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Changes in histamine and white blood cells in the blood, spleen and thymus of magnesium-deficient rat

par Sylvie CLAVERIE-BENUREAU, B. LEBEL *, Francine GAUDIN-HARDING

Centre d'Etudes pharmaceutiques et biologiques, Laboratoire de Biochimie, ERA CNRS 396, Tour D4, rue J.-B.-Clément, 92290 Châtenay Malabry

* *Laboratoire de Pathologie expérimentale, CHU Necker Enfants malades, 75014 Paris, France.*

Summary. Groups of rats were given either a control or a magnesium-deficient diet. The well-known allergy-like crisis, characterized by vasodilatation with redness of the ears and dermatosis, occurred spontaneously in the magnesium-deficient groups. The histamine (H) content and the distribution of the various white blood cells (WBC) were studied as a function of time. During the acute phase, there was a transitory elevation of total blood H and WBC, mainly affecting the polymorphonuclear (PMN) cells, eosinophils (EO) and basophils (BAS). The EO peak preceded that of H. The EO count was especially high during the first part of the acute phase, while H and BAS were higher during the second part of that phase. There were no BAS's in the blood of the controls and those in the Mg-deficient animals were only partly granulated.

H, EO and mast cells (MC) were elevated in the spleen but not in the thymus during acute deficiency. The high H level in the spleen corresponded to the same high level in the blood. When the spleen suspensions were centrifuged, wide differences in supernatant and pellet histamine appeared, according to the deficiency period.

Introduction.

A well-known allergy-like crisis occurs in rats of different strains after a few days on a magnesium-deficient diet (Kruse *et al.*, 1932 ; Lowenhaupt *et al.*, 1950 ; Santillana *et al.*, 1974). During this period, peripheral vasodilatation is visible on rat ears. Soon after, dermatosis also appears on different parts of the body, and serotoninemia (Itokawa *et al.*, 1972) and histaminemia (Bois *et al.*, 1963) rise. The total amount of white blood cells (WBC) also increases and the proportions of various cell types are altered (Battifora, 1971 ; Hungerford and Karson, 1960).

Recently, it was shown that spleen blastic transformation after phytohemagglutinin (PHA) stimulation, was greater in control than in magnesium-deficient rats. The decrease in the stimulation index (cpm with PHA/cpm without PHA) was much higher during the hyperemic period. A negative correlation was found between the stimula-

tion index and histaminemia (Armier *et al.*, 1979). The histamine level could either be considered as a factor determining the period of the allergy-like crisis or as a direct or indirect mediator modulating mitogenic stimulation, leading to a lower stimulation index. Thus, we first compared the histamine levels in the splenic tissues, as well as in the thymus (another lymphoid organ), of both the control and the magnesium-deficient groups. The present paper reports the distribution of histamine (studied in spleen cells from either control or deficient animals) estimated by comparing total histamine with pellet histamine after the cells, suspended in buffer, were centrifuged under conditions similar to those used prior to the blastic transformation assay after lectin stimulation.

The major part of body histamine appears to be derived from the basophils and mast cells. Several factors are involved at different times during the onset and progression of the immediate hypersensitivity crisis ; immunoglobulin E, cyclic AMP and calcium are known to act on the degranulation process, leading to the release of numerous mediators (histamine, serotonin, slow-reacting substance of anaphylaxis, eosinophilic chemotactic factors, heparin, platelet-activating factor). The eosinophils play a role in these processes (Hubscher, 1977 ; Lalaurie and Modat, 1978 ; Kay, 1979 ; Gleich *et al.*, 1979). We thought it would be interesting to study the changes in the number of eosinophils and other WBC forms occurring parallel to changes in the histamine levels in the blood and in two immune system organs, the spleen and the thymus.

Material and methods.

