The suggested role of the kidney in protecting against the development of hypertension through release of depressor substances may also be applicable(13). Thus, a depletion or exhaustion of these materials in chronic renal hypertension might lead to increased responsiveness to pressor agents. In addition, impairment of the ability of the sympathetic nervous system to buffer a pressor response in the chronic hypertensive state could account for the data.

Summary. Renal hypertensive rats were found to have a pressor response to angiotensin which exceeded that of normal rats in both magnitude and duration. The results would appear to be in keeping with an intrinsic increase in pressor reactivity of the hypertensive organism, but are not compatible with the production of a specific angiotensinase as an adaptive mechanism to prevent chronic angiotensinemia.

1. Page, I. H., Bumpus, F. M., *Physiol. Rev.,* 1961, v41, 331.

2. Wolf, R. L., Mendlowitz, M., Gitlow, S. E., Naftchi, N., *Circulation,* 1961, v23, 754.

3. Wolf, R. L., Mendlowitz, M., Pick, J., Gitlow, S. E., Naftchi, N., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 308.

4. Khairallah, P. A., Page, I. H., Bumpus, F. M., Smeby, R. R., *Science,* 1962, v138, 523.

5. Hickler, R. B., Lauler, D. P., Thorn, G. W., *J. Clin. Invest.,* 1963, v42, 635.

6. Wood, J. E., *Circulation,* 1962, v25, 225.

7. Blaquier, P., Bohr, D. F., Taquini, A. C., Jr., Hoobler, S. W., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 711.

8. Bing, J. A., *Acta Pathol. and Microbiol. Scand.,* 1962, v56, 385.

9. Khairallah, P. A., Bumpus, F. M., Page, I. H., Smeby, R. R., *Science,* 1963, v140, 672.

10. Shapiro, A. P., *J. Lab. and Clin. Med.,* 1960, v55, 715.

11. Mendlowitz, M., Naftchi, N. E., Wolf, R. L., Gitlow, S. E., *Am. J. Cardiol.,* 1962, v9, 680.

12. Shapiro, A. P., Moutsos, S. E., Krifcher, E., *J. Clin. Invest.,* 1963, v42, 978.

13. Dahl, L. K., *Ann. Rev. Med.,* 1963, v14, 69.

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Role of Histamine in Producing the Eosinophilia of Magnesium Deficiency.* (28864)

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Magnesium deficiency results in a blood and tissue eosinophilia(1,2). The blood eosinophilia is especially prominent in the acute phase of the deficiency syndrome when there is also a marked dermal hyperemia. It has been reported that mast cells degranulate in the acute phase of magnesium deficiency (2,3). The release of endogenous histamine may, therefore, be responsible for the blood eosinophilia as well as the dermal hyperemia.

However, a blood eosinophilia does not occur in rats after administration of commercial histamine. It has, in fact, been reported that histamine administration stimulates the pituitary-adrenal system(4) resulting in a blood eosinopenia.

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The present experiments were designed to explore the effect of magnesium deficiency, histamine, heparin, serotonin and the pituitary-adrenal system on rat blood eosinophil levels.

Methods and materials. Male, Holtzman rats, weighing 100-150 g were placed on purified diets, either with complete salts or deficient in magnesium. The composition of these diets has been reported(2). Direct eosinophil counts were made from tail vein blood using the Speirs-Meyer diluent(5) in a Fuchs-Rosenthal hemocytometer 0.2 mm deep.

Groups and experimental conditions are presented in Tables I and II. Histamine phosphate (Lilly Co.) injections were calculated as the base. Administered pharmaceutical

TABLE I. Effect of Histamine, Heparin, Serotonin, 48/80 or Pyribenzamine on Eosinophil Count of Intact Rats Compared with the Effect of Magnesium Deficiency.

Group	No. rats	Experimental conditions	Final eosinophil count/mm³
1	6	25 γ histamine i.p. Final count at 24 hr.	146 ± 19*
2	6	1.0 mg histamine subcut daily for 2 days. Final count 24 hr after last inj.	257 ± 82
3	7	4.0-8.0 mg histamine/day for 3 days. Final count 24 hr after last inj.	183 ± 40
4	6 (3 died)	1000 U heparin i.p. and 5 mg histamine subcut. Final count 48 hr after single treatment.	208 ± 53
5	8	250 U heparin i.p. (1 injection) and 5.0 mg histamine daily for 3 days. Final count after 72 hr.	141 ± 35
6	8	2.0 mg serotonin i.p. and 5.0 mg histamine subcut daily for 3 days. Final count 24 hr after last inj.	185 ± 37
7	11	No treatment. Final count on 7th day of control diet.	159 ± 18
8	11	No treatment. Final count on 7th day of magnesium deficient diet.	2145 ±208
9	10	Pyribenzamine 3.75 mg i.p. daily for 1st 6 days in rats on magnesium deficient diet. Final count on 7th day.	280 ± 51
10	6 (3 died)	48/80 i.p. 2.0 mg/100 g B. W. Final count 24 hr after one inj.	562 ±202
11	6	48/80 subcut in 3 daily doses of .64 mg, .96 mg and 1.6 mg. Final count 24 hr after last inj.	3095 ±490

* Eosinophils/mm³ ± S.E. All rats received a control diet unless otherwise stated.

TABLE II. Effect of Hydrocortisone, ACTH or Adrenaline on the Blood Eosinophil Count in Intact, Splenectomized or Magnesium Deficient Rats.

