Effects of magnesium deficiency on reproduction in the white rat

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1. Groups of rats were maintained from weaning on magnesium-deficient (30 mg Mg/kg) and control (460 mg Mg/kg) diets.

2. Mg deficiency for a period of more than 3 months resulted in sterility of both males and females.

3. A Mg deficiency of less than 3 months caused abortions or resorptions *in utero* of foetuses in pregnant females before other physical signs appeared.

4. Mg deficiency during lactation rendered the survival of young rats uncertain. The mother rat died of exhaustion if she did not devour her litter.

5. The growth of surviving young rats born of Mg-deficient mothers was noticeably retarded when they were nursed by mothers receiving the Mg-deficient diet.

It has been known for many years that pregnancy and lactation both require the contribution of numerous substances held in reserve, notably mineral salts. Jacquot (1960) showed that during early pregnancy the female rat stores the materials necessary for lactation. What happens when these indispensable elements are no longer furnished? Various authors have determined that a deficiency in calcium leads to premature births and a decrease in the amounts of Ca and phosphorus in the bone (Hart, McCollum, Steenbock & Humphrey, 1911; Toverad & Toverad, 1931). Bodansky & Duff (1941) showed that foetal growth in the rat depended on the amounts of Ca and P in the diet, and that iron, manganese and copper deficiencies gave rise respectively to anaemia, stunted limbs and degeneration of the central nervous system in the newborn rats.

Le Roy (1926) asserted that magnesium was essential to reproduction in the mouse, and Tufts & Greenberg (1937–8) sought to determine the minimum level of food Mg that would permit normal reproduction in female rats. Sell, Cox & Hajj (1966) further showed that when given less than 30 μg Mg/d per bird, hens remained sterile. Aikawa (1960) measured the Mg flow through the placenta of the rabbit and concluded that the foetus received a large quantity of Mg from the reserves in the mother's bone. Lactation has been studied in several kinds of mammals, especially ruminants, which show signs of tetanus due to Mg deficiency (L'Estrange & Axford, 1964; Lowrey & Grunes, 1968).

The following study was undertaken to examine the effects of Mg deficiency on reproduction in the rat, in particular on fertility, gestation and lactation – three periods during which this metal has proved indispensable.

Table 1. *Composition of the diets (g/kg)* (*From Le Breton*, 1960)

Control (magnesium content 460 mg/kg)

Devitaminized casein	230
Sucrose	570
Lard	100
Salt mixture*	50
Vitamin mixture†	25

Mg-deficient (Mg content, 30 mg/kg)

The composition of this diet was similar to that of the control diet except that the content of Mg sulphate in the salt mixture was reduced and replaced by an equal amount of sucrose.

* Containing (g/kg): sodium chloride 95; dipotassium phosphate 290; monosodium phosphate 140; monocalcium phosphate 290; magnesium sulphate 100; ferric lactate 25; potassium iodide 0·85; potassium alum 0·1; manganese sulphate 0·075; sodium fluoride 0·03.

† Providing (per kg diet): menaphthone, 5 mg; retinyl acetate, 2 mg; cholecalciferol, 250 μg; biotin, 1 mg; vitamin E, α-tocopherol equivalent, 300 mg; cyanocobalamin, 20 μg; thiamin 20 mg; riboflavin, 20 mg; pyridoxine, 20 mg; nicotinic acid, 25 mg; pantothenic acid (as calcium salt), 50 mg; pteroylmonoglutamic acid, 2 mg; *myo*-inositol, 850 mg; choline, 2 g; made up in agar.

EXPERIMENTAL

Wistar rats were used, the females being 3 months old, an age which, according to Hafez (1963), corresponds to the period of maximum fertility. Two different diets were prepared: one, the control, was complete and balanced with a Mg content of 460 mg/kg; the second diet was deficient in Mg, its Mg content being only 30 mg/kg. Both diets were devised by Le Breton (1960); their compositions are given in Table 1. The animals were fed *ad lib.*, and they drank demineralized water. In one group, forty-five females and ten males were maintained on the control diet from weaning until the age of 3 months; in a second group, forty females and ten males were maintained on the diet deficient in Mg for 42 d. Twenty-five control females were mated with control males, and the other twenty control females were mated with males on the Mg-deficient diet. Twenty females on the Mg-deficient diet were mated with control males and the remaining twenty females were mated with males on the Mg-deficient diet: four groups of animals were thus obtained.

