low power view of a longitudinal section of kidney in a rat of Group C showing the cortical, cortico-medullary and extensive papillary calcium salt deposits. X 10.

13 A high power view of renal cortex from a rat of Group B showing the calcium salt deposits outlining the glomeruli and peripheral deposits around the proximal and distal convoluted tubules. X 230.

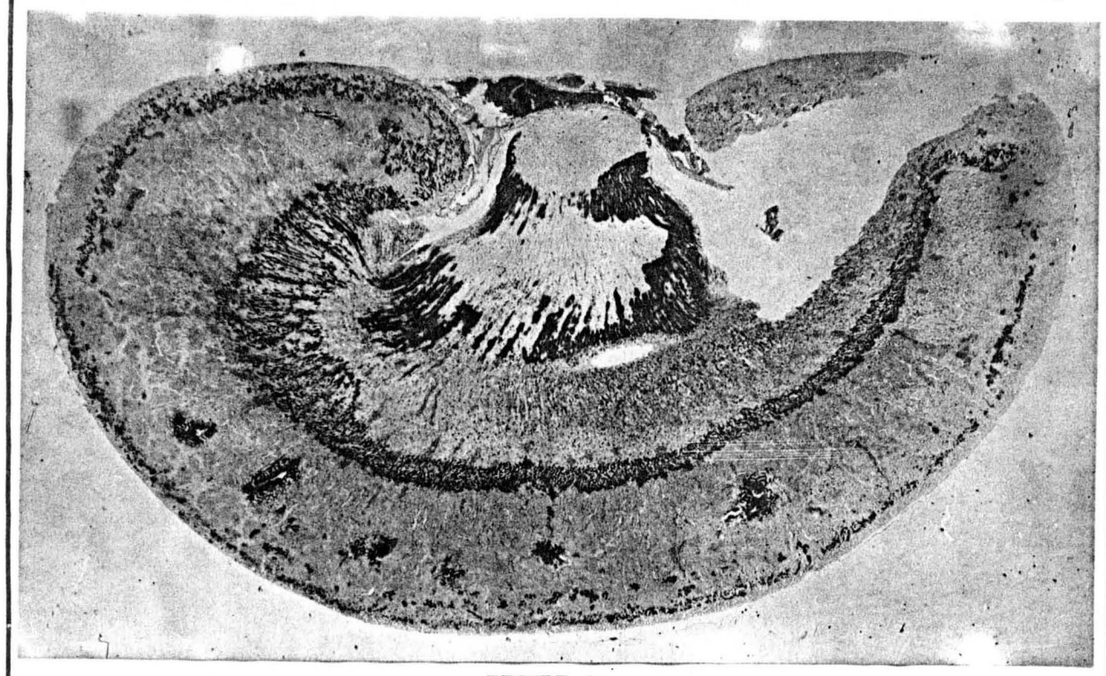

FIGURE 12

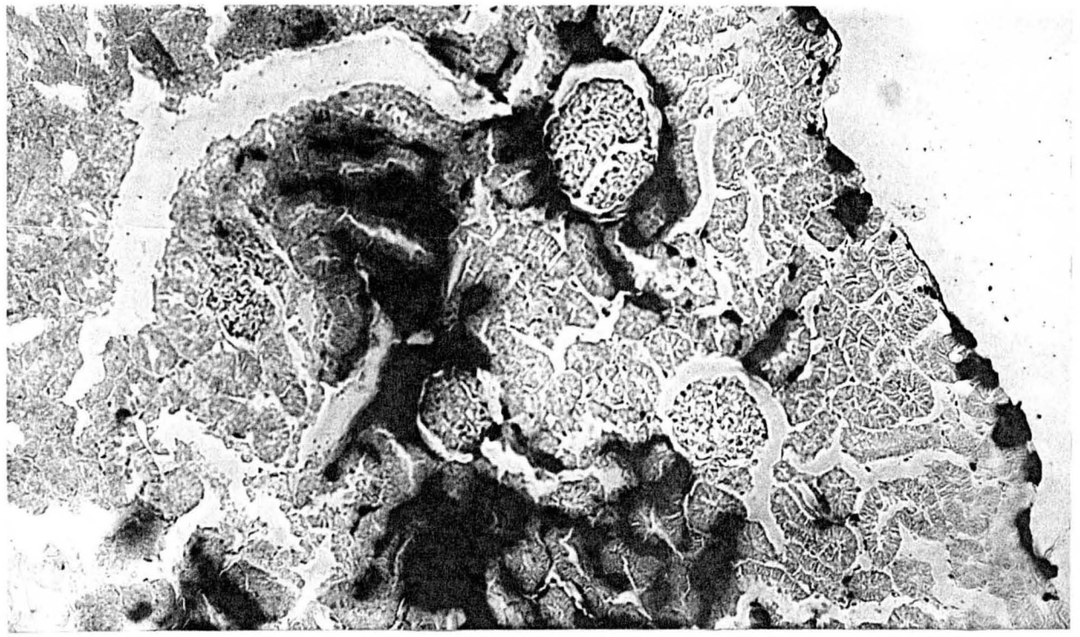

FIGURE 13

PLATE XII

EXPLANATION OF FIGURES

14 A high power view of the renal cortex in a rat of Group C showing the peripheral calcium salt deposition outlining the proximal and distal convoluted tubules. X 230.

