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STUDY ON THE MANIFESTATIONS OF MAGNESIUM DEFICIENCY IN ALBINO RATS

Thesis presented to the Faculty of Arts of the University of Ottawa through the Faculty of Medecine as partial fulfillment of the requirements for the degree of Master of Arts.

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CHAPTER I

INTRODUCTION

SURVEY OF THE LITERATURE

Magnesium deficiency has been a problem interesting research workers for many years[1]. Thus the literature records many phenomena due to this dietary deficiency; but few theories have been advenced to explain them.

The physical and chemical properties of magnesium are well known; but less is known of its metabolism. Magnesium is found in meat, cereals, other plant foods and milk (Stearns '50). Its salts have much in common with the salts of calcium: they are similar in solubility; and probably the same factors govern the absorption of both (Duckworth and Warnock '42-43). Like phosphorus, it is a component of soft tissues as well as of bone; and, with potassium, it furnishes the cations of the intracellular fluid. Magnesium is also a

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1. From Mendel and Osborne in 1917-18, and Leroy in 1926, reports on magnesium deficiency have been published in 1931 (McCollum and Orent, Lavollay), '32 (Kruse et al, Lavollay), '34 (Brookfield), '37 (Watchorn & McCance), '38 (Greenberg et al, Tufts & Greenberg), '41 (Becks & Furuta), '42 (Becks & Furuta, Duckworth and Warnock,) '43 (Duckworth and Godden), '44 (Miller), '46 (Patton & Lazovik), '47 (Lazovik & Patton, Patton), '48 (Barron et al, Bird, Roine et al), '50 (Lowenhaupt et al, Cosla), with the most recent work being that of Dick and Prior in '51.

component of some members of the B group vitamins; and is essential to catalyze certain enzyme reactions, such as that of phosphatase (Stearns '50).

Pearson ('48), Roine et al ('49), Scott et al ('50), Colby and Cheridah ('51), and Stoner ('50) have studied magnesium simultaneously with calcium, phosphorus and potassium, seeking to find the relationship between these important elements.

Coleman et al ('50) produced severe rickets in growing rats with diets very low in phosphorus (.005 to .015 %). Lesions in the kidneys comparable to those from magnesium deprivation were obtained by MacKay and Oliver ('35) with diets excessive in inorganic phosphate. Smith et al ('50) investigated the effects of potassium deficiency and observed necrosis of the myocardium and dilatation of the renal loops of Henle.

These findings when compared with those of magnesium deficiency establish the very close relationship of these elements; but as yet no complete picture is available.

The aspect most thoroughly investigated is that of the symptoms caused by magnesium deficiency. Most authors agree on the general effects produced, i.e. generalized vasodilatation, hyperirritability and nervousness ultimately leading to convulsions and death. These manifestations vary in severity with the starting age of the animals under experiment, the degree of magnesium deficiency, the length of the experimental period

and the dietary level of calcium and riboflavin (Greenberg et al '38). These factors affect all disturbances caused by magnesium deficiency.

Since most research investigators approached the problem from different angles, descriptions of various phenomena are recorded; these can widely be classified as manifestations involving the skin, the nervous system, the bones and teeth, the viscera, and the reproductive system.

THE SKIN:

The generalized vasodilatation is evidenced by hyperemia of the skin. Watchorn and McCance ('37) have noticed loss of hair in some cases and Barron et al ('48) described lesions of the skin "characterized by erythema, purpural hemorrages and eschars". There was recovery from the skin lesions with the addition of magnesium to the diet.

THE NERVOUS SYSTEM:

Three research reports mention magnesium tetany (Stearns '50, Kruse et al '32, and Greenberg and Tufts '38).

Stearns ('50) states: "Although the amount of magnesium in blood plasma is normally small, 1-3 mg per 100 ml., tetany has been observed in young animals and is reported to have occured in a child (Miller '44)."

Kruse et al ('32); and Greenberg and Tufts ('38) throw a little more light on this condition by establishing that the

syndrome produced by magnesium deficiency constitutes tetany, and differentiating it from other known types. Moreover, Greenberg and Tufts ('38) localized the lesion in the pons or the mid brain.

Barron et al ('49) saw chromatolysis and degeneration of the cells of Purkinje of the cerebellum in rabbits and rats. Bird ('49) working on chickens described swelling, tigrolysis, nuclear alterations and failure of the Purkinje cell dendrites to stain.

THE BONES AND TEETH:

The factors regulating the deposition of magnesium in bones and its withdrawal have been most thoroughly studied by Duckworth and Godden ('43). According to these investigators the skeletal magnesium is highly labile and forms a reserve easily drawn on by soft tissues which appear to have priority. The return of magnesium to bone after depletion is a slower process than its removal.

Deficiency of magnesium disturbs calcium metabolism; in calcium deficiency, when magnesium is available, magnesium can replace the calcium of bone to a limited extent. Watchorn and McCance ('37) noted that rats on diets containing magnesium four milligrams per hundred grams had brittle bones and that their teeth were sometimes white and translucent. The brittleness in the long bones was most marked near the epiphyses;

the bones examined were normal in length but purplish red in colour. The incisors of albino rats were brittle and rather lose in their sockets and occasionally they were chalk-white. The molars were outwardly normal.

Dick and Prior ('51) upon histologic examination of the long bones and joints, noted atrophy of the bone with replacement by fibrous tissue and deficient formation of bone and cartilage. The magnesium deficient animals toward the latter part of the experiment were noticeably smaller than their controls.

Becks and Furuta ('42) studied the condition of the teeth when giving a diet containing thirteen parts of magnesium per million parts of food. They reported histopathologic changes in dentin and pulp which increased in severity with the duration of the experiment. These changes were more pronounced in the constantly growing anterior teeth than in the molars and consisted mostly of degeneration and calcification. They had previously concluded (Becks and Furuta '41) that magnesium is an essential mineral for enamel formation in rats.

THE VISCERA:

Lowenhaupt et al ('50) described an inflammatory lesion of extremely general distribution, involving loose connective tissues in perivascular locations in magnesium deficient rats. This lesion occured within two weeks in the young rats. In the early acute stage, it is evidenced by a collec-

tion of inflammatory cells; it progresses to necrosis and to scar formation if the animal survives. In addition, obstructive casts of calcium salts are found late in the deficiency, in the distal portions of convoluted and collecting renal tubules.

The only reference to liver changes that could be found in the literature, is the mention by Brookfield ('34) of degenerative changes which involved the Kupffer cells in the liver of rats. This finding was not confirmed by Greenberg et al ('38).

Degenerative changes in the kidneys have been reported by many. According to Greenberg et al ('38) the process is similar to that found in nephrosis. In the earlier stages degenerative changes in the tubules is a more constant finding than is calcification; which is later found in the corticomedullary zone, in the pyramids, and, at a still later stage, in the cortex. Greenberg et al ('38) mention also that Cramer ('32) found marked changes in the kidneys of rats: extensive degeneration of the tubules and glomeruli most marked at the junction of medulla and cortex, where there were necrosis and calcareous deposits in the tubules and glomeruli. But Cramer ('32) also found calcification of the controls which was attributed to excess of vitamin D.

Brookfield ('34) saw similar changes in the kidneys. Watchorn and McCance ('37) mention that male rats were affected more than females after ninety days on the deficient diet. The subcutaneous and abdominal fat was diminished; and there were

small hemorrhages in the bladder and stomach of some males. The appearance of the kidneys was variable, some practically normal, others grossly abnormal. In two males kidney stones were present; and there were stones in the bladder of three males. More recently, Dick and Prior ('51) described experiments on albino rats in which the deficiency of magnesium produced in the kidney calcification without inflammation of the lower nephron, occuring particularly at the cortico-medullary junction. This was always present in their experimental animals by as early as nine days, and always absent in each of their controls.

THE REPRODUCTIVE SYSTEM:

An interesting point is brought up by Tufts & Greenberg ('38) and investigated by Cosla ('50), i.e.: that magnesium deficiency causes certain disturbances in pregnancy. Tufts and Greenberg ('38) in their investigation on the dietary level of magnesium required in rats, noticed that rats which on a diet containing 5mg/100 g. did not get convulsions, became hyperexcitable when subjected to the added stress of pregnancy and lactation. Rats about 80-120 days old, were bred successfully on this diet; they gave birth to youngs normal in weight and magnesium content. But as most of the mothers either refused to nurse their youngs; and let them starve, or ate them; only a few survived for a month or more.

It was found that these youngs developed magnesium deficiency during lactation. From this fact the authors concluded that the mothers' milk was deficient in magnesium.

Cosla ('50) on the other hand states that magnesium is an element indispensable for conception and progression of normal pregnancy of rats. He observed that total absence of magnesium in the ration of the animal caused an increase in the frequency of sterility; or in the case of pregnancy, lack of normal progression with resorptive abortion. The females which received one-fifth of the normal requirement of magnesium could conceive and go to term; but delivered offsprings which were born dead or died shortly after birth. The percentage of sterility was increased. Females which were for an interval of time submitted to a ration deficient in magnesium, and then were placed on a normal ration again, had normal pregnancies and delivered normal offsprings. Cosla ('50) attributes these disturbances in pregnancy to a direct relationship between magnesium ion and sex hormone activity.

Thus as Kruse et al ('32) concluded, it has been established that magnesium is an essential element for certain bodily activities, growth and life.

PURPOSE OF EXPERIMENTS

From the survey of the literature, it is evident that magnesium, even though a trace element in the body, plays a

very important role in the functions of life. We know some of the manifestations of magnesium deficiency but we are far from understanding the whole import of its role.

It seems that two aspects of investigation thus far did not receive adequate attention, namely:

1. Very few observations describing histological changes in magnesium deficiency have been recorded. 2. Only two reports concerning effects on the reproductive system have been found (Tufts and Greenberg '38 and Cosla '50).

Thus it has seemed advisable to repeat and extend investigation along these lines, in the hope of contributing to their better understanding, and perhaps of suggesting an explanation for the general manifestations of magnesium deficiency.

CHAPTER II

MATERIAL AND METHODS

Most of the work done on magnesium deficiency has been conducted on rats. Since these animals are very adept to experimentation, and easy to manage and since they were available to these laboratories, they were chosen for the present investigations. Forty-four albino rats were used in the course of these experiments.

DIET

The diet used throughout was the same as that used by Dick and Prior ('51).

Vitamin-free casein	15.
Cocoanut oil	2.
Wesson oil[1]	2.
Sucrose	77.
Philips & Hart salt mixture[2]	4.

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1. Since Wesson oil is not sold in this country, it was replaced by Mazola oil, the Canadian equivalent.

2. PHILIPS & HART SALT MIXTURE:

NaCl	335.	KI	1.6
K_2HPO_4	645.	$MnSO_4 \cdot 4H_2O$	3.5
$CaHPO_4$	150.	$ZnCl_2$	0.5
$CaCO_3$	600.	$CuSO_4 \cdot 5H_2O$	0.6
Fe citrate	55.	$MgSO_4 \cdot 7H_2O$	204.

Thiamin chloride.....	0.0003
Riboflavin.....	0.0004
Pyridoxine hydrochloride.....	0.0003
Calcium pantothenate.....	0.0010
Nicotinic acid.....	0.0010
Choline.....	0.1000
Inositol.....	0.0375
Para-amino benzoic acid.....	0.0375
Vitamin A.....	100. units
Vitamin D.....	100. units
Vitamin E.....	0.001
Vitamin K.....	0.001

In the magnesium deficient diet, the $MgSO_4 \cdot 7H_2O$ was replaced by sucrose. Distilled water was fed to all rats ad libitum.

This diet contains not more than 0.65 per cent (16.3 mEq/L.) calcium and 0.47 per cent (15 mEq/L.) phosphorus (Dick and Prior '51); which is adequate for growth, but less than that required for production of metastatic calcification (Barnes '42, Greenberg et al '38). The control diet contains 0.0404 per cent magnesium.

NUMBER OF ANIMALS

Five series of experiments were completed.

SERIES I:

This first series was run to verify and adjust the experimental conditions. Ten young albino rats were divided into two groups; three males and two females received the magnesium deficient diet, three males and two females received the control diet (Table 1). One male on deficient diet died after twenty-three days, a control was sacrificed at the same date. The remaining animals were killed after twenty-eight days of experimentation.

The results proved that the experimental conditions were adequate, since the physiological and histological manifestations were essentially the same as those described by Dick and Prior ('51).

TABLE I

DATA ON ANIMALS OF SERIES I

INFORMATION	CONTROL	DEFICIENT
Animal No.	441-443-444-445-446	442-447-448-449-450
No. of males	3	3
No. of females	2	2
Initial weights in gms.	88- 88- 76- 56- 68	72- 72- 98- 68- 64
Av. initial wt.	75.2 g	74.8 g
Final weights in gms.	200-148-188-155	94- 78-162-102
Av. final wt.	172.7 g	109 g
Approx. No. slides	50	50
Tissues studied	kidney and liver	kidney and liver

SERIES II:

Series II was carried out on nine possibly pregnant females; these animals having been in contact with males for fifteen days prior to the beginning of the feeding experiment. Three of these were put on control diet for twenty-five days, i.e. for the whole length of the experiment; the other six were put on the diet deficient in magnesium at different intervals, and for varying lengths of time during the experimental period (Table II). When not on the deficient diet they were fed the control diet.

One of the control animals had an abnormal pregnancy and was sacrificed after giving birth to eleven dead offsprings. This animal is disregarded in the results of these experiments.

One control and five deficient animals were pregnant. The control gave birth to eleven youngs; the embryos of the deficient group varied in number and physical condition, in proportion to the degree of magnesium deficiency of the mothers.

Due to transfer to another department for laboratory facilities, some of the microscopic slides of the first and second series are not available now. Of the first series, general observations and weight charts are supplied; of the second series experimental data and microscopic slides of adult tissues are available (Table II).