During the mating period, each mating couple was maintained on the Mg-deficient diet where one animal in the couple was maintained on the Mg-deficient diet before mating.

Oestrus was ascertained by vaginal smear analysis and the beginning of gestation was verified by the presence of sperms in the vagina.

After 5 d of mating, males were separated from females and each group of females was further subdivided into two; half were given the Mg-deficient diet and half were given the control diet for the remainder of the gestation period. Eight groups of female rats were thus obtained including one control group of fifteen animals. The females were isolated in individual cages 8 d before the end of pregnancy. Some females rats were killed during gestation to study the foetus.

The newborn rats were numbered and weighed and, 5 d after birth, the amount of Mg in the liver of some rats was determined by flame spectrometry (MacIntyre & Davidsson, 1958). During lactation, each group of females was further subdivided,

Table 2. *Effects of magnesium content of the diet on reproduction and lactation in normal female rats mated with normal males given the control diet (diet T), or with males given a Mg-deficient diet (diet TMg) for 6 weeks; half of the females were given diet T and half were given the TMg diet after mating and throughout gestation and lactation*

Diet after mating ...	Diet T (460 mg Mg/kg)		Diet TMg (30 mg Mg/kg)	
Cross and diet before mating ...	♀T × ♂T	♀T × ♂TMg	♀T × ♂T	♀T × ♂TMg
Reproduction				
No. of matings	15	10	10	10
No. of impregnations	11	6	7	6
No. of litters	11	6	6	5
No. of abortions	0	0	1	1
No. of offspring	95	33	29	25
No. of offspring/litter	$8\cdot6 \pm 1\cdot8$	$5\cdot5 \pm 2\cdot2$	$4\cdot9 \pm 1\cdot2$	$5\cdot0 \pm 0\cdot6$
Wt. of newborn rats (g)	$5\cdot4 \pm 0\cdot4$	$5\cdot9 \pm 0\cdot6$	$5\cdot4 \pm 0\cdot6$	$5\cdot6 \pm 0\cdot5$
No. of offspring surviving to 3rd day	95	33	15	13
Offspring devoured by their mother	0	0	14	12
Mg content of liver of offspring in their 5th day $(\mu g/g)*$	220 ± 16	221 ± 15	213 ± 12	210 ± 11
No. of females surviving at the beginning of lactation	11	6	5	4
	↓	↓	↓	↓
Lactation				
Diet during lactation	T TMg	T TMg	T TMg	T TMg
No. of females at the beginning of lactation	6　5	3　3	2　3	1　3
No. of offspring at the beginning of lactation	49　41	15　13	5　6	3　6
No. of females surviving at weaning	6　4	3　2	1　0	1　0
No. of offspring surviving at 3 weeks	49　20	15　6	4　0	2　0

* Mean values with their standard errors.

some being given the control diet and others the Mg-deficient diet. The gain in body-weight of the litter was studied until weaning.

RESULTS

It was observed that the duration of Mg deficiency had a considerable effect on fertility; after 3 months of Mg deficiency male and female rats were so weakened – with such signs as intense keratinization of vagina, oedema of limbs and cataract blindness – that reproduction was impossible. However, very few clinical signs were present after 6 weeks of Mg deficiency before mating, and reproduction was possible.

Mating of normal female rats with normal males or with males given the Mg-deficient diet (Table 2)

Verification of impregnation by vaginal smear analysis showed that with males from the control group a pregnancy rate of 70 % was obtained; this was similar to

Table 3. *Effects of the magnesium content of the diet on reproduction and lactation in female rats given a Mg-deficient diet (diet TMg) for 6 weeks and mated with normal males given the control diet (diet T) or with males given diet TMg for 6 weeks; half of the females were given diet T and half were given diet TMg after mating and throughout gestation and lactation*

Diet after mating ...	Diet T (460 mg Mg/kg)		Diet TMg (30 mg Mg/kg)	
Cross and diet before mating ...	♀TMg×♂T	♀TMg×♂TMg	♀TMg×♂T	♀TMg×♂TMg
Reproduction				
No. of matings	10	10	10	10
No. of impregnations	7	6	7	6
No. of litters	6	4	2	2
No. of abortions	1	2	5	5
No. of offspring	27	19	9	8
No. of offspring/litter*	4·5±1·5	4·8±1·2	4·5±0·8	4·0±0·2
Weight of newborn rats* (g)	5·6±0·4	5·4±0·5	—	—
No. of offspring surviving to 3rd day	20	14	0	0
Offspring devoured by their mother	7	5	9	8
Mg content of liver of offspring in their 5th day (µg/g)*	203±15	202±15	—	—
No. of females surviving at beginning of lactation	6	4	1	1

Lactation

Diet during lactation	T	TMg	T	TMg	
No. of females at the beginning of lactation	2	4	1	3	No lactation
No. of offspring at the beginning of lactation	7	7	4	7	
No. of females surviving 3 weeks after giving birth	2	0	1	1	
No. of offspring surviving at weaning	7	0	4	1	

* Mean values with their standard errors.

the accepted statistical averages. Mating with Mg-deficient males produced a pregnancy rate of only 60 %.