15 A high power view of the renal medulla in a rat of Group B showing calcium salt deposition in the collecting tubules. X 230.

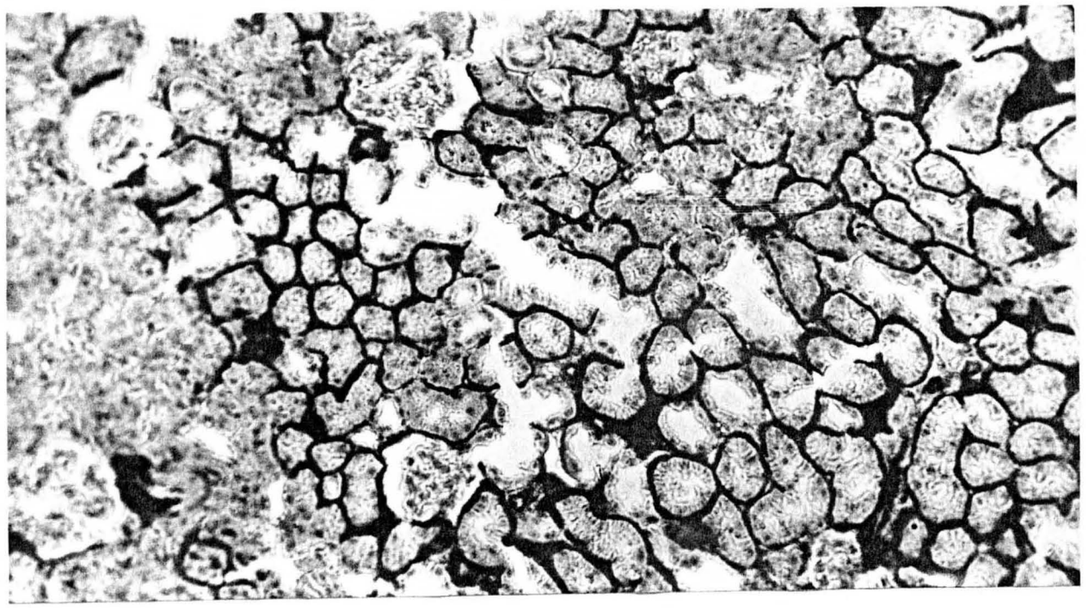

FIGURE 14

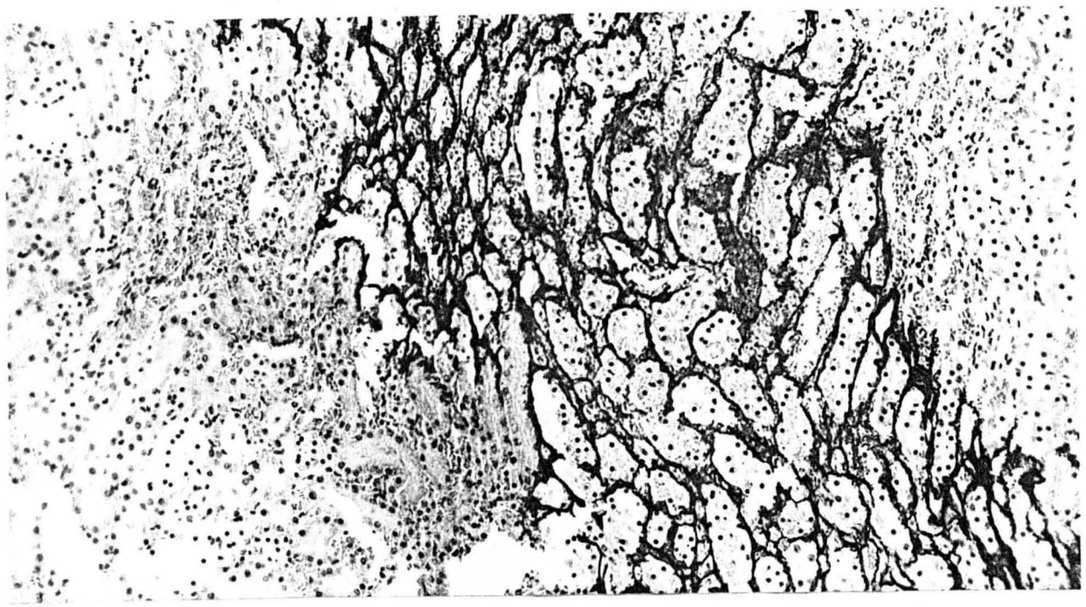

FIGURE 15

PLATE XIII

EXPLANATION OF FIGURES

16 A color photograph of a rat from Group A showing the matted non-glossy coat which resulted from maintenance or a magnesium deficient diet.

