EFFECT OF MAGNESIUM DEFICIENCY ON MAST CELLS AND URINARY HISTAMINE IN RATS*

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THE peripheral vasodilation occurring in rats fed a magnesium deficient diet has been well described some thirty years ago by McCollum and co-workers (McCollum and Orent, 1931 ; Kruse, Orent and McCollum, 1932). Only recently was this change related to modifications in the number of subcutaneous mast cells by Bélanger, Van Erkel and Jakerow (1957). Other workers have also noted that rats fed a magnesium deficient diet develop leucocytosis with eosinophilia (Kashiwa and Hungerford, 1958) as well as an infiltration of eosinophils around small blood vessels (Lowenhaupt, Schulman and Greenberg, 1950) and in submaxillary glands, lungs and mesenteric lymph nodes (Hungerford and Karson, 1960). Furthermore, plasma and urinary histamine concentration increase concurrently with the development of peripheral vasodilation (Bois, Gascon and Beaulnes, 1963) and regeneration of mast cells is impeded in magnesium deficient rats injected with compound 48/80 (Bois, Byrne and Bélanger, 1960).

The syndrome of magnesium deficiency develops more or less rapidly according to age. In young animals (3–4 weeks), the first signs of peripheral vasodilation appear within 1 or 2 days and last for approximately 10–12 days. The erythema is generalized and accompanied by a slight oedema of the ears, snout and paws. This change may subside momentarily if the animals are suddenly frightened by brisk handling (Bois, unpublished). After 2 weeks, convulsive seizures are frequent and often lethal ; only a few animals can survive for 4 weeks. Older rats can withstand the convulsive episodes much better ; rats of 5–6 weeks (100 g. body weight) can be kept chronically deficient for as long as 3 months.

The present report concerns the changes in distribution of mast cells in the tongue and in subcutaneous tissue of chronic magnesium deficient rats. Attempts have been made to correlate urinary histamine excretion and peripheral vasodilation with the decrease in mast cell population. Special attention was also given to modifications in staining affinities of mast cells after prolonged magnesium deficiency.

MATERIAL AND METHODS

Fifty male Holtzman Sprague-Dawley rats (average body weight 110–115 g.) were subdivided into two groups. The first group (30 rats) received a magnesium deficient diet slightly modified from that of Van Reen and Pearson (1953) and demineralized water as drinking fluid. The composition of the magnesium deficient diet was as follows : constituents (per cent) —purified casein (23·0), dextrose (58·7), gelatin (5·0), dl-methionine (0·3), corn oil (5·0), minerals (6·0), vitamin mix (2·0) (obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.) ; minerals (g.)—calcium carbonate (45·0), potassium phosphate dibasic

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(18·0), calcium phosphate monobasic (12·2), sodium chloride (20·0), ferric citrate (4·0), manganous sulphate (0·6), potassium iodide (0·13), zinc carbonate (0·035), copper sulphate (0·035). Controls (20 rats) had the same diet into which had been blended 600 mg. of magnesium sulphate per 100 g. of diet.

Rats were killed after 10, 20 and 60 days ; after autopsy the ears, lower lip, tongue, and specimens of the skin of the abdomen, scrotum and paws were dissected and fixed in alcohol formol acid (Bélanger and Bois, 1960). The tongue was cut transversely at its base immediately anterior to the frenulum linguae and fixed ; another section 4–5 mm. anterior to the first one was made with a razor blade a few hours later thereby producing a small block easily recognizable both macroscopically and microscopically by the absence of a central furrow on the dorsal surface. Paraffin sections were stained with toluidine blue (Bélanger and Hartnett, 1960), Padawer's stain (1959), diluted Wright (Bélanger *et al.*, 1957) and combinations of alcian blue-toluidine blue and alcian blue-safranin (Spicer, 1960). Sections were also stained with Delafield haematoxylin lightly differentiated (3 sec. in 0·2 per cent HCl in 95 per cent alcohol) and phloxine. Finally, a few specimens were frozen and cut in a cryostat (Pearse) and sections stained with diluted Wright, with and without post-fixation with formalin. Mast cell counts in the tongue were performed on every tenth section (8 μ thick) stained with Padawer's stain ; 5 sections per block were counted using the following procedures : the whole transverse section of the tongue was projected on a paper at a magnification of $\times 110$. A central area was demarcated on the projected image (Fig. 1) ; mast cells were identified by a pencil dot on the paper and counted in both central and peripheral regions. Urinary histamine determinations were made with the guinea-pig ileum method on urinary specimens obtained after 1, 5, 10, 15, 20, 25, 35, 45, and 60 days (Bois *et al.*, 1963).

