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Protective Effect of Magnesium Deficiency on Experimental Allergic Encephalomyelitis in the Rat.* (30986)

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Acute magnesium deficiency in the rat is characterized in its earliest stage by a series of clinical and laboratory manifestations, such as pronounced hyperemia of the skin, leukocytosis and eosinophilia(1,2,3). Increased urinary excretion of histamine coincident with mast cell degranulation has also been described at this stage of the acute deficiency, and all the findings have been reported to be preventable by antihistaminic preparations(4, 5). This picture, which develops in 6-9 days, is suggestive of a transient hypersensitive state. If the animals are sacrificed after about 21-28 days on the magnesium deficient regime many microscopic lesions are found in various tissues. In cardiac and skeletal muscle, the lesions are characterized by focal perivascular collections of inflammatory cells which may be associated with muscle cell necrosis. This histological picture often resembles that seen in delayed hypersensitivity reactions. Because these findings are suggestive of an altered immunological state, the response of the magnesium deprived rat to an allergenic stimulus was tested. Experimental allergic encephalomyelitis (EAE), believed to represent a form of delayed hypersensitivity, was chosen for this purpose. When guinea pig spinal cord homogenates plus complete Freund's adjuvant are injected into the foot pads of rats, a predictable number of rats will develop EAE when histological grading of the lesions is used(6,7).

Materials and methods. Dietary regime: A synthetic diet containing less than 1 mg of magnesium per 100 g was used to produce magnesium deficiency. Control rats were fed a similar diet supplemented with 65 mg of magnesium per 100 g. The composition of the diets has been published(8).

Experiments: Male CD rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing between 100 and 120 g were individually housed in metal cages and had free access to their respective diets and distilled water.

The method of Levine and Wenk for production of EAE was utilized(6,7). All rats were pretreated with 0.6 ml of pertussis vaccine (Parke, Davis Co.) given intraperitoneally after dilution to 1.5 ml in normal saline solution. Four days later they were injected with 0.5 ml of a 33% homogenate of guinea pig spinal cord plus complete Freund's adjuvant intradermally into the foot pads. The number of animals used and the dietary regimes are illustrated in Table I. Two experiments were performed. They were designed so that the phase of hyperemia of the magnesium deficiency occurred at several intervals in relation to the administration of the encephalitogenic agent. In Experiment 1 most of the deficient rats were in the phase of skin hyperemia at time of the antigen injection. In Experiment 2 the period of hyperemia occurred at the end of the second week following the injection of Group C, and at the

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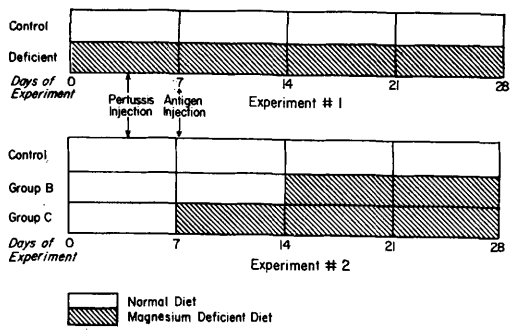
Table I
Experimental Design



end of the third week after the injection in Group B.

Twenty-one days after the antigen injection all the rats were sacrificed by exsanguination by aortic puncture under light ether anesthesia. The blood was collected and sera stored frozen at —10°C. Complete autopsies were performed. The brains and spinal cords were serially blocked and processed for routine histologic examination. Also, every major organ, skeletal muscle and site of injection of the encephalitogenic mixture were examined histologically. A few animals which showed severe paralysis and signs of imminent death were sacrificed prior to the 21-day period. All animals dying spontaneously were discarded. Antipertussis agglutination titers were determined in 5 randomly selected sera in each group.