Three similar experiments were carried out successively using two groups of Wistar CF rats (« Janvier » production) with a mean weight of 90 g and fed either a control diet (40 mg Mg/100 g diet) or a deficient diet (4 mg Mg/100 g diet). The semi-synthetic diets have been described previously (Santillana *et al.*, 1974). The animals were weighed twice a week ; small samples of peripheral blood were taken from the tail and placed on EDTA twice a week during the hyperemic period and once a week otherwise. Total histamine was determined on 0.1 ml by the fluorometric method of Shore *et al.* (1959), as automatized by Lebel (1980). After staining by the usual May-Grunwald and Giemsa (MGG) method, the different WBC's were determined on a drop of blood on a glass slide. The eosinophils were directly counted with the method of Piette (1961), and the total WBC's by the usual method. At different times, groups of rats were sacrificed and several organs taken out for print examination and histamine determination. The spleen cells were obtained by dilacerating the spleen in a RPMI medium. The suspension was left standing 3 min in order to discard the tissue fibers ; the top suspension was centrifuged and the pellets used for histamine estimation after being suspended and centrifuged twice.

Each mean result is expressed $\pm$ SEM (standard deviation/$\sqrt{n}$). The significance of the difference between two means was estimated with Student's t-test when the distribution was appropriate, and otherwise with the non-parametric Wilcoxon-Mann-Whitney test (WMW). Only statistically different means are shown with the probable error.

Results.

Comparison of magnesium-deficient and control rats.

Growth and food intake. — The growth and food intake of the deficient animals were slightly depressed in all experiments, as previously described (Santillana *et al.*, 1974).

White blood cells. — The composition of the different WBC's is given in table 1 as a percent of the total WBC's. The proportion of lymphocytes was depressed, while that of the polymorphonuclear (PMN) cells increased considerably. Three periods were defined during deficiency according to their clinical signs : appearance of redness of the ears (period I), decrease of redness of the ears and increase of dermatosis (period II), disappearance of clinical symptoms (period III). The total WBC's and direct eosinophil counts, together with the histamine levels, are given in table 2 and figure 1.

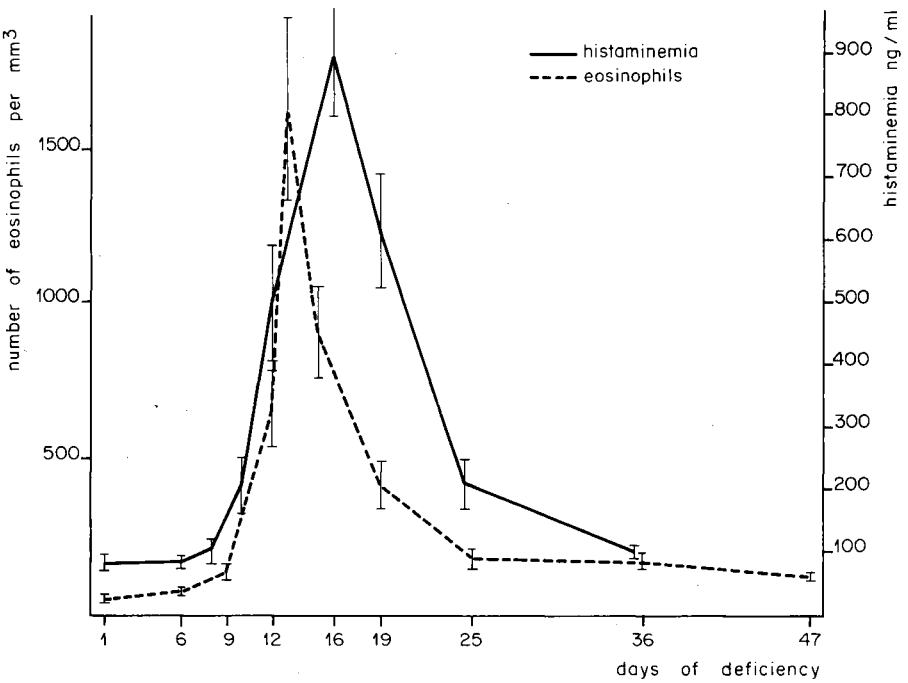

FIG. 1. — *Changes in eosinophils and histaminemia during magnesium deficiency in Wistar rat.*

From these data, it can be assumed that the total WBC's, as well as the histamine levels, mainly increased during period II of the acute deficiency phase. On the contrary, the eosinophil counts were higher in period I, as shown either by direct count (table 1) or by MGG staining. The basophils were more numerous in period II when the histamine levels were high. It is noteworthy that the basophils, exclusively observed during the acute deficiency phase, were only partially granulated.