RESULTS

Magnesium deficiency : 10 *days*

After 10 days of magnesium deficient diet, all animals developed peripheral vasodilation more pronounced in the ears, snout, paws and scrotum. Frequent periods of scratching as well as a few convulsive seizures (4 rats died) were noted. Histological sections revealed a slight change of distribution of mast cells especially in the ears, paws and lower lip ; cells immediately underneath the epidermis were less numerous and partially degranulated. On the other hand, mast cells in the tongue were not significantly influenced (Table I). Eosinophils were seen in sections of all tissues studied ; vasodilation was easily confirmed on cryostat sections showing dilated capillaries, especially in the ears ; eosinophils were also numerous in the capillaries themselves. Urinary histamine was markedly increased (Table II).

Magnesium deficiency : 20 *days*

After 20 days, most magnesium-deficient rats were blanched except for a few animals showing brief and sporadic episodes of hyperaemia mostly limited to the

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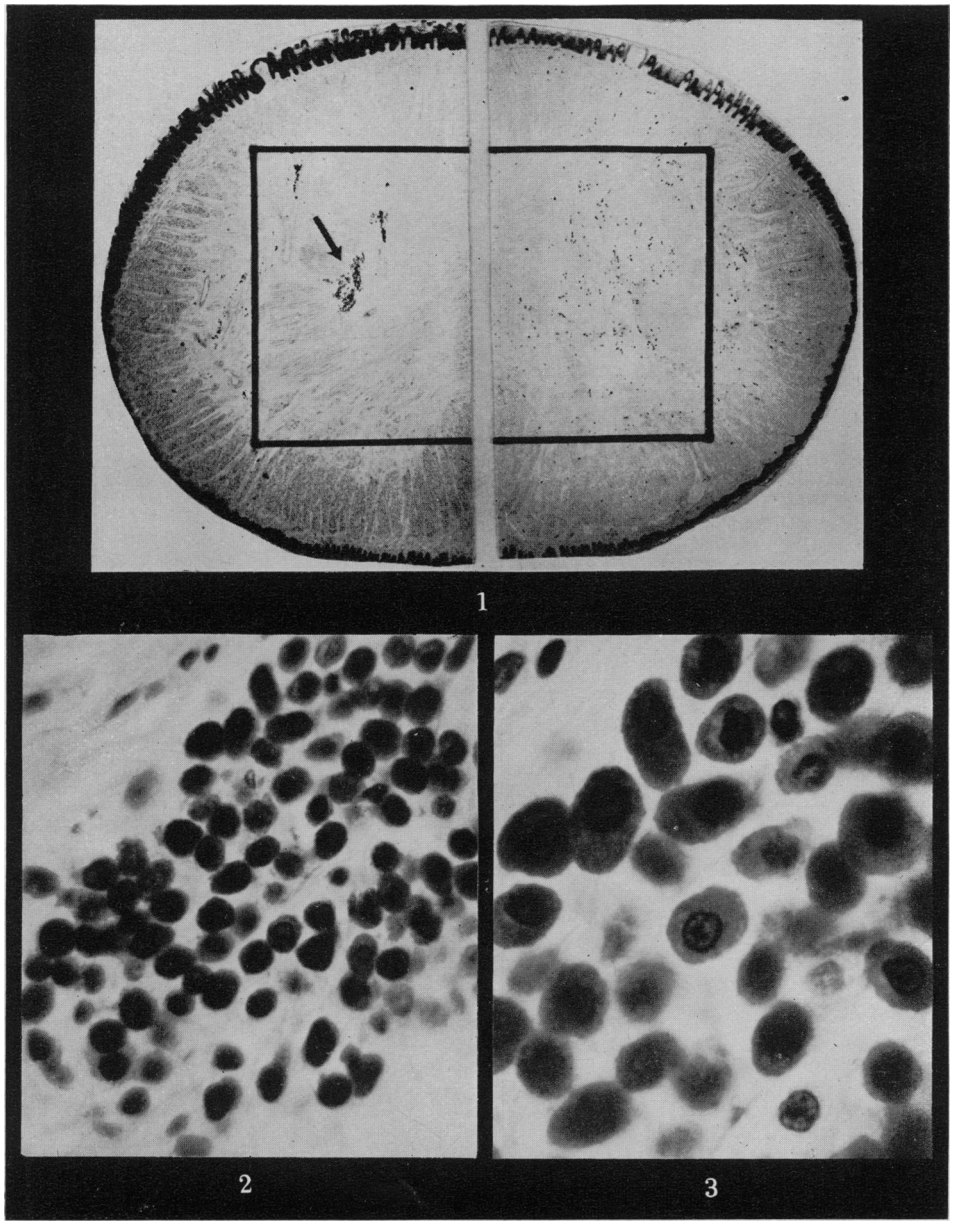
EXPLANATION OF PLATE