TABLE II

DATA ON ANIMALS OF SERIES II

Animal No.	No. days on each diet	Initial wt.	Final wt.	Pregnant	No. embryos	No. slides	Tissues examined
459	25d	177g	182g	?	__	22	kidney-liver
460	15d-10c	204g	220g	no	__	27	kidney-liver-uterus
461	5c-20d	204g	175g	yes	8	30	kidney-liver-uterus
462	5c-15d-5c	173g	228g	yes	2	26	kidney-liver-uterus
463	10c-15d	146g	173g	yes	9	20	kidney-liver-uterus
464	10c-15d	204g	200g	yes	4	25	kidney-liver-uterus
466	25c	193g	180g	yes	11	31	kidney-liver-uterus
467	25c	144g	200g	no	__	28	kidney-liver

Total number of slides: 209

c: control of diet
d: deficient diet

465 had an abnormal pregnancy and was sacrificed before the end of the experiment.

SERIES III:

Six females were put on diet after a period of fifteen days of contact with males. Two were chosen as controls, the other four were put on a feeding plan quite similar to that of the deficient animals of series II (Table III). One animal died four days after being put on deficient diet; the others were sacrificed after an experimental period of twenty-five days. Two were pregnant, one on control diet, the other on the magnesium deficient diet; the embryos were taken at birth, fixed and prepared for microscopic examination.

TABLE III

DATA ON ANIMALS OF SERIES III

Animal No.	No. days on each diet	Initial wt.	Final wt.	Pregnant	No. slides	Tissues examined
468	25d	222g	212g	no		
469	15d-10c	256g	252g	yes	37	transverse cross sections of embryos
470	5c-20d	228g	220g	no		
472	25c	230g	202g	yes	39	transverse cross sections of embryos
473	25c	212g	232g	no		

Total number of slides: 76

c: control diet
d: deficient diet

471 died four days after being put on the magnesium deficient diet.

From these series, it was concluded that for best results, diet totally deficient in magnesium should be fed over the entire period of experimentation; and that the best method to obtain pregnant females, was to put healthy mature females in contact with males for a period of fifteen days, thus covering two ovarian cycles of the females but not a full gestation period.

The next two series were then conducted on possibly pregnant females; the weight of the animals was taken daily, and the mothers were killed at different intervals in an attempt to get the embryos at different stages of resorption.

SERIES IV:

This series comprised thirteen animals; three of these were fed the control diet, the ten others, the deficient diet. One of the control animals which showed definite signs of pregnancy was sacrificed after thirteen days of experimentation, the deficient mothers were sacrificed when their weight, after increasing rapidly, showed a sudden drop; suggesting possible resorption. Five of the deficient animals showed evidence of possible pregnancy on gross postmortem examination, two more were found to be pregnant by histological examination. For data on the animals of this series, see Table IV.

TABLE IV

DATA ON ANIMALS OF SERIES IV

Animal No.	No. days on each diet	Initial wt.	Final wt.	Pregnant	No. slides	Tissues examined
474	17d	196g	188g	yes	29	ovary-tubes and embryonic contents
475	9d	182g	186g	no	12	uterine tubes
476	5d	164g	144g	yes	35	uterine tubes and embryonic contents
477	13d	180g	174g	yes	8	ovary-tubes and embryohic contents
478	16d	160g	178g	yes	8	ovary-tubes and embryonic contents
479	15d	206g	202g	no	9	tubes
480	13d	194g	178g	yes	38	tubes and embryos
481	12d	204g	198g	yes	68	tubes and embryonic contents
482	14d	192g	173g	yes	23	tubes and embryonic contents
483	14d	192g	174g	no	15	tubes
484	16c	164g	184g	no	16	ovary and tubes
485	16c	170g	198g	no	16	tubes
486	14c	200g	244g	yes	67	tubes and embryos

Total number of slides: 342--c: control diet--d: deficient diet

SERIES V:

Six females were put with males; of these, only the ones showing signs of possible pregnancy: enlargement of the mammary glands and increase in weight, were put on diet. There were three of them, two of which were put on the deficient diet, the other being put on the control diet. The control animal gave birth to normal youngs; and embryos were found in the uterine tubes of one of the deficient animals (Table V).

TABLE V

DATA ON ANIMALS OF SERIES V

Animal No.	No. days on each diet	Initial wt.	Final wt.	Pregnant	No. slides	Tissues examined
488	8d	182g	144g	no	17	uterine tubes-ovary
491	12d	198g	228g	yes	12	ovary, tubes and embryos
492	12c	204g	216g	yes	16	ovary, tubes and embryos

Total number of slides: 45

c: control diet
d: deficient diet

487-489-490 were judged not to be pregnant and were discarded.

WEIGHT CHARTS

Weight charts, showing the curves of weight variations over certain periods of time, were prepared from the recorded data and are included in Chapter III.

HISTOLOGICAL TECHNIQUES

The animals were killed with ether, tissues of organs specified in the previous tables with each series, were fixed in Bouin's solution, imbedded in paraffin, cut from 6-10 micra thick, stained with haematoxylin, phloxine and orange G (H.P.O.) and mounted with Permount.

Approximately eight hundred slides were prepared. Examination of the slides was made with a Spencer microscope x100, x440, x950.

CHAPTER III

OBSERVATIONS

SERIES I (see Table I p. 19):

GENERAL OBSERVATIONS

The animals on magnesium deficient diet showed hyperemia and nervousness early in the experiment; their ears and tail were of a markedly darker red than the corresponding parts of the control animals. Convulsive-like movements were elicited later when the deficient animals were handled to be weighed. One deficient animal (number 442) died in convulsive seizure after twenty-two days on deficient diet.

The control animals ate regularly and showed normal behaviour throughout the experiment.

WEIGHT OBSERVATIONS

The weight curves of the deficient and control animals are comparable to the weight chart presented by Dick and Prior ('51) (see comparison in Chart 1).

HISTOLOGICAL OBSERVATIONS

The microscopic slides of the animals of this series not being available presently, let it only be mentioned that upon histological study, degenerative changes in the kidneys and the liver of the deficient animals were found; the changes being cloudy swelling, calcification and vacuolization.

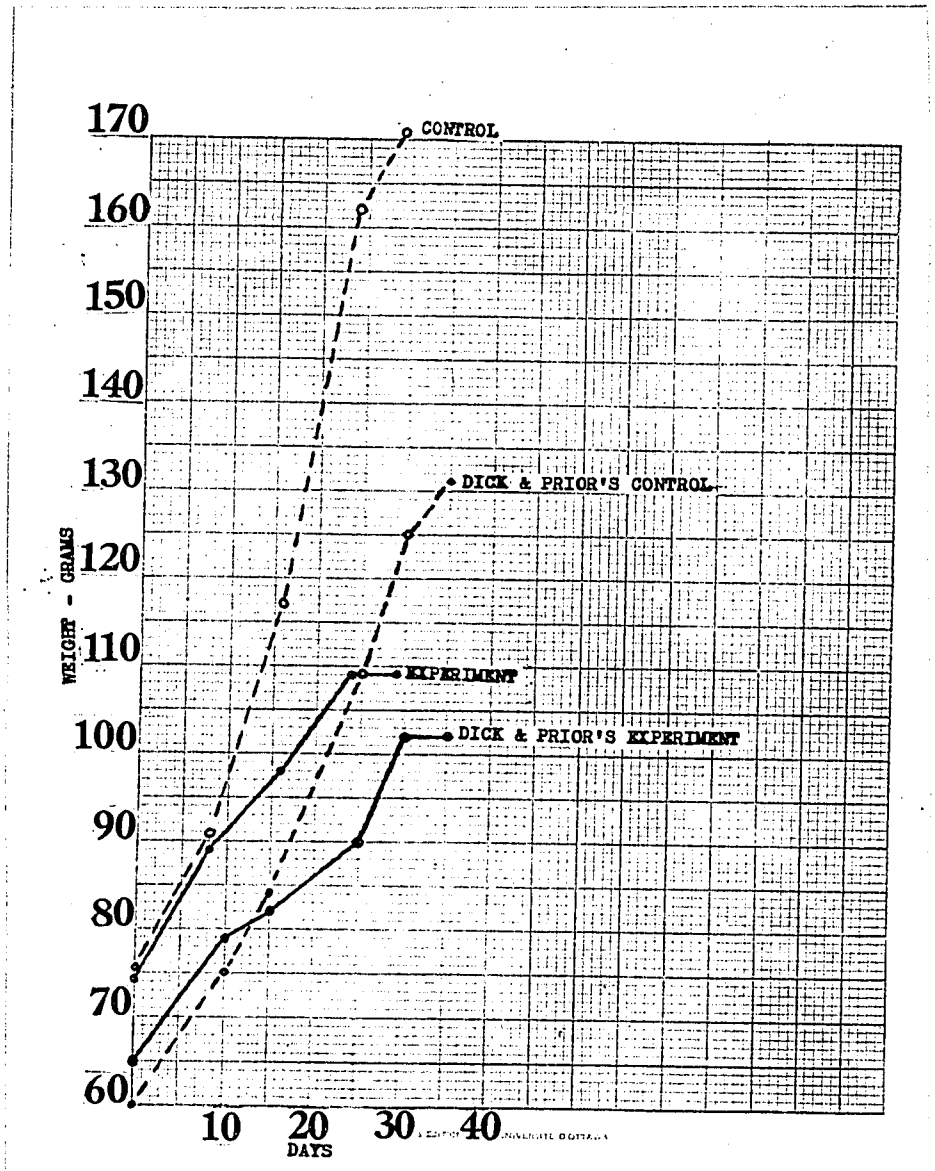

CHART 1: comparison of weight curves of Series I experiments on magnesium deficiency, with the results of Dick and Prior ('51).

SERIES II (see Table II, p. 21):

GENERAL OBSERVATIONS

Deficient animals

Animal number 459: This animal while with the males gained weight rapidly and underwent enlargement of the mammary glands, thus at the beginning of the feeding experiment it was judged to be pregnant. However, it gave birth to no young. The usual symptoms of generalized vasodilatation, nervousness, irritability and loss of weight were seen; no convulsion was witnessed. On gross post-mortem examination, the uterus appeared to be congested but there was no evidence of pregnancy.

Animal number 460: This animal never appeared to be pregnant; and never gave birth to any young. The usual symptoms of generalized vasodilatation, nervousness, irritability and loss of weight were apparent as long as the animal was on the deficient diet. As soon as the control diet was started, the animal gained weight rapidly, a small necrotic spot on one ear gradually healed and disappeared; but the nervousness remained, although slightly diminished, until the end of the experiment. On gross post-mortem examination, there was intense congestion of the uterine arteries, the ovaries were hemorrhagic, and there was no evident discoloration or caries of the teeth.

Animal number 461: After five days on control diet and seven days on the deficient diet, this animal gave birth to eight youngs. The total weight of the litter was 38 grams. Two youngs of an average weight of 4.5g were sacrificed immediately; two others died on the same day; another died two days later; another one disappeared, probably eaten by the mother. The seventh one was sacrificed after a week, its weight being 6 grams. The last one was allowed to live until the end of the experiment, when it was sacrificed, at the thirteenth day of age, its weight then being 14.7 grams.

Animal number 462: After five days on control diet and twelve days on deficient diet, this animal gave birth to two youngs which apparently were born dead. Weights of these youngs were 3.4 grams and 4.4 grams. The mother suffered marked hemorrhage about the nares after nine days on the deficient diet.

Animal number 463: After ten days on the control diet and six days on the deficient diet, this animal gave birth to nine youngs the total weight of which was 38 grams. Three were sacrificed the same day, their average weight being 3.25 grams. Four died; after two, four, six and seven days respectively. One of the remaining two was apparently eaten by the mother and the last one, weighing 7.4 grams, was sacrificed when five days old. The sites of pregnancy were still

present in the uterus of the mother and were observed upon post-mortem examination.

Animal number 464: After twelve days on the magnesium deficient diet this animal gave birth to four youngs, two of which were alive and two of which were dead. The dead ones were fixed for histological examination; the other two were eaten by the mother. Upon post-mortem examination of the mother, six masses were seen in the right tube, none were seen in the left.

Control animals

Animal number 465: This animal underwent an abnormal pregnancy resulting in the birth of eleven dead offsprings. It is therefore disregarded in the results of these experiments.

Animal number 466: This animal gave birth to eleven youngs after being on the control diet for sixteen days. The total weight of the litter was 57 grams. Three were sacrificed on the same day, their average weight being 5.43 grams. Five days later two were sacrificed, their average weight being 9.2 grams. At the end of the experiment, nine days after birth, the total weight of the remaining ones was 75.6 grams, giving an average weight of 12.6 grams for those six.

Animal number 467: This animal had no youngs and progressed normally on the control diet.

WEIGHT OBSERVATIONS

The average weight of the youngs whose mother had been at some time on the magnesium deficient diet, was at birth, 3.7 grams, which is less than the average weight of the youngs whose mother had been on the control diet. These youngs weighing at birth an average of 5.4 grams.

The weight curves of the adult animals (Chart 2) show that the deficient animals did not gain weight as rapidly as the control animals, until the time of delivery. After delivery, the control animals continued to gain weight while the experimental animals lost weight.

As compared with Chart 1, which is of non-pregnant younger animals, Chart 2, shows approximately the same increase in weight for the controls, excluding the loss due to delivery. The deficient animals, show a similar gain; then a levelling of weight as seen in Chart 1; but after delivery, they continued to lose weight.

The changes in weight expressed in Chart 2 occured more rapidly than those seen in Chart 1; which may be due to the fact that many animals in Series II were pregnant.