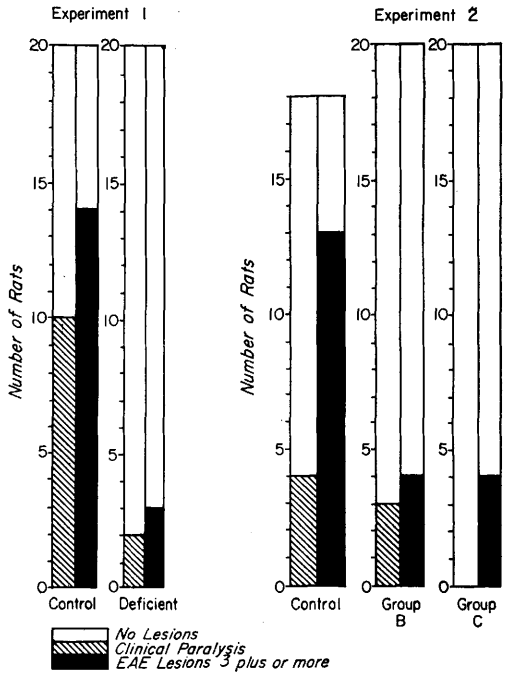
Results. The results are summarized in Table II. There were no significant weight differences in the control and deficient animals. Most paralytic forms of EAE occurred in the early part of the third week after injection of the spinal cord-adjuvant mixture. In Experiment 1 paralysis occurred in 50% of the control rats, whereas, only 10% of the magnesium deficient rats were paralyzed. The differences were even more striking histologically. Significant EAE lesions were found in 70% of the controls and in only 15% of the experimental animals. Not only were fewer lesions found in magnesium deficient rats but those present were usually much less severe than those of the control animals. In Experiment 2 the incidence of paralysis was 22% in the control rats, 15% in Group B and 0% in Group C. The histological evidence of EAE closely correlated with Experiment 1. The incidence was 72% in the controls and 20% in Group B and C. When the myopathy produced by the magnesium deficiency was correlated with the lesions of EAE, it was found that those animals showing moderately severe and severe muscular lesions of magnesium proficiency were generally devoid of EAE lesions and, conversely, that the most severe lesions of EAE occurred in animals with no myopathy. The degree of myopathy, we believe, correlates with the severity of the deficiency state.

There were no differences in the character of "adjuvant disease" outside the CNS. The histopathology of the injected foot pads, lymph nodes and spleen was similar in both experimental and control animals.

Pertussis agglutination titers varied from 1:25 to 1:500 with no significant differences between the various groups and their controls. There was no correlation between the titer and the presence or absence of EAE.

Discussion. The experiments show that magnesium deficiency has a protective effect

Table II
Results of Experiments I and 2



against EAE. This effect is manifested whether the encephalitogenic agent precedes, accompanies or follows the hyperemic phase of the magnesium deficiency. These results suggest that a true hypersensitivity state does not exist in magnesium deficiency and that, on the contrary, there is an apparent increased tolerance to foreign antigens, at least of the type used. Since the experiments were terminated 21 days following injection of the encephalitogenic agent, it could be argued that magnesium deficiency has a delaying rather than a protective role. The period of 21 days was chosen because most paralytic EAE in control and experimental animals occurred in the early part of the third week and because it is known that recovery takes place in most animals not severely affected by the disease. Furthermore, it is difficult to maintain severely magnesium deficient rats alive beyond this period because they tend to die suddenly in convulsive seizures.

The protective effect of magnesium deficiency against EAE is difficult to interpret. Although not completely understood, the pathogenic mechanism of EAE is believed to be a cell-mediated immunological phenomenon, a form of delayed hypersensitivity(9). Some attempts to transfer the process passively have met with partial success only when pooled lymphoid cells were injected into suitable hosts(10). The disease can be prevented by cortisone administration(11) or X-irradiation(12), agents that probably exert their action by virtue of their lympholytic effects. In these conditions, impairment in the production of antibodies also exists(12). In magnesium deficiency the ability to produce antibodies against pertussis vaccine seems to be undisturbed. This, plus the fact that the lymphoid organs appear histologically normal in magnesium deficiency, suggests that the protective effect of magnesium deficiency upon EAE is mediated by a different mechanism than X-irradiation or cortisone administration. Since it has been shown that magnesium ions are necessary for *in vitro* fixation of complement(13) it may be that, although normal amounts of antibodies are produced, their action is altered by low tissue magnesium levels. Complement-fixing anti-brain antibodies occur in animals injected with encephalitogenic mixtures but their role in the pathogenesis of the EAE lesions remains obscure(12,14).