TABLE 1

Changes in the different white blood cells during magnesium deficiency in rat

			Number of data per group	Lymphocytes	Polymorpho-nuclear cells	Eosinophils	Basophils *	Monocytes
				(p. 100 of total WBC)				
Control			20	85.6 ± 3.9	12.0 ± 0.8	0.5 ± 0.1	0	1.9 ± 0.2
Magnesium-deficient	Acute phase	I From day 8 to day 10	10	44.1 ± 4	49.1 ± 5.0	4.2 ± 0.8	0.6 ± 0.2	2.0 ± 0.2
		II From day 16 to day 18	10	51.2 ± 1.9	41.5 ± 2.3	1.8 ± 0.3	3.2 ± 0.8	2.3 ± 0.3
	Chronic phase	III After 30 day	10	82.7 ± 6.0	15.1 ± 1.5	0.6 ± 0.1	0.1 ± 0.1	1.5 ± 0.2

± SEM, * Partly granulated.

TABLE 2

Changes in total white blood cells, eosinophils and histaminemia during magnesium deficiency in rat (± SEM)

			Total WBC's per mm³	Eosinophils	Histamine (ng/ml)
Control			8 398 ± 804 (10)	66 ± 6 (20)	101 ± 10 (15)
Magnesium-deficient	Acute phase	I Day 8 to day 10	24 520 ± 3 100 (10)	1 152 ± 170 (20)	438 ± 114 (10)
		II Day 16 to day 18	41 032 ± 6 300 (10)	538 ± 39 (20)	862 ± 141 (10)
	Chronic phase	III After day 30	13 630 ± 745 (10)	191 ± 16 (20)	174 ± 23 (10)

Weight, histamine content, number of eosinophils and print examination of spleen and thymus. — Table 3 shows the weight and histamine content of the spleen and the thymus as well as blood histamine at the same period. The usual hypertrophy of the spleen in magnesium-deficient animals, confirmed in this experiment, started in period II and became more pronounced in period III. It would have been even more marked, if the values had been calculated per 100 g of body weight.

TABLE 3

Weight and histamine content of spleen and thymus of magnesium-deficient rats at different periods of deficiency

		Number of animals	Weight (mg)		Histamine (ng)		
			Spleen	Thymus	Spleen /100 mg	Thymus /100 mg	Blood /ml
Control	Day 8 to day 40	4	350 ± 28	363 ± 24	150 ± 25	534 ± 59	80 ± 5 (11)
Magnesium-deficient, acute phase	I Day 8 to day 12	4	346 ± 20	332 ± 48	135 ± 14	561 ± 41	248 ± 29 (15)
	II Day 14 to day 18	7	517 [b] ± 54	327 ± 13	660 [a] ± 197	861 ± 81	899 [a] ± 118 (10)
Control	Day 40 to day 56	4	535 ± 55	433 ± 44	101 ± 19	1 004 ± 145	78 ± 8 (5)
Magnesium-deficient, chronic phase	III Day 40 to day 56	6	709 ± 98	292 ± 19	290 ± 88	1 256 ± 49	113 ± 10 (10)

± SEM ; P values : [a] < 0.01 ; [b] < 0.02.