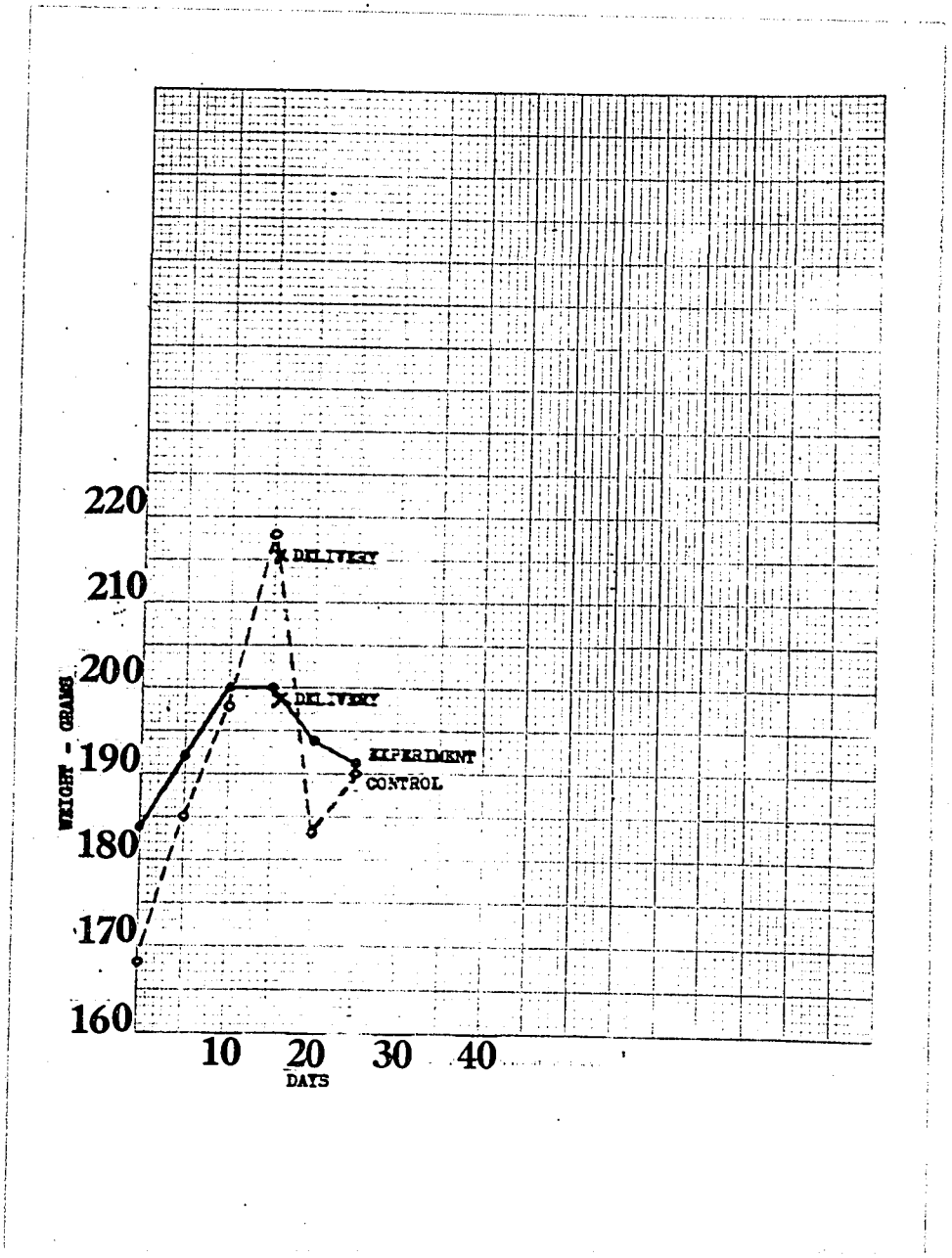

CHART 2: Average weight curves of the animals of Series II. "X" marks the average time of delivery.

HISTOLOGICAL OBSERVATIONS

Deficient animals

Animal number 459: The liver shows a beginning of distortion of its structure, the portal blood vessels are engorged with broken-down red blood cells; and there is a slight periportal fibroblastic infiltration. The parenchymal cells show cloudy swelling and swelling of nuclei. Some fibroblasts are seen throughout; and patches of cells show intracellular fatty infiltration.

Low power examination of the kidney shows small orange-staining masses in the cortex, and along the medullary rays; high power examination reveals these to be engorged blood vessels containing red blood cells in the process of breaking down. There is an accumulation of dark-staining nuclei near some of the glomeruli; there seems to be fibroblastic infiltration around the afferent arteriole, to such an extent as to cause compression of Bowman's capsule; causing a diminution of the capsular space on that side; or proliferation of the epithelium of the capsule on one side forming a crescent as occurs in glomerulomephritis. There seems also to be thickening of the endothelial wall of the glomerular tuft. The cortical arteries show a thickening of all their walls. In a few instances the lumina have actually been occluded by these thickenings, in which cases there is a minor amount of infiltration of fibroblasts about the vessels. In more

numerous instances the lumina have not been occluded, however, in these, there is a profuse periarterial infiltration of fibroblasts.

Animal number 460: There is engorgement of the blood vessels of the uterine tubes, with thickening of the walls of their blood vessels. Stasis in the arterioles, with breakdown of red blood cells is also observed.

The liver is hyperemic; the portal veins are enormously distented with old broken-down red blood cells. Some central veins are also engorged; and the sinusoids are filled with blood. Some vessels contain a pink-staining homogeneous material. Very few fibroblasts are noted in the portal spaces. Parenchymal cells show cloudy swelling, and swelling of the nuclei; some are breaking down, distorting the usual liver structure.

The kidneys show engorgement of the blood vessels in the cortex and at the cortico-medullary junction; but not as many as seen in sections taken from animal number 459. There is also some accumulation of fibroblasts, which here appear to be definitely a periarterial infiltration. There is a generalized distribution of fibroblasts in the cortex and in the medulla. Also in the medulla there are patches of pink-staining homogeneous material between the tubules.

Animal number 461: There is thickening of the walls

of the blood vessels of the uterine tubes. These blood vessels are very much dilated; and contain red and white blood cells.

An acute process is going on in the liver. The blood vessels are engorged; and most of the sinusoids are filled with broken red blood cells. The parenchymal cells are greatly degenerated and cellular debris, broken down nuclei and cytoplasm have replaced the liver cords. Very few fibroblasts are seen. Throughout the section there are areas where parenchymal cells are vacuolated, these areas occur very often close to some blood vessels, giving appearance of fatty infiltration.

Engorgement of numerous blood vessels of the cortex of the kidney is seen and there is a very pronounced infiltration of fibroblasts and round cells. This kidney presents a picture similar to that of the kidneys of all the other deficient animals, and for this reason a section from this animal was used for figure 1; which shows general swelling of the tubular cells with obliteration of all the lumina of the tubules; and accumulation of cells with dark staining nuclei near the glomeruli. There seems to be fibroblastic infiltration around the afferent arteriole, which, as described in the observations on the kidney of animal number 459, exerts compression on Bowman's capsule, causing a diminution of the capsular space. There seems to be also some thickening of the endothelial wall of the glomerular tufts.

Figure 1 can be compared to figure 2, which is of the kidney of the control animal number 466. Some degeneration of

the tubular epithelium is showing in figure 2. This is probably due to post-mortem changes but the lumina of most of the tubules are not occluded and the cell boundaries can be recognized. The glomerulus appears more compact, there is no accumulation of cells near it and the capsular space is present and regular.

Animal number 462: Engorgement and thickening of the walls of blood vessels of the uterine tubes are observed; many white blood cells are seen in the endometrium.

The parenchymal cells and nuclei are swollen, giving a very compact appearance to the liver structure. Fibroblasts and small cells with dark nuclei, most probably lymphocytes, are seen throughout the section.

There is some engorgement of the blood vessels in the cortex of the kidney; and moderate generalized infiltration of fibroblasts in the cortex and medulla. A slight increase in number of fibroblasts is noted about the afferent arteriole but it is difficult to demonstrate a definite periarteriolar infiltration at this point.

Animal number 463: There is engorgement of the blood vessels and thickening of the wall of the arterioles of the uterine tubes.

The liver structure is greatly distorted; the portal spaces are very obvious, because of the dilated portal veins.

These portal spaces are also infiltrated by a pink-staining vacuolated material, and some fibroblasts. The parenchymal cells exhibit cloudy swelling. Their nuclei are swollen and granular; and some have a great amount of fatty inclusions. Occasional fibroblasts and other small cells with dark nuclei may be noted between the parenchymal cells close to the portal spaces. Despite this disrupted architecture, the central veins may be made out in a few instances; and they are unremarkable, excepting for the collection about them of fibroblasts and small cells with dark nuclei.

Profuse engorgement of blood vessels in the cortex and medulla, as well as in the glomerular tufts, is seen in the kidney. There is a slight generalized infiltration of fibroblasts; but no apparent periarterial localization.

Animal number 464: Broken-down red blood cells are seen in the engorged blood vessels of the uterine tube.

In the liver, most blood vessels are engorged with old blood. There are many vacuolated cells, other parenchymal cells exhibit cloudy swelling to the point of obliterating most sinusoids thus greatly distorting the liver architecture. Fibroblasts and small cells with dark staining nuclei are seen throughout the section. This picture of the liver is characteristic and essentially the same as seen in previous deficient animals and therefore, was used for illustration as figure 3, which can be compared with figure 4.

The latter shows a section of liver taken from the control animal number 467, and it illustrates the appearance of a normal rat liver in which a blood vessel, parenchymal cells and sinusoids can be recognized.

There is some engorgement of blood vessels in the cortex of the kidney. In the medulla, there are small patches of blood in the tubules or engorgement of small blood vessels. There is generalized slight infiltration in the cortex, with dense patches; but no definite periarterial distribution.

Control animals

Animal number 466: A more even general distribution of blood is encountered in the uterine tubes, liver and kidneys as compared to the deficient animals.

The liver structure is intact and a microphotograph of the kidney was used as figure 2 to be compared with that of a deficient kidney. The glomeruli are more compact and there is no infiltration of fibroblasts or round cells. The tubular cells show some degeneration which is probably due to post-mortem changes.

Animal number 467: A section of this animal's liver was used in figure 4 as a control to be compared with the micro-photograph of the deficient animal's liver. Both liver and kidneys of this animal presented no abnormalities.

SERIES III (see Table III, p. 23)

GENERAL OBSERVATIONS

Deficient animals

As seen in previous series, the animals on the deficient diet showed vasodilatation, irritability, nervousness and loss of weight. In addition, animal number 468 had marked hyperpnea, discoloration of the fur around the neck and diarrhea.

After eight days on the magnesium deficient diet, animal number 469 gave birth to eight youngs, two of which were dead. The two dead youngs and a live one were taken immediately for histological preparation. The average weight of the youngs was 5.3 grams. Their skin was very dry and plicatured.

Upon gross post-mortem examination, the deficient adult animals were seen to have an increased amount of peritoneal fat as compared to the control animals.

Control animals

The controls behaved normally throughout the experiment. Animal number 472 gave birth to seven youngs, all of which were alive and of healthy appearance.

Two were taken to be prepared for microscopic examination. Their average weight was 5.5 grams.

WEIGHT OBSERVATIONS

The weight curves of the adult animals of this series as seen in Chart 3 show the characteristic gain of the control animal with a sudden drop at the time of delivery.

The experimental animal's curve shows an initial slight loss, then a sudden drop due to delivery followed by a gain, which is mostly due to the increase in weight of animal number 460. This animal seemed to recuperate somewhat after delivery. The following loss is due to animal number 468 and animal number 470; which at that time were markedly affected by the deficiency. The last gain is again due to animal number 469; which by then was on the control diet (see Table III, p. 23).

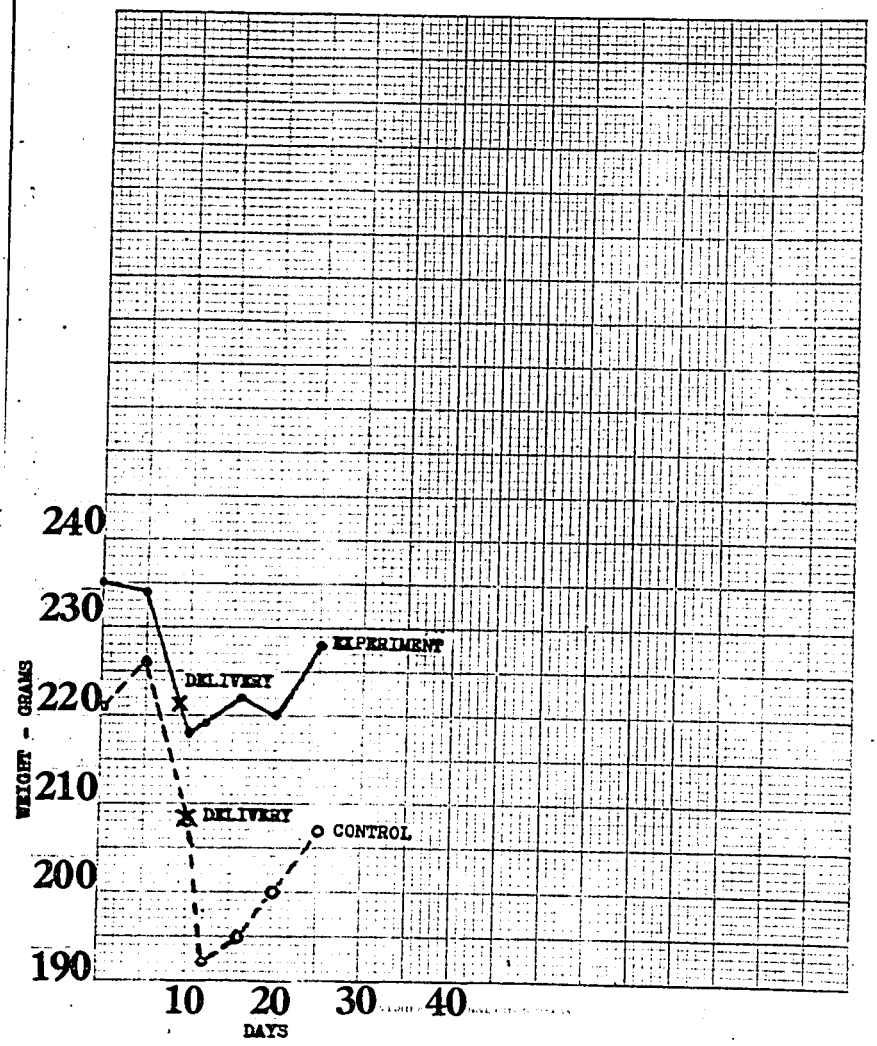

CHART 3: Average weight curves of the animals of Series III, "X" marks the time of delivery.