The results of these experiments do not support the view that a hyperimmune state exists in magnesium deficiency. On the contrary, magnesium deficiency is accompanied by increased tolerance to at least the EAE allergenic stimulus. Further study designed to clarify the reason for resistance of the magnesium deficient rat to EAE may aid in understanding the pathogenic mechanisms responsible for production of EAE lesions.

Summary. Acutely magnesium deficient rats exhibit hyperemia of the skin, leukocytosis, eosinophilia, degranulation of mast cells and histaminuria. These changes are preventable with antihistaminic therapy. The histological lesions of more prolonged magnesium deficiency bear a resemblance to those of delayed hypersensitivity. Because of the suggestion of a hyperimmune state in magnesium deficiency, an attempt to produce experimental allergic encephalomyelitis (EAE) in magnesium deficient rats was made. The results show that, rather than enhancing the development of EAE, magnesium deficiency exerts a protective effect. Histological evidence of EAE occurred in 70% of control rats fed normal diets and in only 18% of rats started on a magnesium deficient diet at various intervals before and after injection of the encephalitogenic mixture. Furthermore, the degree of EAE in magnesium deficient rats was inversely proportional to the severity of the myopathy characteristic of magnesium deficiency. The reason for the resistance of the magnesium deficient animal to EAE remains obscure.

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Effect of Large Doses of Subcellular Fractions on Skin Graft Survival in Mice. (30987)

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Injection of large doses of disrupted cells has been successfully used for induction of tolerance to skin graft in mice across the weak sex histocompatibility barrier(1-5).

The distribution of transplantation antigens in the different subcellular fractions was tested by the ability of the latter to produce sensitivity to a subsequently placed skin graft (6). The purpose of this paper is to describe the distribution of transplantation antigens as manifested by their ability to induce tolerance to skin graft across the Y, H_3 and H_2 histocompatibility barriers. Additional pretreatment of the recipients with sublethal doses of irradiation and continuous administration of antigen were also investigated in some of these animals.

Materials and methods. Preparation of antigenic material. The donor animals were sacrificed by ether inhalation. The spleen and liver were immediately removed and put in a 0.25 M sucrose solution at pH 7.2 and 5°C in the ratio 1:4 by volume. The organs were homogenized in an Omni mixer at 16,000 r.p.m. The homogenate was then frozen and thawed 3 times in acetone-CO_2 snow. This treatment resulted in complete disruption of all the cells. The homogenate is then centrifuged in a Lord centrifuge at different speeds depending upon the preparation used. The refrigerated Spinco-ultracentrifuge was used to prepare the 100,000 *g* sediment and supernate. The material was stored at —20°C until used. The time of storage did not exceed one week.

Administration and dosage. In the experiments across the Y histocompatibility barrier (C_{57}BL male to female) the 2,000, 10,000, and 30,000 *g* sediments were given intraperitoneally in doses of 0.25 ml daily for 10 days which corresponded to 9, 3 and 10 mg of dry tissue weight respectively. The 30,000 *g* and 100,000 *g* supernate were administered intraperitoneally in doses of 1 ml daily for 10 days. The 100,000 *g* sediment was diluted with sucrose solution to its original volume and injected I.P. in doses of 1 ml daily for 10 days.

In experiments designed to cross the H_3 histocompatibility barrier difference, the 10,000 *g* and 30,000 *g* sediments were given together as 0.25 ml intravenously for the 1st dose and thereafter daily intraperitoneally for 15 days. The 10,000 *g* supernate which excludes the nuclear and mitochondrial fraction was given in a 1 ml dose intravenously followed by intraperitoneal daily injection of 1 ml for 15 days.

In experiments designed to overcome the H_2 histocompatibility barrier the antigen was injected in a similar manner. The number of daily injections was increased to 20 days.

X-ray irradiation. A dose of 300 r (measured in air) was delivered with a General Electric Maximar 220 X-ray machine under the following conditions: 15 MA, 220 KVP, 60 cm FSD, ¼ mm Cu + 1 mm AL filter. Dose rate under these circumstances was 47 r/min measured in air. Antigen injections