The histamine content, higher at stages II and III of deficiency when blood histamine was high (10 times the control content), showed greater differences (4 times higher) with the controls at period II. Although the histamine content of the thymus was higher than that of the spleen, the deficiency had little or no effect on it. Table 4 shows the histamine patterns in the spleen and the thymus and the remaining pellet histamine when the cells were suspended in a RPMI medium and centrifuged. The ratio (pellet histamine/total histamine) × 100 has been calculated. These data show that, as in the previous experiment (table 3), the splenic histamine content was particularly high at period II of deficiency when half of the histamine remained in the cells. The thymic histamine content, higher than in the spleen and unrelated to the deficiency, showed large intra-group variations. The same proportion of histamine remained in the cells when they were suspended in RPMI and centrifuged. It can thus be assumed that splenic

TABLE 4

Pattern of histamine content in spleen and thymus cell suspensions during magnesium deficiency in rat

			Spleen				Thymus				
			Histamine			Millions of cells per total organ	Histamine			Millions of cells per total organ	Number of animals per group
			1	2	3		1	2	3		
Control		Day 7 to 16	538 ± 103	86 ± 26	16 ± 2	201 ± 26	1 420 ± 140	284 ± 28	20 ± 4	675 ± 97	3
		Day 40 to 56	475 ± 82	114 ± 24	24 ± 1	306 ± 76	4 785 ± 837	622 ± 120	13 ± 0,6	856 ± 112	4
Magnesium-deficient	Acute phase	Days 4 and 5	350 ± 33	85 ± 11	25 ± 1	214 ± 19					5
		Days 7 and 8	1 500 ± 333	480 ± 101	32 ± 4	422 ± 72	1 576 ± 242	283 ± 36	18 ± 2	537 ± 58	4
	Chronic phase	Day 8 to 16	4 735 [a] ± 354	2 320 [a] ± 395	49 ± 5	395 ± 31	1 868 ± 286	392 ± 21	21 ± 2	478 ± 96	3
		Day 40 to 56	1 026 ± 234	308 ± 128	30 ± 2	443 ± 75	2 885 ± 405	432 ± 37	15 ± 1	655 ± 44	6

± SEM, P values : [a] < 0,01.
1 : histamine (ng in the whole organ) ; 2 : histamine (ng remaining in pellet after two sets of centrifugation resuspension) ; 3 : 2/1 × 100.

histamine was influenced by magnesium deficiency and that 50 p. 100 remained in the cellular part during the acute deficiency phase. The variations in the thymus were not significant at the same age : after centrifugation, the cells represented only 13 to 21 p. 100 of the total histamine.

Data on the examination of the spleen prints and eosinophil count on a spleen suspension are given in table 5. Numerous eosinophils and mast cells were present in the spleen of magnesium-deficient animals, while they were rare in the control group. This has been confirmed for the eosinophils by direct count of a spleen cell suspension : the number of eosinophils in the whole spleen after 10 days on the diet was 750 times higher in the deficient than in the control rats at the same age.

TABLE 5

Print examination and eosinophil (EO) count of spleens from magnesium-deficient and control rats

	Print examination (1)	Days on diet	Number of EO *	
			$\times 10^{-3}$ per 100 mg spleen	$\times 10^{-3}$ in the whole spleen
Control	rare EO	4	11.26	35.92
	rare mast cells	10	25.80	118.71
Magnesium deficient	very numerous EO	4	41.78	123.24
	numerous mast cells	8	319.09	1 512.50
		10	11 626.00	88 465.76

(1) 4 observations in this particular experiment ; numerous similar observations in previous experiments.

* In one typical sample of each group, the sample being selected after print examination.

Discussion and conclusion.

The usual allergy-like crisis of the magnesium-deficient rat is easy to reproduce, but the reasons for the onset of this crisis in the rat, and not in other species, are still not understood. But whatever the initial onset, the patterns of the different parameters involved are worth studying, and the rat allergy-like crisis is perfectly reproducible. Among these parameters are basophils and mast cells, histamine and eosinophils. The easiest place to study the changes in these parameters, using a non-destructive method, is in the blood. However, this study does not necessarily reflect the actual situation in the various tissues, as suggested by Scheinmann *et al.* (review 1979), who state that for one eosinophil in the blood of normal rats, there are 200 in the bone marrow and 200 in the tissues. We have shown that the peak for blood eosinophils occurs before the histamine and basophil peaks. It should be noted that, under normal conditions, basophils have been rarely observed in rats of different strains (Mitruka and Rawnsley, 1977). In magnesium-deficient rats, the basophils, appearing at the acute phase and showing a peak in period II of the acute phase, were only partly granulated and looked immature, like those described by Combs *et al.* (1965). Would these basophils be compensating for the drop in mastocytes observed by Bois (1963) in tissues such as dermis ? In the duode-

nal submucosa, Kraeuter and Schwartz (1980) noticed that a number of immature-looking mast cells increased with progressive deficiency, but that, at the same time, the histamine content in the peritoneal mast cells was low compared to that of the controls.

