Aggravation by Magnesium Deficiency of Pulmonary Lymphosarcomatosis Occurring Spontaneously in a Strain of Sprague-Dawley Rats

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Zusammenfassung

Männliche, 124 ± 11 g schwere Sprague Dawley-Ratten (HsdIF:CFY, Interfauna, Tuttlingen) erhielten während 28 Tagen eine Mg-Mangel- (Mg-d; 90 ppm Mg, n = 40) oder Mg-Überschuß-Diät (Mg-s; 9000 ppm Mg als Mg-Asp.HCl, Verla-Pharm, Tutzing; n = 30). Untergruppen erhielten 0; 37,5; 75 oder 150 ppm Nickel (Sulfat) im Trinkwasser. Unabhängig von der Ni-Belastung fanden sich bei 47,5% der Mg-Mangel-Tiere über alle Lungenlappen verteilte weißliche, verhärtete und in die Tiefe gehende Bezirke; die Feuchtgewichte betrugen 7,4 ± 3,3 g gegenüber 1,7 ± 0,4 g bei den makroskopisch unauffälligen Lungen, die Mortalität lag bei 17,5%. Lichtmikroskopisch fand sich in 14 von 36 Proben (39%) eine Lymphosarkomatose von höchstem Schweregrad mit z.T. vollständig aufgehobener Struktur des Lungengewebes. – In der Mg-Überschuß-Gruppe trat keine Mortalität auf, die Lungen (2,2 ± 0,4 g) waren makroskopisch unauffällig. Überraschenderweise fanden sich histologisch aber auch hier in allen Fällen Veränderungen im Sinne einer Lymphosarkomatose, wenngleich von beträchtlich geringerem Schweregrad (p = 0,00023, Chi²-Test). Bei Verwendung desselben Stammes, jedoch nach Schaffung von SPF-Zuchtbedingungen (n = 40) waren bei Mg-d bzw. Mg-s Tieren mit einer Ni-Belastung von 0 bzw. 75 ppm Ni (Sulfat) weder makroskopisch noch mikroskopisch Zeichen einer Lymphosarkomatose erkennbar.
Die Pathogenese der Lymphosarkomatose ist unbekannt; nichtsdestoweniger ist es überraschend, daß ein subakuter Mg-Mangel diese Veränderungen dramatisch verstärken kann.

Summary

Male Sprague Dawley rats (HsdIF:CFY, Interfauna, Tuttlingen, Germany) weighing 124 ± 11 g received either a Mg-deficient (Mg-d; 90 ppm Mg; n = 40) or a Mg-sufficient (Mg-s; 9000 ppm Mg as Mg-Asp.HCl, Verla-Pharm, Tutzing, Germany; n = 30) diet during 28 days. The drinking water of subgroups was enriched with nickel (sulfate) at 0; 37.5; 75 or 150 ppm. Independently of Ni, 47.5% of Mg-d rats developed whitish, indurative, subjacent foci of all lobes of the lung; their wet-weight amounted to 7.4 ± 3.3 g in contrast to 1.7 ± 0.4 g of the remainder. Mortality amounted to 17.5%. Microscopic examination revealed lymphosarcomatosis of the highest degree of severity in 14 of 36 samples (39%), partly with completely destroyed lung structure. – No mortality occurred in Mg-s rats and the lungs, weighing 2.2 ± 0.4 g, looked macroscopically normal. Surprisingly, disseminated spots of lymphosarcomatosis became visible on microscopic examination, however at a considerably lower degree of severity (p = 0.00023, Chi²-test). Using the same strain of Sprague Dawley rats, however after providing SPF breeding conditions (n = 40), no signs of pulmonary lymphosarcomatosis were detectable macroscopically or microscopically in Mg-d or Mg-s animals receiving drinking water enriched with 0 or 75 ppm Ni (sulfate).
The etiology of the pulmonary lymphosarcomatosis remains unsolved. Nevertheless it is surprising that subacute Mg deficiency can strongly aggravate these alterations.