FIG. 1.—Two halves of transverse sections of the rat tongue stained with toluidine blue. On the left after 60 days of magnesium deficiency ; on the right, control tongue. The central rectangular frame was used for counting the mast cells and delimits the central from the peripheral zone. Note on the left the presence of an especially dense mast cell cluster (arrow) in the central zone. Compare with the uniform distribution of cells in the control section (right). Similar clusters were found only in a few sections of magnesium deficient tongues ; other sections were very poor in mast cells. $\times 20$.

FIG. 2.—Section through the same cell cluster indicated by the arrow in Fig. 1 at higher power. Note the spheroidal appearance of the cells. Delafield haematoxylin and phloxine. $\times 230$.

FIG. 3.—Higher power of Fig. 2. The comparatively large nucleus of the cells is clearly outlined. Because of the cluster arrangement and the size of the nucleus one might question the identification of these cells with mast cells ; on the other hand they are well filled with granules that stain with the classical metachromatic dyes.



Bois.

ears. Five more rats had died : sections of the ears, lower lip, skin of the abdomen, paws and scrotum stained with toluidine blue, Padawer's stain or Wright showed a change in distribution and shape of mast cells ; most of the cells closer to the surface of the skin had disappeared, leaving a few poorly granulated and pleomorphic cells. Mast cell counts in the tongue revealed a slight decrease in number and a change in distribution of the cells (Table I). The decrease was as much important in the peripheral zone as in the central zone of the tongue. Partially degranulated cells were seen in both zones and in some cases cells were found possessing both granules stained with alcian blue and granules stained with toluidine blue or safranin. Tissue eosinophils were still present though less numerous than after the 10 days period. Urinary histamine had considerably decreased in comparison with the previous period (Table II).

TABLE I.—*Mast Cell Counts in Transverse Sections of the Tongue of Magnesium Deficient Rats. Mean ± S.E.*

Groups	Time	No. of rats	Zone*		Total
			Peripheral	Central	
Controls	10 days	4	537±23	953±58	1490
Deficient		4	504±11	846±68	1350
Controls	20 days	4	400±20	886±20	1286
Deficient		4	331±34	536±83	867
Controls	60 days	5	380±33	903±17	1283
Deficient		5	34±9	206±83	240

* See Fig. 1.

TABLE II.—*Effect of Feeding a Magnesium Deficient Diet on Urinary Excretion of Free Histamine* in Male Rats*

	Days of magnesium deficiency								
	1	5	10	15	20	25	35	45	60
Controls	4·4 ±1·7	2·9 ±0·9	3·4 ±0·2	4·2 ±0·4	3·4 ±0·8	4·6 ±0·5	5·3 ±1·6	4·9 ±0·7	5·9 ±1·1
Deficient	4·7 ±1·7	10·7 ±3·6	19·5† ±5·0	29·8† ±5·3	4·0 ±0·8	4·8 ±0·9	7·4 ±1·0	7·8 ±1·7	8·0 ±1·8

* Mean values from 6 rats per group ±S.E. expressed in μg. of histamine per rat/24 hr.
† $P < 0{\cdot}01$.

Magnesium deficiency : 60 days

After 60 days, all the rats were blanched and appeared anaemic ; the peripheral hyperaemia was replaced by a more or less important white oedema, which was more conspicuous in the paws. Four more rats had died. Numerous skin lesions of the snout and ears were noted, possibly from scratching. The total number of mast cells was greatly reduced in the tongue (Table I) and in the other tissues. With regards to the distribution of the cells the tendency observed after 20 days was more pronounced ; mast cells were sometimes grouped in isolated clusters in the central zone of the tongue (Fig. 1). In some sections, only 15–20 cells could be counted. With the low magnification used for counting, only those cells well stained with Padawer's stain could be marked on the paper. Padawer's stain was especially useful for this purpose due to its fine selectivity for metachromatic granules. It is noteworthy that most of the cells in the clusters were rounded with a large nucleus. This was clear when stained with Delafield haematoxylin ; both

the granules (light blue) and the nuclei (dark blue) showing as on Fig. 2 and 3. A few cells exhibited dual colorations with alcian blue-toluidine blue and alcian blue-safranin.