We have particularly studied the spleen which hypertrophies in magnesium-deficient rats, and spleen cells are frequently assayed in immunological studies. Our team has already investigated the comparative blastic stimulation effect of PHA on spleen cells (Armier *et al.*, 1979) at different periods of magnesium deficiency and has shown a negative correlation between histaminemia and the stimulation index in the hyperemic period. We now demonstrate that when histaminemia is high, histamine is also high in the spleen. When the cells are isolated under conditions similar to those used for PHA stimulation assays, 49 p. 100 of the total histamine (2 320 ng for the whole organ) remains in the cells during period II of the acute phase, while only 20 p. 100 (86 ng for the whole organ) remains in the cells of control animals. The increased impairment of splenic cell stimulation by PHA might thus possibly be attributed to histamine. The *in vitro* work of Wang and Zweiman (1978) on human lymphocytes shows that an addition of histamine impairs lymphocyte response to mitogen, and PHA has been reported to be active in the release of histamine from mast cells (Hook *et al.*, 1974). However, these authors used relatively high concentrations, while at lower concentration histamine has a mitogen-stimulating effect (Morgan *et al.*, 1975). The histamine remaining in the spleen cells might be mainly located in the mastocytes, or be bound to a subpopulation of B and T lymphocytes, as suggested by Ballet and Merler (1976) studying human cells.

During the acute phase, the histamine content is fairly constant in the thymus of magnesium-deficient rats and is not significantly different from that of controls of the same age.

During the acute deficiency phase, the eosinophils and mast cells are numerous in the spleen of magnesium-deficient animals. The role of the eosinophils is not easy to define because of the many possibilities of these cells, but they are likely to play an active part in the modulation of the allergic inflammation reaction. Numerous enzymes are present in eosinophils : histaminase, inactivating histamine ; arylsulfatase, inactivating the slow-reacting substance of anaphylaxis (SRSA) ; phospholipase D, degrading the platelet-activating factor (PAF) ; peroxydase, differing from neutrophil peroxydase (review of Weller and Goetzl, 1979). Moreover, a major basic protein (MBP), neutralizing heparin, was found in the granule of eosinophils (Gleich, 1977). Another important eosinophilic factor, shown by Hubscher (1975), is the eosinophil-derived inhibitor (EDI) of released histamine which might also play a role.

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Résumé. Des groupes de rats reçoivent soit un régime témoin soit un régime carencé en magnésium. La crise d'apparence allergique connue depuis longtemps apparaît spontanément dans les groupes carencés. Cette crise est caractérisée par une vasodilatation péri-

phérique, rougeur des oreilles puis dermatose. La teneur en histamine (H) et la distribution des différents leucocytes sont étudiées en fonction du temps. Dans le sang, pendant la période aiguë, il y a une élévation transitoire de H, des leucocytes totaux, affectant particulièrement les polynucléaires, les éosinophiles (EO) et les basophiles (BAS). Le pic des EO précède celui de H. Le nombre des EO est particulièrement élevé pendant la première partie de la phase aiguë tandis que H et BAS sont plus élevés pendant la deuxième partie de la phase aiguë. Il n'y a pas de BAS dans le sang des témoins et les BAS des animaux carencés en magnésium ne sont que partiellement granulés.

Dans la rate, mais pas dans le thymus, une élévation des mastocytes est observée pendant la phase aiguë de la carence. H est particulièrement élevée dans la rate, quand elle est élevée dans le sang. Il y a de grandes différences dans la distribution de H dans les surnageants et culots de centrifugation des cellules sphéniques provenant soit de rats carencés, soit de rats témoins.

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