Résumé

Des rats de l'embranchement Sprague Dawley (HsdIf: CFY, Interfauna, Tuttlingen), d'un poids de 124 ± 11 g, étaient administrés un régime pauvre en magnésium (Mg-d; 90 ppm Mg, n = 40) ou un régime excédant en magnésium (Mg-s; 9000 ppm Mg comme Mg-Asp. HCl, Verla-Pharm, Tutzing, n = 30) pendant 28 jours. Des sous-groupes recevaient 0; 37,5; 75 ou 150 ppm de nickel (sulfate) dans leur eau potable. Indépendent de l'apport de nickel, on trouvait chez 47,5% rats Mg-d des régions blanchâtres, durcies et profonds, distribuées sur tous les deux lobes du poumon; leur poids humide se totalisait à 7,4 ± 3,3 g vis-à-vis 1,7 ± 0,4 g chez les poumons modestes en sens macroscopique; la mortalité se chiffrait à 17,5%. Chez 14 de 36 (39%) des épreuves l'examination photo-microscopique révélait une lymphosarcomatose au suprême degré, la structure pulmonaire en partie étant détruite complètement. – Dans le group des rats Mg-s, il n'y avait pas des mortalités; les poumons (2,2 ± 0,4 g) étaient macroscopiquement modestes. A notre surprise nous trouvions dans tous ces cas aussi des changements histologiques en sens d'une lymphosarcomatose, quoique à un degré considérablement moins suprême (p = 0,00023, test Chi²). En utilisant le même embranchement des rats Sprague Dawley, pourtant après réalisation des conditions d'élevage (n = 40) SPF, nous ne pouvions pas discerner des signes d'une lymphosarcomatose, ni macroscopiquement et ni microscopiquement, chez les animaux Mg-d et Mg-s avec une charge de Ni de 0 resp. 75 ppm Ni (sulfate).
La pathogenèse de la lymphosarcomatose est inconnue; nonobstant c'est surprenant d'apprendre qu'une carence en Mg subaigue peut redoubler ces changments dramatiquement.

1 Introduction

A large volume of mostly experimental but also of clinical data has been collected so far concerning the potential carcinogenicity of metals, their role in cancer therapy and prevention or concerning their potential to modulate chemical carcinogenesis.

Jacobs and *Pienta* emphasize the often inconsistent results: "It is interesting to note that while many minerals inhibit chemical carcinogenesis, mineral supplementation often increases the growth of established experimental tumors. Furthermore, it is not uncommon for deficiencies in many minerals to result in increased neoplasia [8]." Accordingly the interactions of Mg and cancer are also complex: both Mg load

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and Mg deficit may modulate carcinogenic or anticarcinogenic effects. So far, Mg deficiency has been shown to induce benign lesions or even malignant defects only in rats, and if so, only exceptionally in a minority of animals of a particular strain [3].
With these data in mind it seemed worthwhile to report on pulmonary lymphosarcomatosis aggravated by Mg deficiency, although the primary aim of these investigations was to study interactions between Mg and nickel.

2 Material and Methods

2.1 Animals

In the first series of experiments, 70 male Sprague Dawley rats (HsdIF:CFY) with an initial body weight of 124 ± 11 g (mean ± SD) were purchased from Interfauna, Tuttlingen, Germany, the traditional source of animals used in the Institute since approximately 20 years. Data obtained after the experiments were finished revealed for the first time the presence of several pathogenic viruses and bacteria in this lot of rats, namely SDAV/RCV (Sialodacryadenitis Virus), KRV (Kilham Rat Virus), OPV (Orphan Parvovirus) and Tyzzer's Clostridium piliformis.
In the second series of experiments 40 male Sprague Dawley rats (115 ± 7 g) were purchased from Harlan-Winkelmann, Borchen, Germany. These rats were essentially derived from the Interfauna strain, however after providing SPF conditions.
The animals were randomly distributed into groups of n = 10 in both experimental series.

2.2 Diets and drinking water

A Mg-deficient diet (Altromin C 1035) was used as basal diet, containing 90 ± 5 ppm Mg (Mg-d) as determined by analysis (n = 7). This diet was enriched with magnesium-aspartate-hydrochloride (Verla-Pharm, Tutzing; lot No. 9104-397-411) yielding a final Mg concentration of 8426 ± 96 ppm (Mg-s) as determined by analysis. The diets were offered as pellets ad libitum.
Drinking water (deionized water) was also offered ad libitum and contained different concentrations of nickel (Ni) as sulfate. In the first experiment, Ni concentrations amounted to 0; 37.5; 75 and 150 ppm, and in the second experiment to 0 and 75 ppm Ni.
Animals were observed during 28 days.

2.3 Section and histology

On day 29, all survivors were anesthetised with CO_2 and pentobarbital (60 mg/kg b.w., i.p.). Arterial blood and samples of skin and fur were taken for electrolyte determinations. Abdomen and pleural cavity were carefully inspected. Then the lungs were taken, weighed and transformed into 8% formalin solution.
Using standard techniques histological sections were prepared (n = 6/lung) and stained with hematoxylin-eosin or according to van Gieson. Microscopic evaluation was done under blind conditions. The severity of lymphosarcomatosis was estimated using an arbitrary scale of 0–5 and statistically evaluated using the Chi^2-test.
The photos of the microscopic sections were taken at a magnification of 0.32 × 12.5 × 2.5 (camera × ocular × objective).