Regions with white oedema contained very few mast cells with rare monocytes, neutrophils and histiocytes ; eosinophils had almost totally disappeared. Urinary histamine was still slightly higher than normal (Table II).

DISCUSSION

The induction of magnesium deficiency in rats by dietary means produces peripheral vasodilation associated with a number of manifestations typical of histamine release such as mast cell degranulation (Bélanger *et al.*, 1957), elevation of plasma and urinary histamine (Bois *et al.*, 1963) and eosinophilia (Kashiwa and Hungerford, 1958). The present report confirms and extends these observations. It is noteworthy that after 10 days of magnesium deficiency, during the period of maximal hyperaemia, changes in the number and morphology of mast cells were less important than the eosinophilia and the marked increase in plasma (Bois *et al.*, 1962) and urinary histamine. In other words, histamine would be liberated with little mast cell degranulation as proposed by Smith (1958) or conversely regranulation would proceed as fast as degranulation. Our previous experiments do not support this last hypothesis since mast cell regeneration appears to be inhibited in magnesium deficient rats (Bois *et al.*, 1960). It must be recalled that no erythema appeared in magnesium deficient rats previously treated with a potent mast cell degranulating agent, compound 48/80 (Bois *et al.*, 1960). These observations indicate that mast cells must be present at their normal sites for the initial development of peripheral vasodilation in magnesium deficient rats. After 20 days or more of magnesium deficiency the progressive decrease in mast cell population is considerably more important than the urinary increase of histamine excretion and peripheral vasodilation has been replaced by blanching. Therefore, there does not appear to be a strict parallelism between mast cell degranulation, peripheral vasodilation and level of urinary histamine excretion. Further studies on tissue histamine and serotonin in these animals should help clarify this situation.

Another explanation for the high plasma and urinary histamine might be that newly synthesized histamine is not " bound " in magnesium deficient rats, or might also be the result of an abnormal absorption of bacterial histamine from the gastro-intestinal tract ; however, use of antibiotics in the diet did not modify the syndrome (Bois, unpublished).

The change in distribution and shape of subcutaneous mast cells is yet difficult to interpret. According to Bélanger *et al.* (1957), pleomorphism would represent an increased amoeboid activity of the cells.

The presence of isolated clusters of mast cells in the central zone of the tongue was an unexpected finding. These are apparently young cells as judged from their large rounded nucleus ; the dual staining of some of these with alcian blue-toluidine blue or safranin reveals a modification in the cell constituents but does not permit an interpretation as to a maturation or a degranulation phase. It must be recalled however, that the total number of mast cells is considerably reduced.

The development of subcutaneous white oedema after 60 days of magnesium deficiency could have a variety of causes. The most important being probably the decreased protein synthesis (Menaker and Kleiner, 1952) and the prolonged secondary electrolyte disturbances (MacIntyre and Davidsson, 1958).

Finally, the changes in number and distribution of mast cells after prolonged magnesium deficiency cannot be ascribed to a non-specific effect of the deficiency since severe calcium deficiency does not produce it (Bois, unpublished) and the number of mast cells is even increased, in some tissues such as the bone marrow in calcium deficient rats (Urist and McLean, 1957).

Magnesium deficiency presents a special interest because it creates a situation of histamine liberation without the use of exogenous liberators. Such an experimental situation may provide a new approach to the study of histamine release and mast cell function.

SUMMARY

The changes in number and distribution of mast cells in the tongue and in subcutaneous tissue of the rat, after 10, 20 and 60 days of magnesium deficiency have been studied. Mast cell counts in the tongue revealed a constant decrease in the total number of the cells. After 60 days, isolated clusters of mast cells were abnormally distributed in the central zone of the tongue ; some cells possessed both granules stained with alcian blue and granules stained with toluidine blue or safranin. Attempts have been made to correlate urinary histamine excretion and peripheral vasodilation with the decrease in mast cell population. Apparently, no strict parallelism exists among these three phenomena.

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