The females that were given the control diet during gestation gave birth to viable young; but those that were mated with Mg-deficient males, produced smaller litters, four to five young instead of eight or more. When these same females were given the Mg-deficient diet during lactation they either died at the end of this period, or killed their offspring without, however, devouring them. When females were given the Mg-deficient diet during gestation, the percentage of live births remained the same, but about 50 % of the litter was either abandoned or devoured by the mother, and when the Mg-deficient diet was continued, the female rats died during, or at the end, of lactation.

The concentration of liver Mg in young rats (5 d old), was slightly lower in those born of mothers who were given the Mg-deficient diet during gestation.
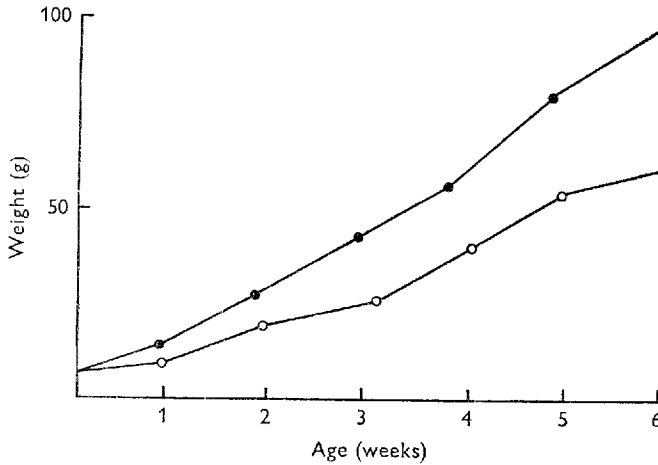
Fig. 1. Rate of weight gain of newborn rats (produced from mating control male rats with female rats which received the control diet during gestation) nursed by females given the control diet (●—●), or nursed by females given a magnesium-deficient diet (○—○), during the lactation period.

Mating of Mg-deficient females with normal or Mg-deficient males (Table 3)

Vaginal smear analysis showed that there was a 60-70 % pregnancy rate, but the pregnancies reached full term only when the Mg-deficient diet was withdrawn immediately after separation of the males and the females. Nevertheless, litters were smaller, four to five young rats, and 20 % of the litters were devoured by the mother. The weights of the newborn rats were normal, but the Mg concentration in their livers was slightly lower than in those born in the control group (200 μg/g fresh liver compared to 220 μg/g for the controls).

On the other hand, when Mg-deficient females were maintained on the Mg-deficient diet after mating, numerous abortions were observed and all the newborn rats either were abandoned by their mother and died, or were devoured by her. Because of the large number of abortions and the reduced size of the litters, it was decided to kill several additional females to study the contents of the uterus. It was observed that gestation began normally at the opening of the uterus and the number of implantations was similar to the normal figure obtained with rats on the control diet. Towards the end of pregnancy, however, the number of foetuses was reduced, although the number of implantation sites remained the same. The condition of the foetuses showed considerable variation: some were fully developed, but others had stopped developing or were already partly resorbed.

Effects of Mg deficiency on lactation

It has been noted that the females given the Mg-deficient diet before and during gestation devoured their young; the females given the Mg-deficient diet before mating and then placed on the normal diet during gestation had a normal though smaller litter, and they suckled their young until weaning. However, when females in the latter group were placed again on the Mg-deficient diet at the beginning of

lactation, two of them died at the beginning of this period and all died before weaning. By then, they weighed much less than normal females. At autopsy their kidneys were seen to be injured, appearing nephritic; the Mg concentration in the kidneys – about 16 μg/g fresh tissue in the rats given the control diet – reached 18 μg/g. This increase in concentration of Mg in the kidneys is one of the characteristics of Mg deficiency in the adult rat (Andrieux-Domont & Le Van Hung, 1969). In contrast we observed that the Mg concentration of the liver in female rats given the Mg-deficient diet decreased.