3 Results

3.1 First series of experiments

The animals kept on the Mg-deficient diet gained less body weight than the Mg-sufficient rats (Table 1). Increased irritability together with skin alterations became overt after approximately 7 days. Since Ni exerted no obvious effects – neither in Mg-d nor in Mg-s rats – these subgroups were not analyzed separately. Instead of this, only Mg-d and Mg-s groups were compared. Main data are summarized in Table 1.
In the Mg-d diet groups mortality occurred in the 4th week and

Tab. 1: Data of the first series of experiments.

	Mg-d	Mg-s
Initial body weight (g) (day 1)	125 ± 12 (n = 40)	122 ± 10 (n = 30)
Final body weight (g) (day 29)	257 ± 37 (n = 33)	380 ± 25 (n = 30)
Mortality (%)	17.5	0
Macroscopically visible lung tumors (%)	47.5	0
Lung weights (g)		
with macroscopically visible lung tumors	7.4 ± 3.3	–
without macroscopically visible lung tumors	1.7 ± 0.4	2.2 ± 0.4

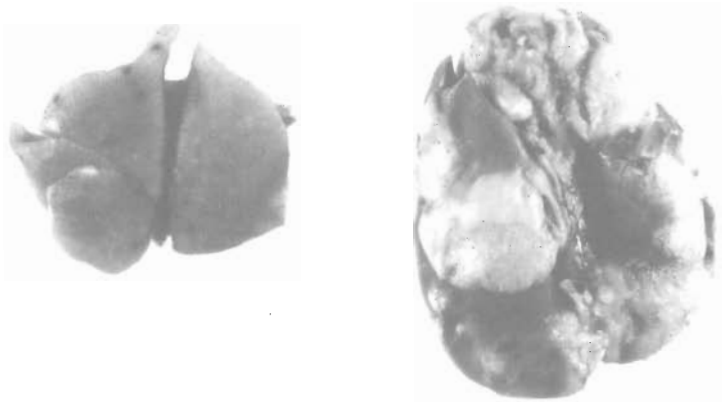

Fig. 1: Rat lung with macroscopically visible lung tumor in comparison to a normal rat lung.

amounted to totally 17.5% in contrast to the Mg-s groups with no mortality. Nearly half of the Mg-d rats (47.5%) showed large macroscopically visible lung tumors, probably inducing mortality. An example is given in Figure 1, in comparison to a normal rat lung.

Microscopical alterations were evaluated according to their severity, where

+: beginning lymphosarcomatosis: isolated sites of the lung show an infiltration of round cells; the lung marking is completely kept; the alveolar walls show mainly no abnormality (Fig. 2),

++: the process is advanced, larger areas are affected; blood vessels showing strong infiltration of round cells are always the initial foci; the progression disseminates to peripheral lung tissues; most alveolar walls are concerned (Fig. 3),

+++: the total lung tissue is affected of diffuse and partly focal infiltrations of round cells; the pulmonary alveoli are scarcely pneumatic (Fig. 4),

+++++: the structure of the lung tissue is totally destroyed; there are only areas of infiltrated round cells and partly secondary accumulation of pus (polymorphonuclear neutrophil leucocytes, based on an inflammation) and necrotic foci (Fig. 5).

Frequencies at which pathogenic findings occurred are summarized in Table 2. Obviously, Mg-deficiency significantly aggravated pulmonary lymphosarcomatosis ($p < 0.00023$).

3.2 Second series of experiments

Using the same strain of Sprague Dawley rats, however after providing SPF breeding conditions, no mortality occurred. Main data are summarized in Table 3. Microscopically, any signs of lymphosarcomatosis were detectable.