HISTOLOGICAL OBSERVATIONS

Transverse cross sections of two whole embryos were examined; one from deficient mother number 469, the other from control mother number 472.

Deficient embryo from animal number 469: The skin is highly keratinized, the epidermis is heaped into papillary-like projections (Figure 5) in contrast with the moderate keratinization and flattened epidermis of a control embryo (Figure 6).

There is calcification of the vertebra (Figure 7) and of some of the ribs. No such calcification is encountered in the control (Figure 8).

At the periphery of the heart muscle, there is an engorgement of the capillaries just underlying a thickened visceral pericardium overlying which is a hemorrhagic exudate (Figure 9). Embryo of a control animal (Figure 10) does not exhibit such changes.

The microscopic appearance of the lungs suggests a diminution in size of the alveoli; the inter alveolar septa being greatly thickened and irregular with break-down of the lining cells (Figure 11); these changes are obvious when compared with the lung of a control embryo (Figure 12). All the layers of the wall of the bronchioles are in a state of degeneration with fragmentation of the cells; the blood vessels are engorged with degenerating red blood cells and all the

layers of their walls are thickened by degenerating cells. There are free red blood cells in the pleural space.

The liver architecture is markedly altered, the organ being greatly congested. The sinusoids are intensely engorged causing distortion of the liver cords. The central veins are dilated and filled with blood cells which in some places invade the liver tissue. There is picnosis and karyolysis of the parenchymal cells. There is a generalized infiltration of white blood cells and macrophages in the organ (Figure 13). Figure 14 of control embryo from animal number 472 serves for comparison.

There is degeneration of the tubular epithelium of the kidneys with escape of the cytoplasm into the lumen.

Control embryo from animal number 472: The skin is keratinized (Figure 6) but less than that of the embryo of deficient animal number 469 (Figure 5). There is no calcification of the vertebra (Figure 8) and ribs; there is a more even distribution of blood to the heart wall (Figure 10) than there was in embryo of deficient animal number 469 (Figure 9). The lungs do not look so compact (Figure 12); the inter alveolar septa are thinner and the lining cells are intact. The walls of the bronchioles are also intact. The liver cells are organized into a normal liver architecture; there is no engorgement of the blood vessels and the parenchymal cells are not degenerating (Figure 14).

SERIES IV (see Table IV, p. 25):

GENERAL OBSERVATIONS

Deficient animals

Animal number 474: This animal was put on the magnesium deficient diet. Six days later signs of nervousness and hyperemia were present. It was sacrificed after being on the deficient diet for fifteen days. The abdomen contained a lot of soft fatty pinkish tissue; small dark masses were present in both tubes.

Animal number 475: At the beginning of the experiment, this animal weighed 182 grams and gave no evidence of pregnancy. Signs of hyperemia appeared five days later. This animal was sacrificed after being on the deficient diet for eight days; the abdominal muscles were in fair shape. There was no evident pregnancy but the uterine tubes were very congested, there were some small skin ulcerative lesions.

Animal number 476: At the beginning of the experiment this animal seemed to be in advanced pregnancy and weighed 164 grams; during the first days on the diet, she lost 18 grams, then 4 grams; then gained 6 grams and again lost 4 grams, making a total loss of 20 grams over a period of five days. At this point, the animal was sacrificed. The skin was thin and soft, the abdominal muscles were very soft as if in a preliminary

state of decomposition. In the abdomen, the intestines and tubes were all imbedded in a soft mass of pale fatty pinkish tissue, the uterine tubes were hard to find except on the animal's left side where there were two prominent masses.

Animal number 477: This animal was put on the magnesium deficient diet; it soon had diarrhea and five days after the beginning of the experiment, it showed nervousness and some hyperemia. Four days later, it had diarrhea again and was sacrificed after twelve days on the deficient diet. The tubes were small, there was little evidence of pregnancy on gross examination, but later, histological examination revealed the presence of pregnancy. Some soft fatty pinkish tissue was in the abdomen.

Animal number 478: This animal was put on the magnesium deficient diet; five days later, there were signs of nervousness and hyperemia, eleven days later, this animal was sacrificed having been on the deficient diet for sixteen days. Some fatty pinkish tissue was present in the abdomen and very small masses were seen in both tubes.

Animal number 479: This animal was put on the magnesium deficient diet and soon afterwards had diarrhea and hyperemic congestion of the eyelids. Five days after the beginning of the experiment, there was marked hyperemia and

an ulcerative lesion of the skin behind the ear; four days later, the palpebral lesions were markedly aggravated, and the animal was sacrificed six days afterwards. She was in a debilitated state, there were lesions of the skin around the neck, the nose was running, the eyelids were red and scally, the muscles were very soft, the uterine tubes were very small and there was no evidence of pregnancy.

Animal number 480: This animal also showed nervousness and hyperemia five days after being put on the deficient diet; it was sacrificed twelve days after the beginning of the experiment having lost sixteen grams. The tubes were congested and contained developing embryos .

Animal number 481: This animal was put on the magnesium deficient diet and showed some hyperemia and was excitable five days later; she was sacrificed after eleven days on the deficient diet and some small masses were seen in the uterine tubes.

Animal number 482: This animal was put on the deficient diet and showed nervousness and hyperemia five days later; on the eleventh day of the experiment, she bled on the food dish. This animal lost weight during the first four days because of lack of water, the pipette being defective. She was sacrificed after thirteen days on the deficient diet; the skin was tough, the tubes congested, three small separate masses were in the left tube, no inclusions were evident on the right side.

Animal number 483: This animal was put on the magnesium deficient diet and showed nervousness and hyperemia after five days; on the sixth day, the skin of the end of the tail was ripped off when trying to catch the animal for weighing, the rat ate the uncovered part of the tail. This animal was sacrificed after thirteen days of experimental feeding. There was a moderate amount of fatty soft pinkish tissue in the abdomen, there were no evidence of pregnancy and no gross abnormality.

Control animals

Animal number 484: This animal was put on the control diet and progressed normally. She was sacrificed after fifteen days; there was no evidence of pregnancy and the tissues were normal.

Animal number 485: This animal was put on the control diet and sacrificed fifteen days later. The tissues were normal, the tubes were small and there was no evidence of pregnancy.

Animal number 486: This animal was put on the control diet and sacrificed thirteen days later. She was pregnant, the tubes were transparent and vascular and contained well developed embryos.

WEIGHT OBSERVATIONS

The weight curves of animals of series IV are recorded in Charts 4 and 5.

Chart 4 expresses the average results, the control animals gaining weight and the deficient animals losing weight. The initial loss of all animals was due to the fact that the animals did not eat well during the first few days of the experiment while they were getting accustomed to the new diet.

Chart 5 shows the weight curves of the pregnant animals only; the variation in weight being much more remarkable than those of Chart 4 which is an average of all the animals of Series IV, whether pregnant or not.

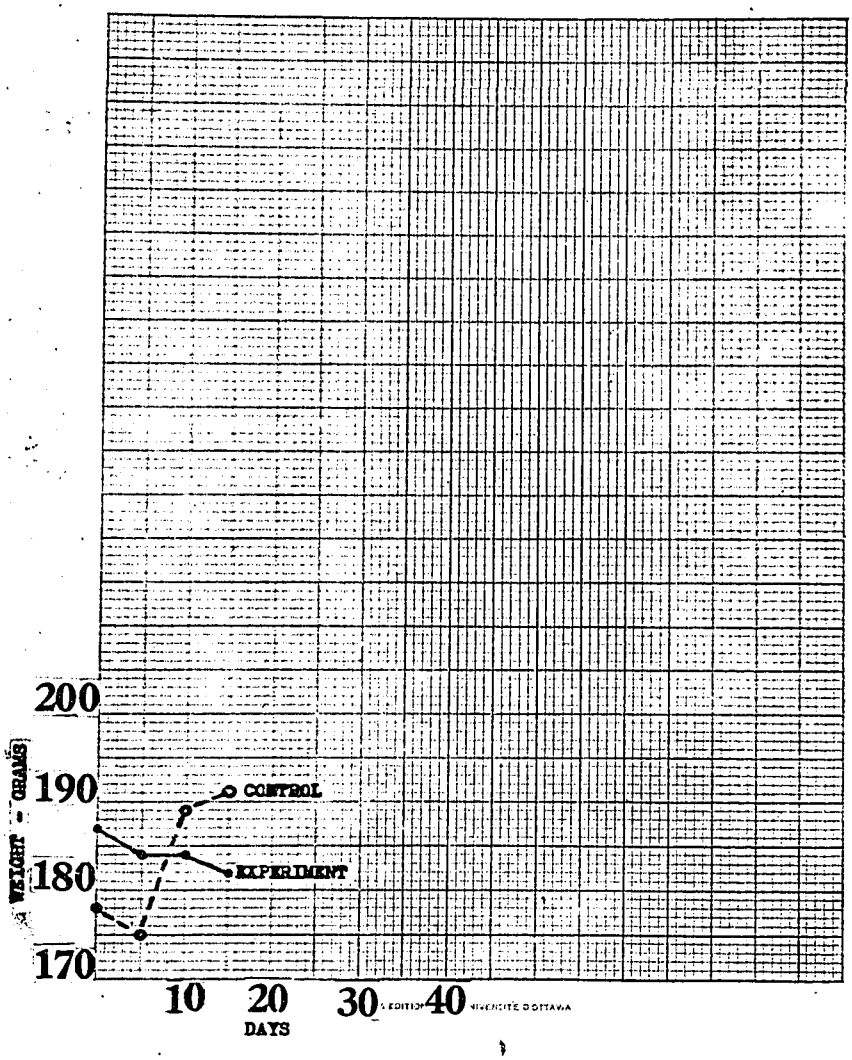

CHART 4: Average weight curves of the animals of Series IV.

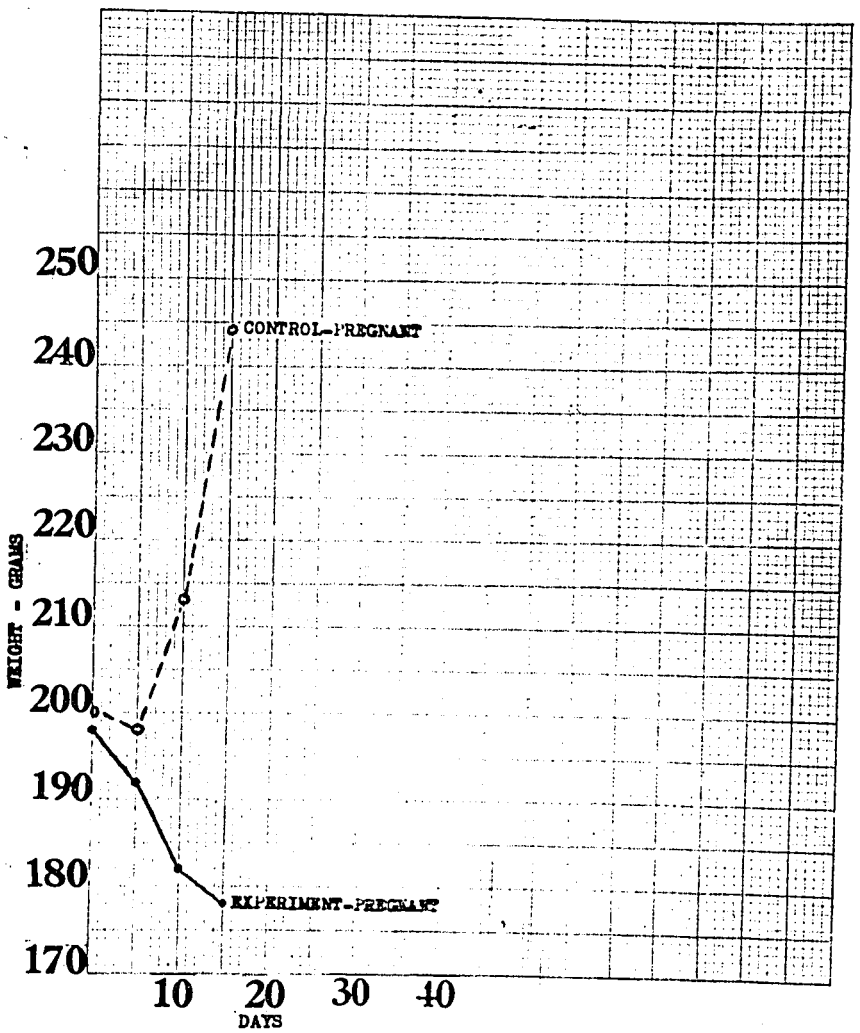

CHART 5: Average weight curves of the pregnant animals of Series IV.

HISTOLOGICAL OBSERVATIONS

Deficient animals

Animal number 474: The uterine tube is congested, in some portions, there are inflammatory changes in the endometrium with accumulation of inflammatory cells and edema fluid in the lumen, other portions show fatty degeneration of the myometrium.

At the junction of the uterus and placenta there is an accumulation of cellular debris; inside the tube is a necrotic mass; there is stasis in the engorged blood vessels in the placenta (Figure 15) with break-down of the red blood cells and release of pigment. This is not encountered in the placenta of control animals as is illustrated in the microphotograph of animal number 486 shown in Figure 16.