17 A color photograph of a rat from Group A showing the lesions of scaly dermatitis resulting from a lack of the minimum dietary magnesium content of the diet.

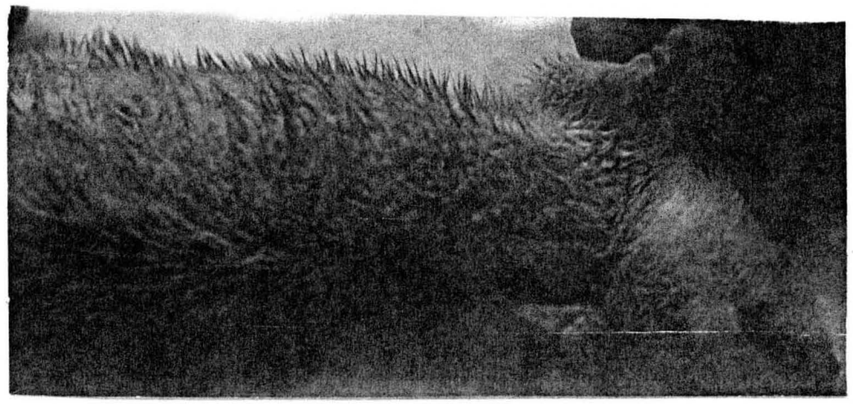

FIGURE 16

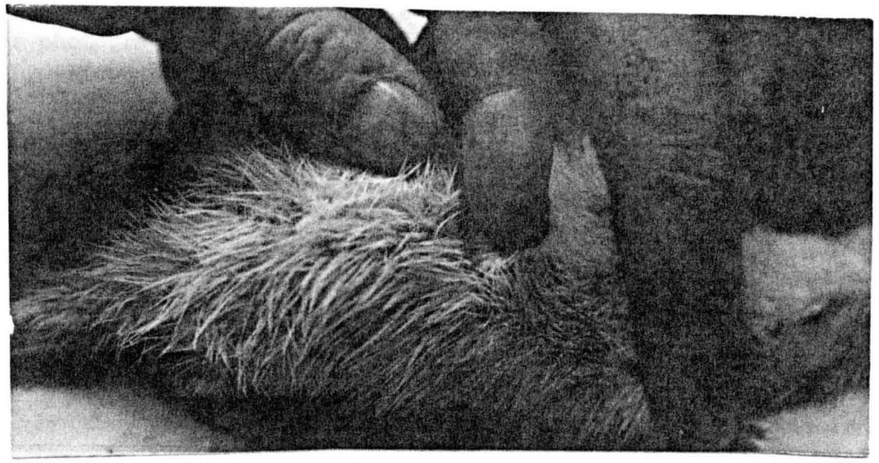

FIGURE 17

PLATE XIV

EXPLANATION OF FIGURES

18 A color photograph of a rat of Group A showing one of the earliest signs of Mg-deficiency. Note the vasodilatation of the ears, foot pads, and conjuctivitis.

19 A color photograph showing the grossly visible particles of Mg-triplephosphate which have settled to the bottom of the test tube. Urine specimen from a rat of Group C.

FIGURE 18

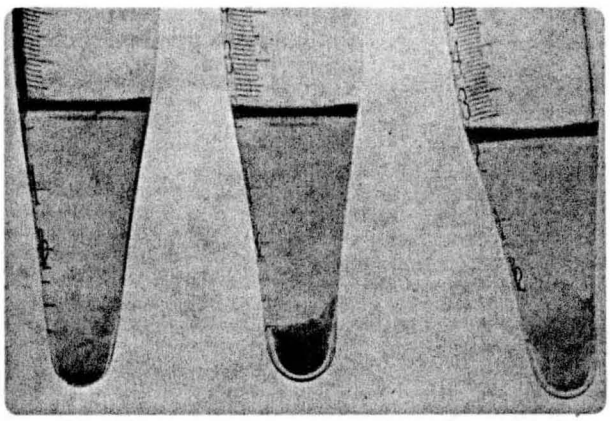

FIGURE 19

APPROVAL SHEET

The thesis submitted by John Harry Fournier has been read and approved by four members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 29, 1967
Date

Signature of Advisor