Discussion

It is known from the literature that in the rat only – and moreover only in certain strains – Mg deficiency may produce tumor-like proliferations of the intestine [10], periostal desmoid tumors (*Belanger, L.F.*, cited in [3]), thymic lymphoma [1, 2], sarcoma [9], and leukemia [6, 7]. The underlying mechanisms are not yet fully understood, but cell-mediated immunoincompetence probably plays a central role [7]. The data presented in this

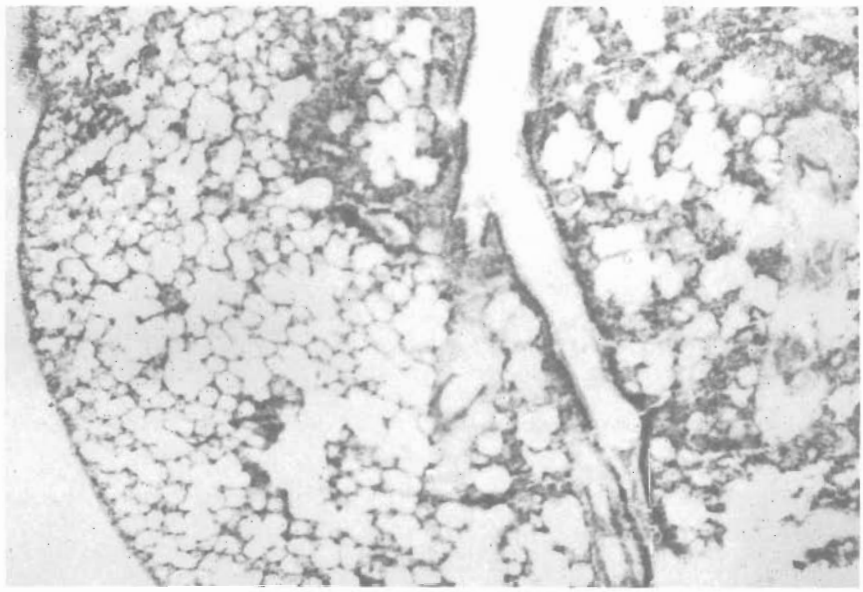

Fig. 2: Lymphosarcomatosis, degree of severity +.

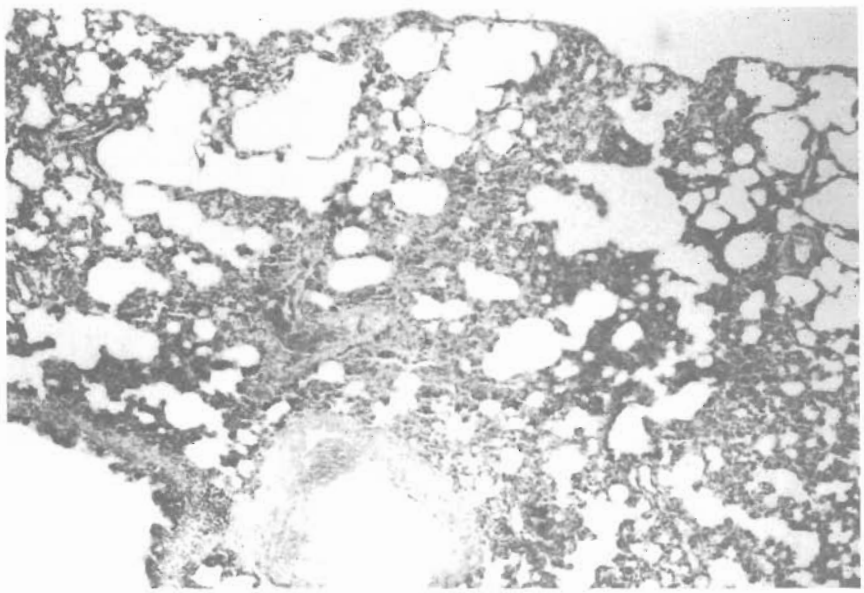

Fig. 3: Lymphosarcomatosis, degree of severity ++.

Tab. 2: Evaluation and frequency of the microscopical alterations. Chi^2-Test: $p = 0.00023$

Degree of severity of the microscopic findings	Mg-d n = 36 (100%)	Mg-s n = 30 (100%)
+	3 (8.3%)	1 (3.3%)
++	13 (36.1%)	9 (30.0%)
+++	6 (16.7%)	19 (63.3%)
+++++	14 (38.9%)	1 (3.3%)