In one site of the tube, a recently implanted embryo was found (Figure 17). The placenta shows engorgement with, and break-down of red blood cells, with resultant mucoid degeneration of the animal pole.

The ovary contains many engorged blood vessels.

Animal number 475: The uterine tube has engorged blood vessels, and is empty.

Animal number 476: The uterine tube shows marked changes, the blood vessels are engorged and their walls are thickened by an infiltration of fibroblast and white blood cells.

These inflammatory cells are spread throughout the myometrium which also shows fatty infiltration and in some places contains edema fluid. Numerous eosinophils can be seen throughout the uterine wall; within the tube is a necrotic mass which shows some calcification. This animal also has some encapsulated tumors, cyst adenoma with signs of malignancy, originating probably from the ovary.

Animal number 477: The uterine tube contains engorged blood vessels, and many eosinophils and white blood cells are invading the connective tissue; inside the tube are debris of degenerating placenta. The ovary has engorged blood vessels.

Animal number 478: The tube has engorged blood vessels and contains edema fluid in some regions and necrotic mass in others. The placenta is degenerating and contains many pigmented cells, there is edematous degeneration of an early embryo.

The ovary has engorged blood vessels (Figure 18), giving a picture comparable to the ovaries of all the other deficient animals. Ovaries of control animals (Figure 19) show no such engorgement.

Animal number 479: The uterine tube is small and not pregnant, its blood vessels are engorged (Figure 20). This is a picture similar to that of the tubes of all the deficient animals. The uterine tubes of controls (Figure 21) show lack of vascular engorgement.

Animal number 480: The tube in non-pregnant area has engorged blood vessels and extra uterine hemorrhage.

The embryo in the tube is showing early organization, esophagus and trachea can be recognized with a beginning of formation of thyroid, the blood vessels around the spinal cord are engorged. The muscle formation seems to be somewhat retarded. The liver contains engorged blood vessels, the veins and sinusoids are dilated and contain broken down red blood cells, the trabeculae are spread apart and there are areas of necrosis and infiltration of eosinophils and lymphocytes. There seems to be some retardation in the development of the alimentary canal.

Animal number 481: The uterine tube has engorged blood vessels and contains many embryos in various states of degeneration. The embryonic tissues show first engorgement of the blood vessels then break-down of the red blood cells and production of a colloidal fluid which spreads into the tissues. At the same time there is infiltration by inflammatory cells, lymphocytes, eosinophils, macrophages and fibroblasts; the placental cells break down and there is then only a necrotic mass inside the tube, which itself shows degeneration of the myometrium.

Animal number 482: The uterus has engorged blood vessels which have greatly thickened walls, a large hematoma separates the foetus from the uterus, there is vacuolization and degeneration of the placenta and embryonic debris were seen.

Animal number 483: The empty uterus has engorged blood vessels and some white blood cells infiltration is detected in the connective tissue.

Control animals

Animal number 484: The uterus is normal in appearance, there are no engorged blood vessels like there were in the tubes of the deficient animals, the ovary is not congested.

Animal number 485: The tubes present normal picture, there is a regular distribution of muscles, glands and blood vessels. Figure 21, a microphotograph showing the uterine tube of this animal can be compared with Figure 20 which shows the uterine tubes of a deficient animal.

Animal number 486: The tubes are normal and contain well organized embryos. The ovary appears normal (Figure 19) and is not engorged as were those of the deficient animals, an example of which can be seen in Figure 18.

The skin of the embryo is not as keratinized as that of the deficient embryos, and normally developing salivary glands are seen. The liver is well organized, the kidneys are in formation but even at this stage, there is no calcification of the vertebra.

The placenta (Figure 16) presents normal structure and is vascular but not engorged as shown in Figure 15 which is the placenta of a deficient animal.

SERIES V (see Table V, p. 27):

GENERAL OBSERVATIONS

Deficient animals

Animal number 488: After a period of fifteen days with males, showed an increase in weight which hinted to the possibility of pregnancy, she was then placed on the magnesium deficient diet and was sacrificed after eight days because she was suffering from diarrhea and had lost 38 grams since the beginning of the experiment. There was no evicence of pregnancy on gross post-mortem examination.

Animal number 491: This animal was put on the deficient diet and was sacrificed after eleven days. Upon gross post-mortem examination, embryos were seen in the uterine tubes.

Control animal

Animal number 492: This animal was fed the control diet and delivered after eleven days.

WEIGHT OBSERVATIONS

The weight curves of these animals are illustrated in Chart 6. The control animal shows the characteristic gain interrupted by delivery while the loss of weight of deficient animal number 488 is masked by the gain due to the progression of pregnancy of deficient animal number 491.

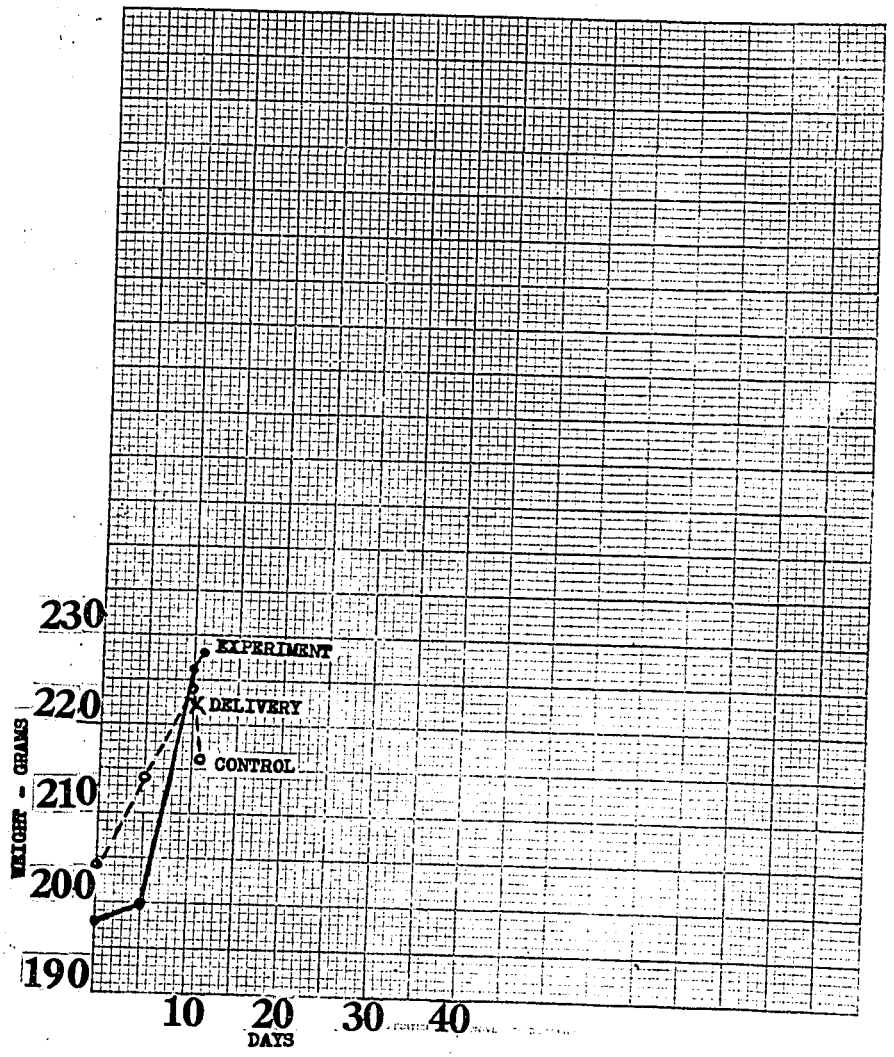

CHART 6: Average weight curves of the animals of Series V.

HISTOLOGICAL OBSERVATIONS

Deficient animals

Animal number 488: The uterine tube has engorged blood vessels, there is thickening of the wall of the arterioles and hemorrhage into the uterine tube; there is no embryonic nor placental tissue.

Animal number 491: The uterus has engorged blood vessels and contains necrotic placental and embryonic tissue.

The embryo's skin is keratinized and its blood vessels are engorged. The liver is congested distorting the liver architecture, there is an infiltration by white blood cells. The kidneys are underdeveloped and contain many fibrocytes. There seems to be retardation of muscular development.

Control animal

Animal number 492: All the youngs are normal in appearance, well developed and their skin is properly keratinized without signs of overkeratinization, so characteristic for embryos of deficient animals. Liver and kidneys exhibit proper development and regular structure.

GENERAL RESULTS FROM SERIES I - V:

Hyperemia, irritability and nervousness were observed in all deficient animals. Only a few were seen in a convulsive state. Some had hyperpnea and ulcerative skin lesions were noted around the ears and neck of a few. These signs appeared after approximately five days on the deficient diet, all the symptoms except nervousness disappeared when the animals were fed the control diet again.

The histological examination of the tissues revealed generalized inflammatory changes in the deficient animals with engorgement of the blood vessels, thickening of their walls, break-down of the red blood cells and degeneration of the parenchymal cells of the liver and of the kidneys. No calcifications were observed in the kidneys but there was early calcification of the vertebrae and ribs of the embryos of deficient mothers.

The uterine tubes and ovaries of the deficient animals exhibited engorged blood vessels.

Embryos were observed in different stages of degeneration. Uterine tubes in which early embryos were found showed a necrotic mass which in some instances degenerated to the point of liquefaction. More developed embryos showed engorgement of blood vessels and retardation in the development

of the muscles, alimentary canal and kidneys. The liver was degenerated.

The average weight of the youngs of deficient mothers was lower than that of the embryos of control mothers.

From the weight curves shown in the six charts, it is evident that the adult deficient animals did not progress normally.

CHAPTER IV

DISCUSSION

Ever since it was established by Osborne and Mendel ('18) and Leroy ('26) that magnesium is essential for animal life, investigators have probed the question of magnesium deficiency and described its symptomatology so that now, it is accepted as a specific entity. Few theories however, have been advanced to explain the physiological basis for the symptoms.

Kruse et al ('32) mention the possibility of ionic disturbances as the cause of local nervous irritation. This seems plausible and could possibly be extended to explain the whole picture. From the findings of the experiments described above, it seems very probable.

Magnesium is an intracellular cation. Its lack causes fluid and ionic imbalance. A change in concentration of magnesium affects the ions of calcium and phosphorus (Forbes '31, Duckworth and Godden '43, and Stoner '50). Moreover, magnesium is an integral part of alkaline phosphatase (Roche et al '50); and thus is needed for the completion of all metabolic reactions involving phosphatase.

Magnesium deficiency could therefore manifest itself in many ways, impairing the general metabolic processes.

The ionic imbalance also probably causes acidosis; which disrupts the metabolism of the cells and, to a certain degree, damages them.

HYPEREMIA

Another fact mentioned previously is that of local irritation to the nerve endings in the tissues (Kruse et al '32), which probably is the initiating cause of the dilatation of the blood vessels. This generalized vasodilatation could cause a lowering in blood pressure; and with a reduced flow of blood, stagnant anoxia would develop (Barcroft '20). The lack of oxygen in turn would lessen the arteriolar tone (Fulton '47) thus leading to further dilatation of blood vessels; and initiating a vicious circle, preventing all compensatory efforts of the body.

MANIFESTATIONS OF THE NERVOUS SYSTEM

The concept of anoxia seems very reasonable, since the nervous symptoms, observed as being specific to magnesium deficiency, are the same as those of anoxia, i.e. occasional hyprepnea, irritability, nervousness and convulsions. These symptoms have been observed in the course of the experiments and are described in the chapter on observations. In the literature, hyperpnea is mentioned by Watchorn and McCance('37), irritation, nervousness and convulsions by Kruse et al ('32), Watchorn and McCance ('37), Greenberg and Tufts ('38), Barron et al ('48) and Dick and Prior ('51).

These symptoms are indicative of malfunction of the central nervous system; and in fact, in cases of magnesium deficiency, Barron et al ('49) and Bird ('49) described chromatolysis and nuclear alterations of the Purkinje cells of the cerebellum; and Greenberg and Tufts ('38) localized lesions in the midbrain and the pons.

Heretofore in this discussion, hyperemia, irritability, nervousness and convulsions have been related to ionic imbalance; the hyperemia being initiated by the ionic imbalance, in turn causing anoxia which results in central nervous system disturbances.

That the nervous symptoms are secondary to brain damage is illustrated by the fact that animals having suffered from magnesium deficiency and then being fed the control diet, recover from other symptoms but not from the nervousness; as described in Chapter III especially in animal number 460 of Series II.

A similar observation was made by Barron et al ('48).

SKIN

The next symptom observed is that of ulcerative skin lesions mostly about the neck and ears. These healed with the return of magnesium to the diet. Similar results were observed by Barron et al ('48) but no explanation of this phenomenon was attempted.

It appears to be a local reaction which can be explained by oxygen lack causing a lessening in the arteriolar tone, whereupon the arterioles become engorged, stasis sets in, the red blood cells break down, and necrosis of the area occurs. Addition of magnesium to the diet would reestablish the ionic balance, the blood flow would be restored, fluid balance improved and the damaged tissue would heal.

LIVER

The changes in the liver except for one observation by Brookfield ('34) who described degeneration of the cytoplasm and nuclei of many liver cells, have been omitted by the investigators of magnesium deficiency.

The engorgement of all the blood vessels in the liver as described in Chapter III seems to be part of the generalized vasodilatation; but since the hepatic veins are so engorged, there is also the possibility of heart failure; which fits well in the picture of anoxia, as do the histological findings of distortion of liver structure with damage and degeneration of the parenchymal cells and infiltration of fibrocytes and white blood cells.