When the females in the control group were given the Mg-deficient diet during pregnancy and lactation, they died before the pups were weaned; but females which were replaced on the control diet after giving birth produced milk normally and the gain in body-weight of the litters was satisfactory – growth was retarded when the newborn rats were nursed by females receiving the Mg-deficient diet (Fig. 1).

DISCUSSION

It is evident that Mg deficiency has repercussions on reproduction. The males become sterile only when they are kept on a Mg-deficient diet for a long period: between 1 and 6 weeks the number of successful matings, as verified by the presence of sperms in vaginal smears, is normal. Although no abortions were seen in females of the control group mated with Mg-deficient males, the number of young in each litter was nevertheless smaller, and though the foetuses showed no malformations, it was not possible to conclude, therefore, that their development was normal and that they were all viable. It was shown earlier, that the Mg concentration in the tissues of adult animals given the Mg-deficient diet decreased noticeably. It is not unlikely, therefore, that the testicles of rats given the Mg deficient diet have a smaller Mg content and that in consequence spermatogenesis becomes abnormal.

In females, it was necessary to distinguish between gestation and lactation – two periods during which there is a great loss of Mg (Coumel, 1967; Greenwald, Dubin & Cardon, 1963). In females given the Mg-deficient diet before gestation, and the control diet during gestation, the abortion rate was only 10 %. When they were maintained on the Mg-deficient diet, however, the abortion rate was 50 %. In normal females placed on the Mg-deficient diet during gestation, the abortion rate was the same as that of the control group. These findings agree with those of Doiseau (1954) and Dumont & Bernard (1966), emphasizing the efficient action of Mg salts in human cases where there is a risk of abortion. In addition, Chein & Kelly (1967) demonstrated that the sensitivity of the uterine muscle to oxytocin depended on the concentration of Mg in the serum.

It is important to note, however, that the signs of deficiency as such are not immediately obvious in the pregnant rat. The different stages of Mg deficiency in the adult rat were noted in a previous article (Andrieux-Domont & Le Van Hung, 1971): the first cutaneous symptoms are vasodilatation and a red secretion staining first the muzzle, then the neck and finally the entire fur. This secretion was identified as porphyrin by Raoul & Marnay (1945). These visible symptoms were not observed

in the pregnant female rats: but at autopsy, the kidneys were seen to be injured. In the kidneys, the concentration of Mg had risen by 2 μg/g fresh tissue, and it had fallen by the same amount in the liver. Distinction should therefore be made between the sudden increase in the need for Mg during gestation and lactation, and the chronic insufficiency which ensues as a result of a prolonged period on a Mg-deficient diet.

Administration of the Mg-deficient diet during gestation did not appear to have a direct effect on the foetus and on the newborn rat; their survival depended on the condition of the mother. However, in contrast to the results of Tufts & Greenberg (1937–8), who did not report any difference in the Mg content of newborn rats, our experiment showed that the concentration of Mg had decreased in the livers of young rats born of mothers placed on the Mg-deficient diet. A decrease in the concentration of serum Mg in the newborn human baby may cause convulsions (Salet, Polonowski, De Gouyon, Pean, Melekian & Fournet, 1966; Friedman, Hatcher & Watson, 1967; Paunier, Radd & Sang Whay Kooh, 1968). Wang, Wang, Khairallah & Schwartz (1971) reported this same phenomenon in female rats given a diet deficient in Mg. In particular, they noted a considerable fall in the concentration of Mg and proteins in the milk of rats on the Mg-deficient diet and the rapid exhaustion of these rats during lactation. In our experiments, when the rats were maintained on the Mg-deficient diet during lactation, the rate of gain in weight of their young slowed down even though they had normal weight at birth, fur growth was retarded and their appearance remained sickly. It was not possible to ascertain whether this retardation of growth was the direct result of the Mg deficiency alone or whether it arose from insufficient nourishment due to the poor quality of the mother's milk. This last possibility appears more likely, for the few animals whose Mg-deficient mothers were given the control diet after parturition had a normal rate of growth.

This experiment makes it possible to state unequivocally that Mg is an essential element in reproduction. A prolonged deficiency of the mineral led to sterility, and a shorter deficiency of 6 weeks following weaning, though it did not prevent reproduction, nevertheless caused abortions or the resorption of the foetuses.

Mg appears even more important in lactation. A deficiency in the mineral during this period slowed down the rate of gain in weight in young rats and led to rapid exhaustion of the mothers.

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