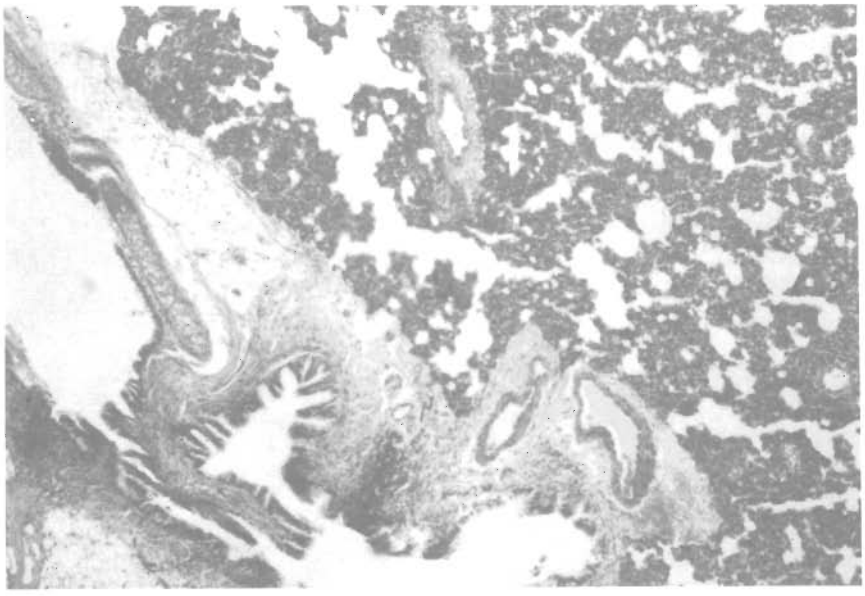

Fig. 4: Lymphosarcomatosis, degree of severity +++.

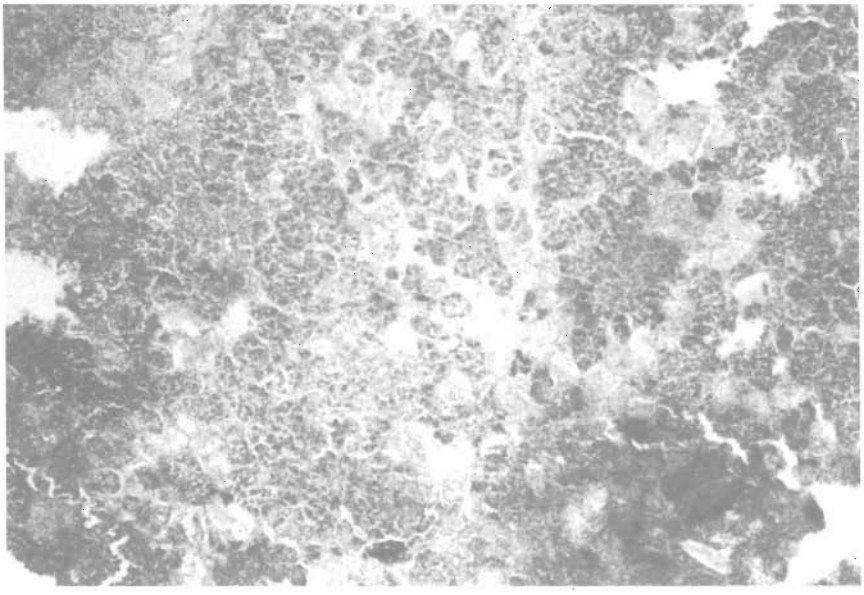

Fig. 5: Lymphosarcomatosis, degree of severity +++++.

Tab. 3: Data of the second series of experiments.

	Mg-d	Mg-s
Initial body weight (g) (day 1)	116 ± 7 (n = 20)	114 ± 7 (n = 20)
Final body weight (g) (day 29)	216 ± 18 (n = 19)	285 ± 18 (n = 20)
Mortality (%)	5	0
Macroscopically visible lung tumors (%)	0	0
Lung weights (g)	1.5 ± 0.2	1.5 ± 0.2

paper are unique since pulmonary lymphosarcomatosis was dramatically aggravated by Mg deficiency within 3–4 weeks inducing 17.5% mortality due to asphyxia. The pathogenesis remains open again. However it seems important to know that Mg deficiency may be induced by chance in toxicological studies when large amounts, e.g. of Mg-poor carbohydrates or modified carbohydrates, are added to a basal diet. As shown not only nephrocalcinosis [4] can be induced under these conditions, but cytotoxicity is generally increased under these conditions [5]. Consequently such effects are 'unspecific' and of course not specifically related to the substance under study. The Sprague Dawley rats used in the first experiment did not fulfill SPF criteria as became overt later. As shown rat-pathogenic viruses and bacteria were present, however, no retroviruses. No pulmonary lymphosarcomatosis in rats has been associated with lung tumors so far. Since these findings could not be reproduced using healthy rats of the same strain the present investigations also stress the necessity to provide optimal SPF conditions for animal experiments. Basal sciences, on the other hand, could also profit if well-defined infected animals were used in combination with other factors.

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