KIDNEYS

The kidneys are also the site of extensive degeneration, due to the fact that the kidneys, because of their role in the maintenance of the fluid and acid-base balance, are

overworked; and also, poorly nourished due to diminished circulation of blood. Histologically, the kidneys are the only site where the findings of the present study differ from those described in the literature. Calcification which is mentioned by Watchorn and McCance ('37), Greenberg et al ('38), Lowenhaupt et al ('50) and Dick and Prior ('51) was not observed in the kidneys during the present experiments. Greenberg et al ('38) however, state that in the earlier stages, degenerative changes in the tubules are a more constant finding than is calcification. They also mention that animals fed on a diet containing less than 1.0mg per cent of magnesium lived for only twenty-five to thirty days. In these cases renal damage was observed in a smaller number of animals and in a less severe form than in animals given a somewhat larger allowance of magnesium and who therefore lived a longer period of time.

The observations described in Chapter III are those of degeneration, it is reasonable to think, that these experiments were of too short a period and of too low a content of magnesium to have produced calcification. The length of these experiments was purposely made short for the purpose of obtaining embryos at different stages of development.

DISCUSSION

OVARIES AND UTERINE TUBES

The ovaries and the tubes were seen histologically in the present study to be much engorged with blood in accordance with the general vasodilatation. No description of ovaries and tubes is available in the literature on magnesium deficiency.

EMBRYOS

Since the diet used in the experiments was totally deficient in magnesium, as was that of Cosla ('50), the embryos were the results of disturbed pregnancy and the experiments of Series II, III, IV, V, were in complete accord with Cosla's ('50) findings. Magnesium is an indispensable element for the normal progression of pregnancy in rats. The results, however, are at variance with the findings of Tufts and Greenberg ('38) who used diets containing 5mg of magnesium per 100 grams; they did not observe changes in the progression of pregnancy but the young of their animals developed symptoms of magnesium deficiency during the suckling period.

Since all animals in the experiments of the present study were put on the deficient diet only after conception, the need of magnesium for conception was not verified, and the mothers having been put on the deficient diet from one to fifteen days after conception, embryos affected at different stages of development were observed. The number and condition

of the offsprings seem to be directly dependent upon the degree of magnesium deficiency of the mothers.

Cosla ('50) does not record any histological description; his observations were statistical based on vaginal smears and count and on gross observation of the foetuses.

Different stages of embryonic degeneration have been observed histologically in this study. In the cases of resorptive abortion, from the histological observations of the tubes and embryonic contents, it may be postulated that the hyperemia and degeneration of the tube led to subsequent degeneration of placenta as described in Chapter III. This would be responsible for the death of an early embryo. The injury to the placenta affects the nutrition of the embryos; the blood cells breaking down in the engorged vessels of the tube and placenta deprive the embryos of nourishment and cause their death.

Other stages of degeneration were seen in older embryos, the mothers of which had been put on the deficient diet at a later stage during their pregnancy. The placenta even though degenerating, because of its greater size provides enough blood supply for a longer survival of the embryo. Also, an older embryo has its own reserve of magnesium which when depleted causes the same disturbances as in the deficient adults. This results in the dilatation of the blood vessels of the embryo which leads to retardation in development and distortion of the

structure of organs such as the liver.

From the degree of damage as revealed by histological study of the uterine tubes and embryos, it would seem that the disturbance in embryonic development is secondary, due to the hyperemic state of the uterine tubes of the mother. The corpora lutea of pregnancy do not show appreciable changes and this does not support Cosla's ('50) assumption of a direct relation between magnesium ions and sex hormone activity, as far as the corpora lutea are concerned. The sterility observed by Cosla ('50) in deficient animals could be due rather to the disturbance in the blood supply of the ovaries and tubes in the deficient animals, than direct action on sex hormone activity.

It would be interesting to investigate further on this problem and to study the ovaries and endometrium of deficient animals at the different stages during the ovarian cycle, in correspondance with biochemical studies of the sex hormones present.

METABOLIC DISORDER

Magnesium deficiency producing ionic imbalance, deficiency of activity of alkaline phosphatase produces a circulatory disturbance which in turn gives degenerative changes in tissues and especially parenchymatous organs. As the reaction to the tissue injury, infiltrations by fibrocytes and round cells are encountered in liver, kidneys, placenta and around

the blood vessels. Fatty infiltration in the liver, uterine tubes and mucoid degeneration in the tubes and placenta are observed.

The injury to the parenchymatous organs and disturbances in circulation produce in turn metabolic changes as illustrated by the weight charts. These metabolic changes, their scope and meaning did not receive as yet full attention and appreciation. Therefore, with the help of modern scientific means, it seems that it would be most interesting to investigate the physiological conditions; such as pH, oxygen and carbon dioxide contents, of the blood in the animals deprived of magnesium and to compare the findings with those of other important ionic deficiencies, especially those of potassium, calcium and sodium.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. Five series of experiments were conducted on forty-four albino rats. The experimental diet was totally deficient in magnesium; the control diet was the same as the experimental diet supplemented by 0.0404 per cent magnesium.

2. All the deficient animals showed signs of irritability, nervousness and hyperemia; some also had hyperpnea, diarrhea and skin lesions. These signs appeared after approximately five days on the deficient diet, all the symptoms except nervousness disappeared when the animals were fed the control diet again.

3. From the weight curves it is evident that the deficient animals did not progress normally.

4. The histological examination of the tissues revealed generalized inflammatory changes in the deficient animals with engorgement of the blood vessels, thickening of their walls, break down of the red blood cells and degeneration of the parenchymal cells of the liver and of the kidneys.

5. Pregnant females on the deficient diet, either resorbed or gave birth to a lesser number of offsprings than those on the control diet, some of the youngs were born dead and, in general, the average weight of these was lower than that of the control embryos. When the deficient diet was started at a late stage of pregnancy retardation of fetal development was encountered and histological signs of magnesium deficiency similar to those of the adult animals were seen in the embryos.

6. The suggested conclusion is that magnesium deficiency produces primary ionic imbalance with alkaline phosphatase inhibition which leads to a sequence of changes namely: vasodilatation, hyperemia, tissue anoxia, degenerative and infiltrative tissue injury with final general metabolic disturbance.

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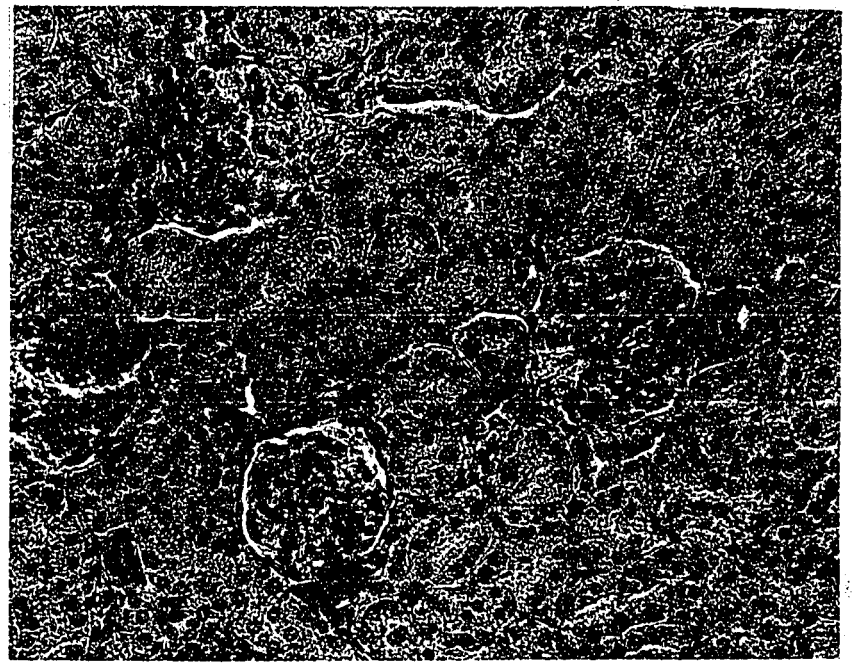

FIGURE I: Kidney of deficient animal number 461. x 430

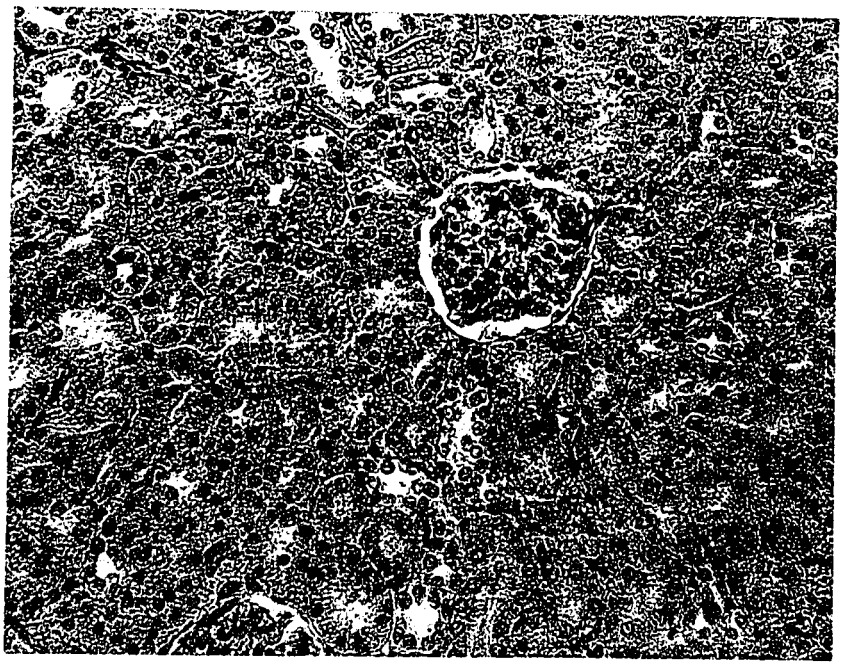

FIGURE 2: Kidney of control animal number 466. x 430.

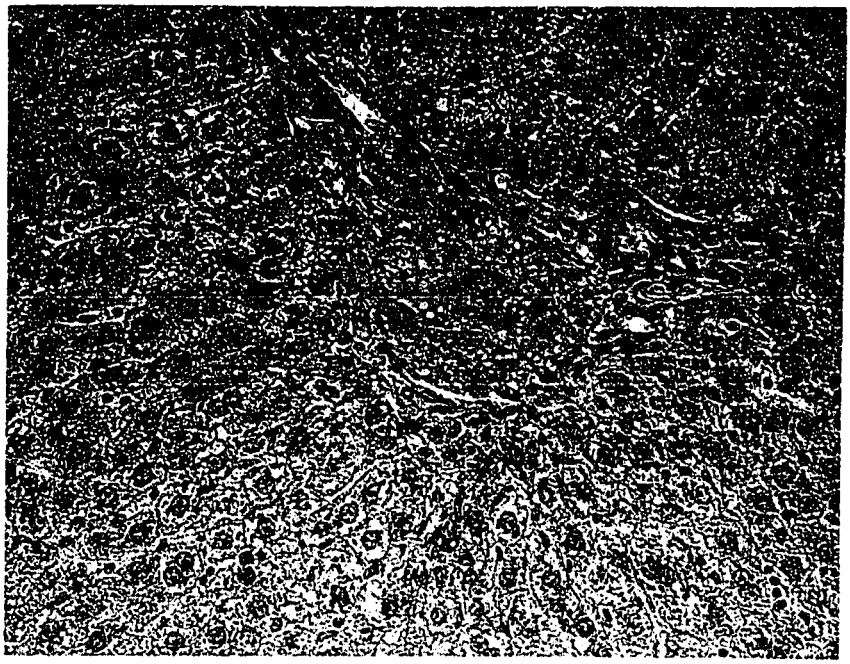

FIGURE 3: Liver of deficient animal number 464. x 430.

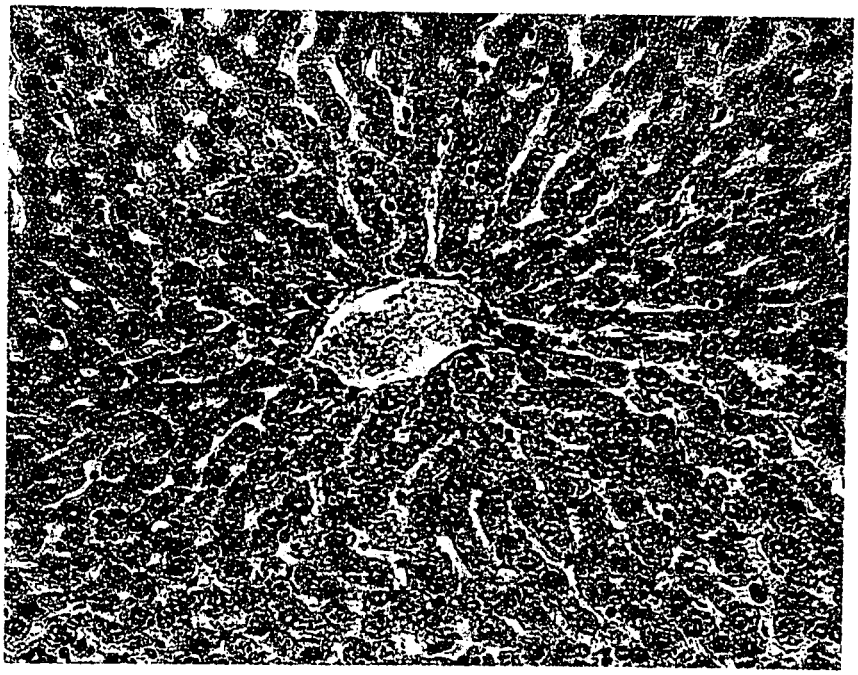

FIGURE 4: Liver of control animal number 467. x 430.

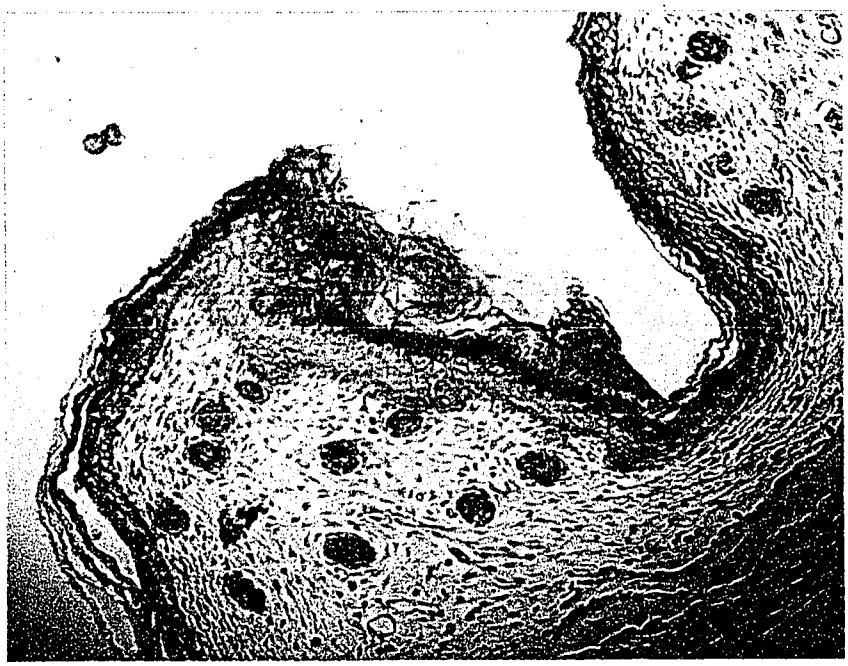

FIGURE 5: Skin of embryo of deficient animal number 469. x 215.

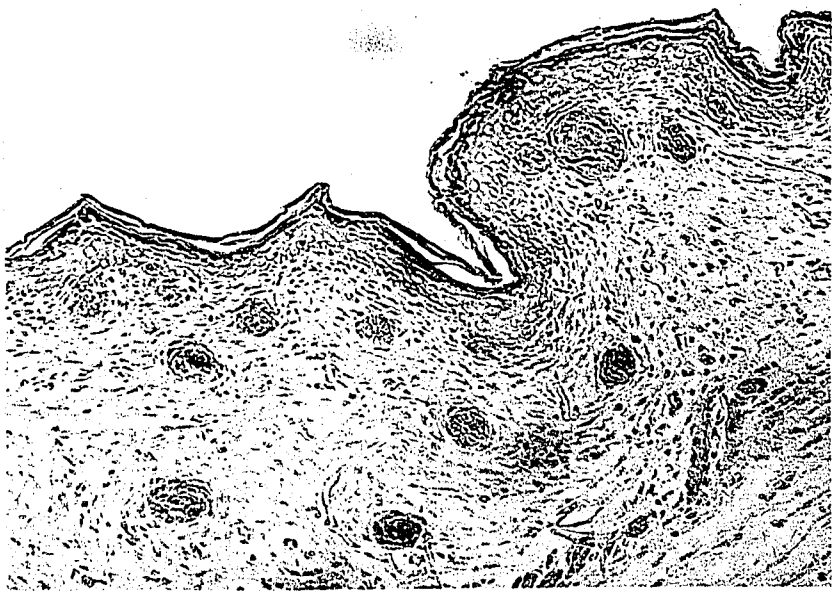

FIGURE 6: Skin of embryo of control animal number 472. x 215.

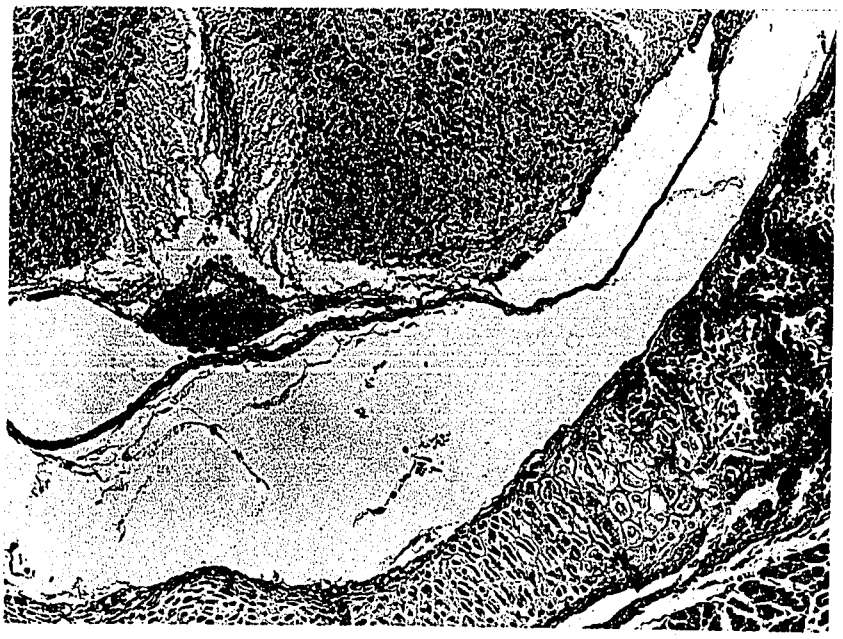

FIGURE 7: Vertebra of embryo of deficient animal number 469. x 215.

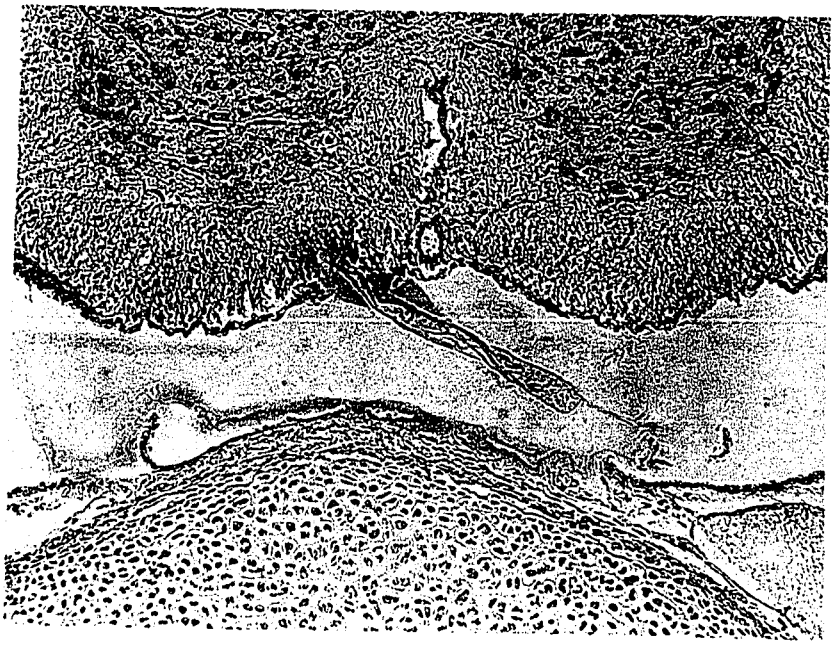

FIGURE 8: Vertebra of embryo of control animal number 472. x 215.

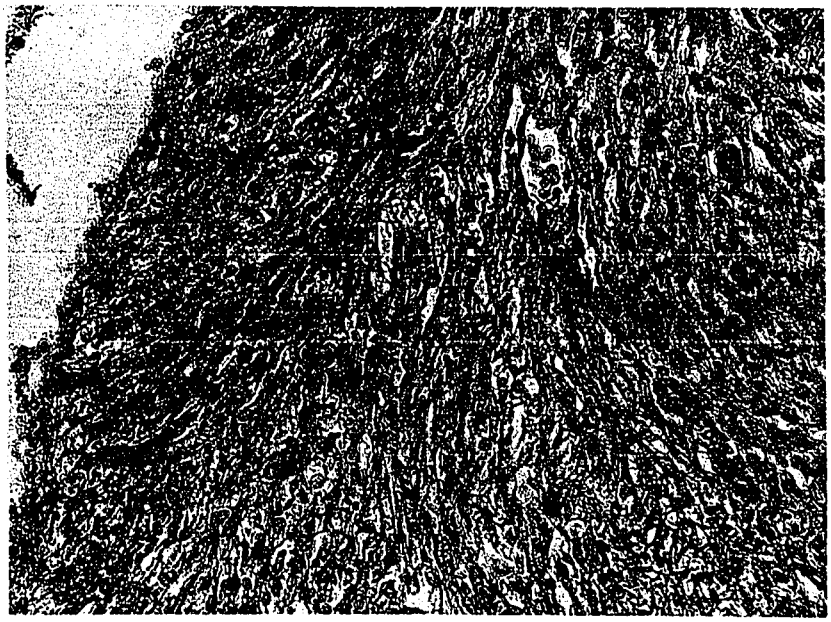

FIGURE 9: Heart of embryo of deficient animal number 469. x 430.

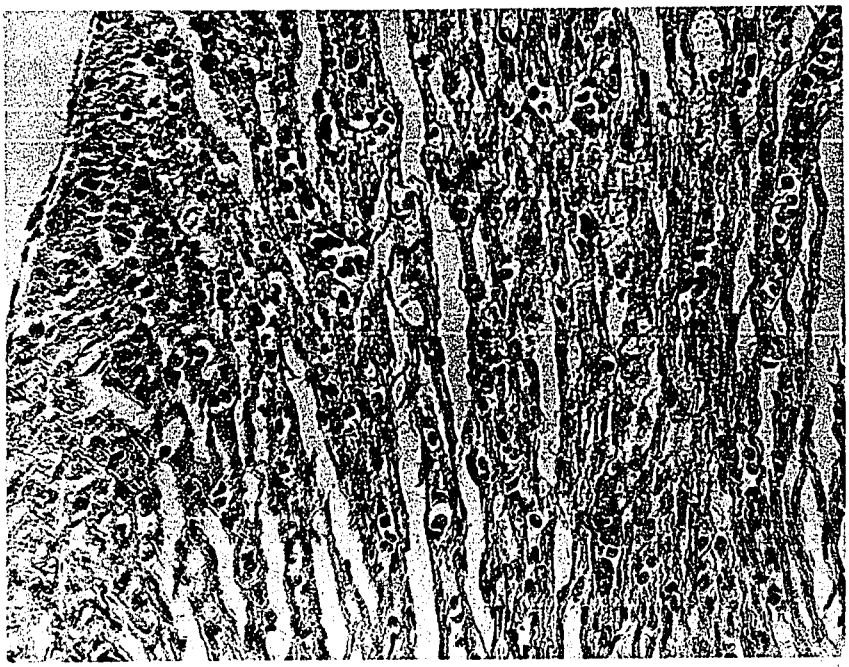

FIGURE 10: Heart of embryo of control animal number 472. x 430.

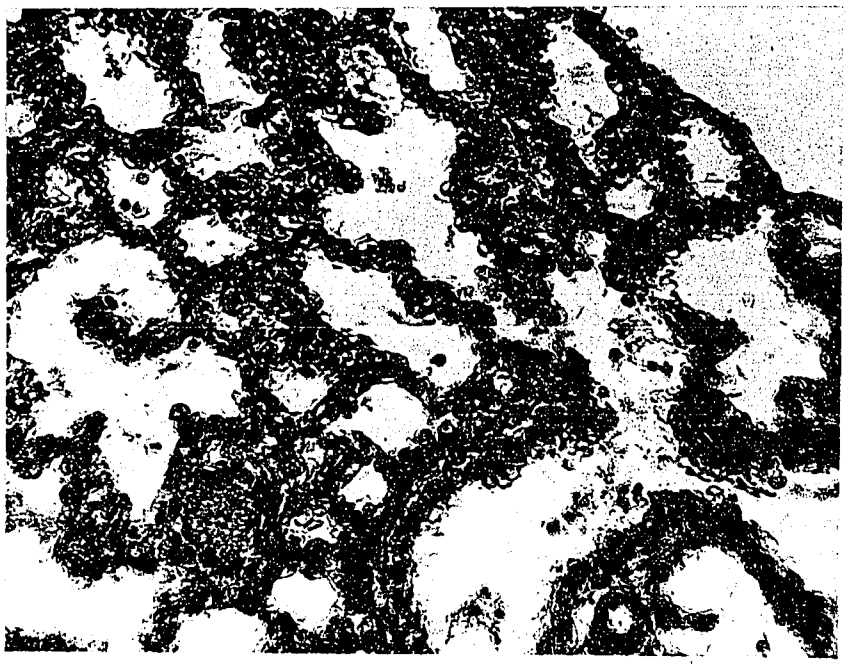

FIGURE 11: Lung of embryo of deficient animal number 469. x 430.

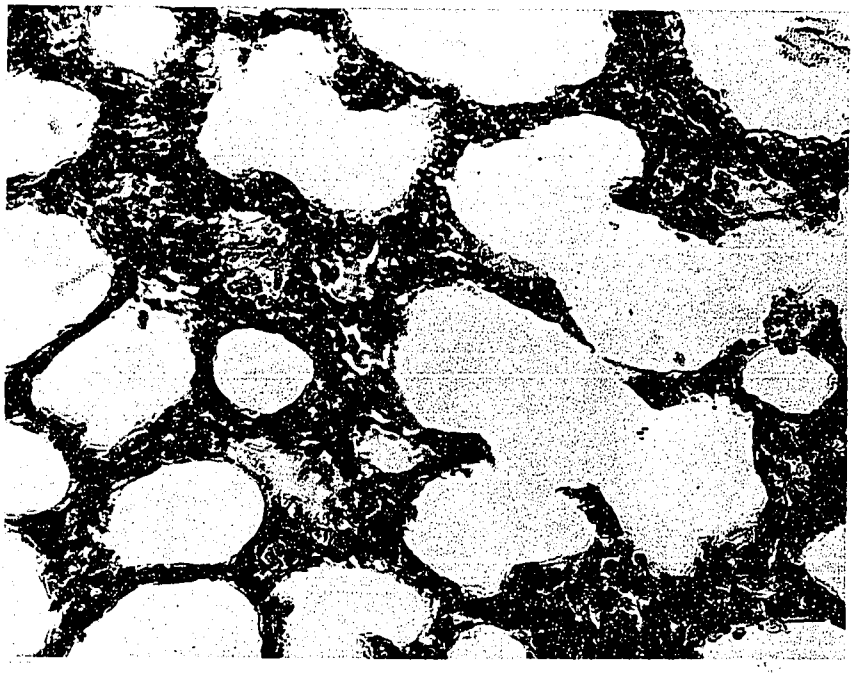

FIGURE 12: Lung of embryo of control animal number 472. x 430.

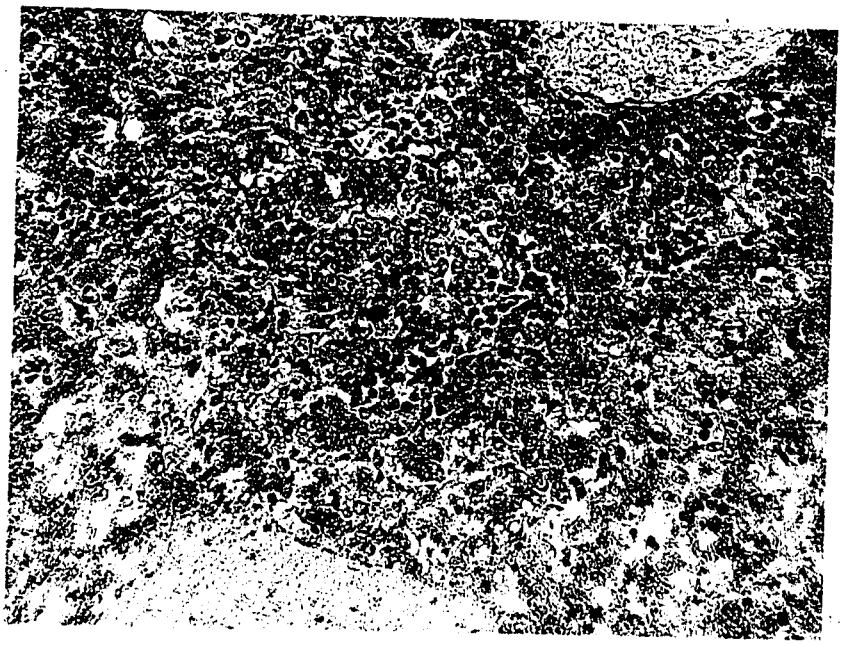

FIGURE 13: Liver of embryo of deficient animal number 469. x 430.

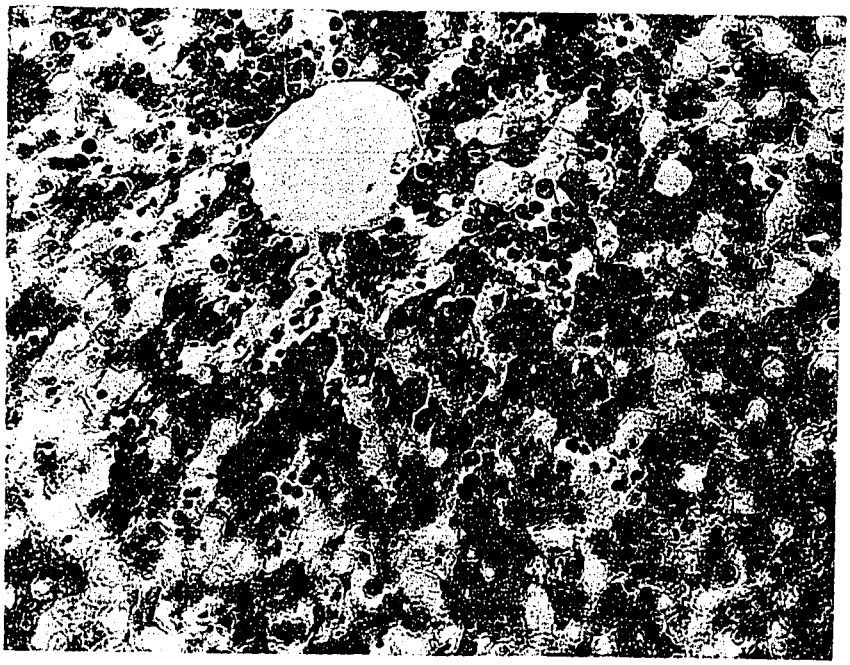

FIGURE 14: Liver of embryo of control animal number 472. x 430.

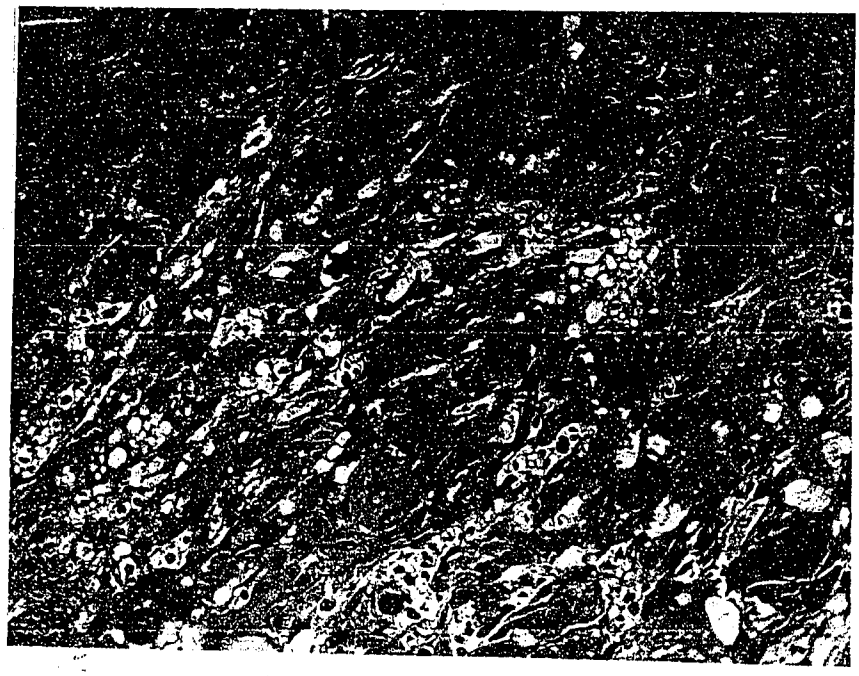

FIGURE 15: Placenta from deficient animal number 474. x 215.

FIGURE 16: Placenta from control animal number 486. x 215.

FIGURE 17: Degenerating early embryo of deficient animal number 474. x 60.

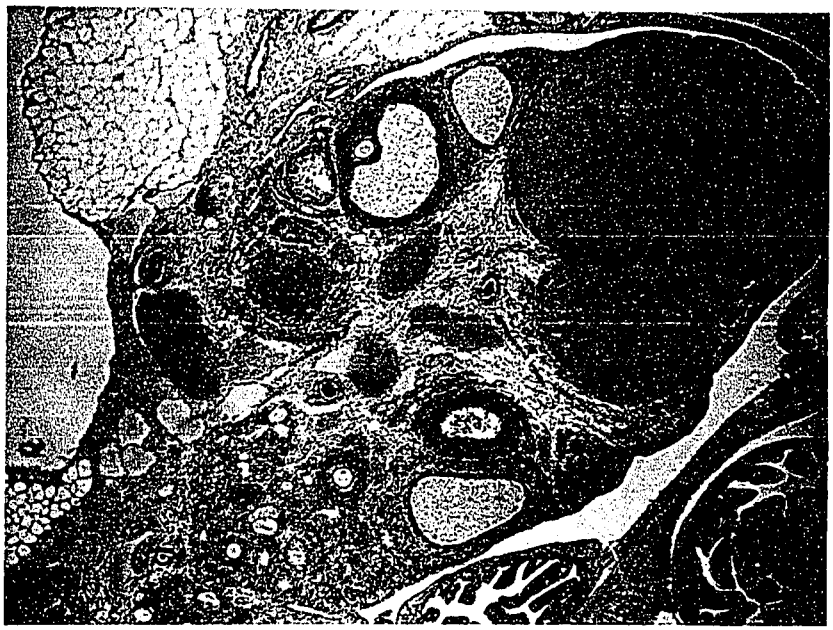

FIGURE 18: Ovary of deficient animal number 478. x 65.

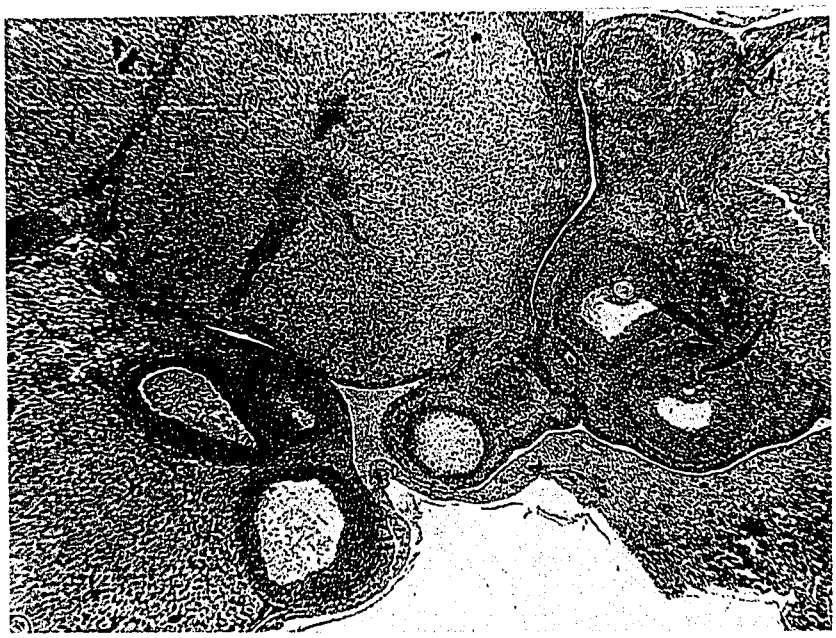

FIGURE 19: Ovary of control animal number 486. x 65.

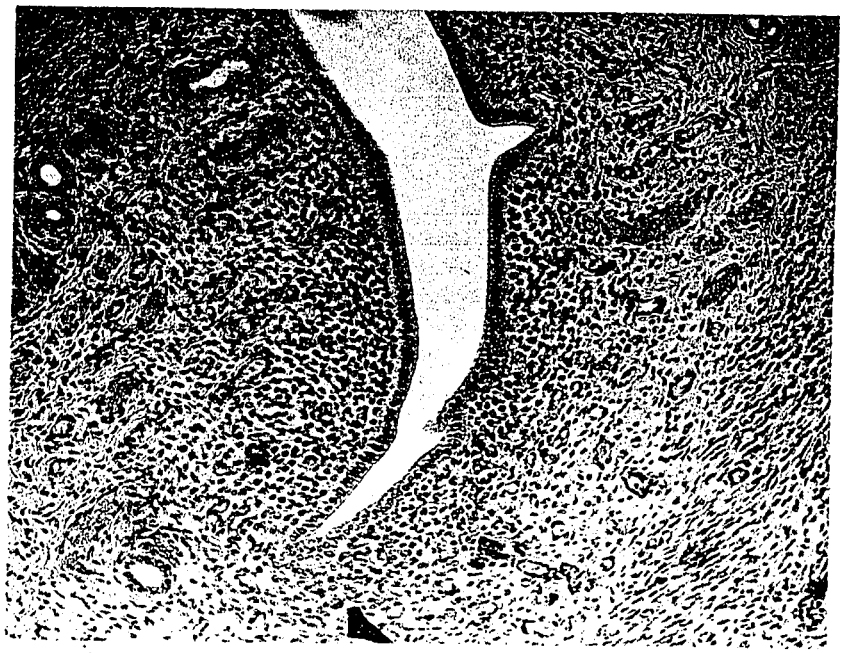

FIGURE 20: Uterine tube of deficient animal number 479. x 215.

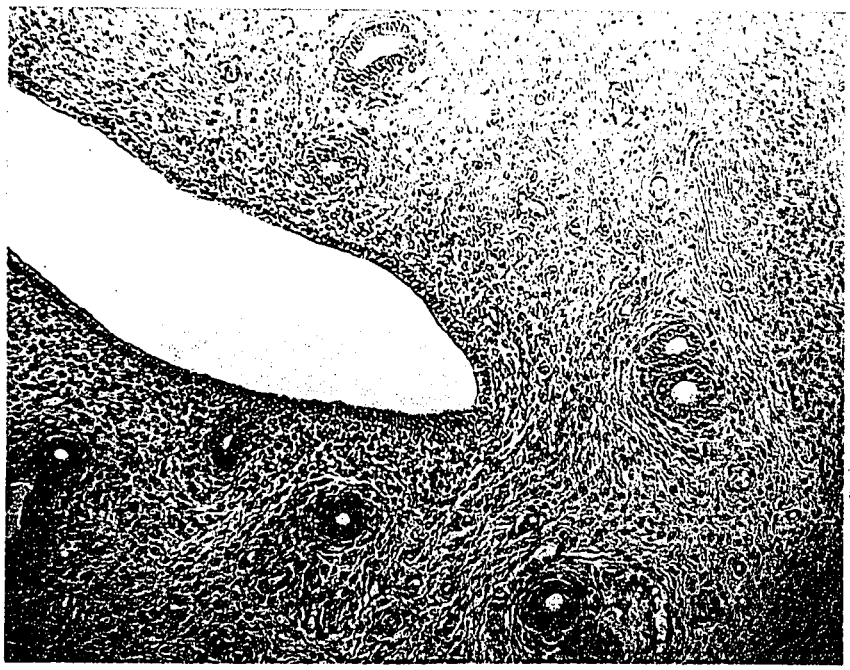

FIGURE 21: Uterine tube of control animal number 485. x 215.