Type of rat	No. rats	Substance tested	% change in blood eosinophils after 4 hr
Intact Mg^{++} deficient diet*	5	.2 cc saline i.p.	− 9 ± 10
Idem	10	5.0 mg hydrocortisone .2 cc i.p.	− 36 ± 15
"	6	16 units ACTH i.p.	− 63 ± 4
Intact control diet	6	adrenalin 70 γ subcut	− 21 ± 12
Intact Mg^{++} deficient diet (2 died)	8	*idem*	+52 ± 26
Splenectomized control diet	10	"	− 18 ± 5
Splenectomized Mg^{++} deficient diet (2 died)	10	"	+91 ± 25

* All magnesium deficient rats were in the acute stage with initially high eosinophil counts. Deaths indicated were caused by injected adrenalin in magnesium deficient rats. Response of eosinophils reported as % change 4 hr after injection ± standard error.

preparations were obtained as follows: heparin sodium, Abbott Labs.; serotonin creatinine sulfate, Regis Chemical Co.; pyribenzamine HCl, Ciba Co.; 48/80, a formaldehyde polymeric condensation product of p-methoxyphenethylmethylamine, Burroughs Wellcome Co.; hydrocortisone acetate, Merck & Co.; ACTHar gel, Armour Co. and adrenalin chloride, Parke Davis & Co.

Results and conclusions. The acute phase of the magnesium deficient syndrome is characterized by a dermal hyperemia(6) in rats and dermal mast cells degranulate in this phase(3). The blood eosinophilia which occurs in the acute phase of magnesium deficiency (Table I, Group 8) is highly significant ($P = < .01$) and may be caused by the endogenous release of mast cell secretory products. However, administration of commercial histamine, (Table I, Groups 1, 2, 3) in a variety of doses, routes and time intervals (some not reported here) failed to duplicate the eosinophilia produced by magnesium deficiency. Administration of 48/80 in gradually increasing doses for 3 days produced a significant ($P = < .01$) eosinophilia

(Table I, Group 11) of comparable magnitude to that produced by dietary magnesium deficiency. One large dose of 48/80 (Table I, Group 10) proved fatal to 50% of the animals. Since 48/80 is supposed to be a fairly specific mast cell degranulating agent (7), one would assume that a mast cell product or products were responsible for the blood eosinophilia. Administration of heparin in combination with histamine also failed to significantly elevate the number of circulating eosinophils (Table I, Groups 4, 5). Curiously, the large dose of heparin administered with histamine (Table I, Group 4) killed 50% of the rats. Massive subcutaneous edema was observed and tail vein blood appeared watery and pale pink. This reaction was less apparent when the smaller dose of heparin was administered. Serotonin administered with histamine was without effect on circulating eosinophils. An antihistamine, pyribenzamine, when administered daily to rats while on the magnesium deficient regimen (Table I, Group 9) prevented the eosinophilia which should have occurred at this time ($P = < .01$ when compared with Group 8).

The above observations suggest that the endogenous release of histamine is probably responsible for the rise in circulating eosinophils during the development of a magnesium deficient state. Magnesium deficiency may also interfere with a histamine-inactivating system thus allowing the accumulation of released histamine. The failure of commercial histamine to produce an eosinophilia suggests that histamine may be rapidly inactivated under normal conditions in the rat. The natural resistance of rats and mice to the effects of histamine is well documented (8) but not understood. It has been suggested that these animals may have a more rapid rate of inactivation of histamine by any one of several proposed mechanisms such as binding by a gamma globulin(9) or acetylation or inactivation by histaminase(10). Histamine produces an eosinophilia in sensitive animals such as the guinea pig(11). The experiments reported here suggested that the magnesium deficient rats and rats receiving 48/80 possessed classical symptoms (including eosinophilia) of acute endogenous excess of histamine. Since parenteral administration of histamine in large doses did not cause these effects, it would seem that magnesium deficiency and possibly also 48/80 interfere with the inactivation of histamine as well as cause the release of histamine from mast cells.

The pituitary-adrenal system has been widely studied for its influence in controlling the level of circulating eosinophils(12). Although the magnesium deficient regimen seemed stressful, it has been reported that indicators of adrenal function such as adrenal and thymic weights were not altered(2). Both hydrocortisone and adrenocorticotropic hormone (ACTH) when administered to eosinophilic rats (Table II) during the acute phase of magnesium deficiency resulted in a lowering of circulating eosinophils within a 4-hour test period. This suggests that adrenal function is normal in the magnesium deficient rat. Adrenalin administered to magnesium deficient rats was uniformly lethal in doses higher than 70 γ. At the 70 γ level adrenalin produced only a 21% fall in blood eosinophils in normal rats (Table II) but increased eosinophils by 52% in magnesium deficient rats ($P = <.01$). Splenomegaly occurs in magnesium deficient rats(2) and the possibility that adrenalin caused splenic contraction thus forcing entrapped eosinophils into the circulation was ruled out by repeating the above experiment in splenectomized rats. Adrenalin caused a 91% increase in circulating eosinophils in magnesium deficient, splenectomized rats but an 18% fall in control splenectomized rats ($P = <.01$). These experiments suggest that the pituitary glands of magnesium deficient rats were unable to respond to sudden stress with an increased output of ACTH, although some ACTH must have been produced in order to maintain a normal adrenal weight.

Although the pituitary may be unable to respond to sudden stress with increased ACTH output in magnesium deficient rats, the main cause of the eosinophilia accompanying magnesium deficiency is probably the endogenous release of histamine possibly

combined with the inadequate functioning of the histamine inactivating system.

Summary. Magnesium deficiency produced a massive blood eosinophilia which could be prevented by administration of pyribenzamine and duplicated by 48/80 administration. Commercial histamine, heparin or serotonin did not produce an eosinophilia of this magnitude. Pituitary function was subnormal in magnesium deficient rats while adrenal function was unimpaired. It was concluded that the eosinophilia of magnesium deficiency is primarily due to the endogenous release of histamine and possibly also to an interference with the histamine inactivating system.

1. Kashiwa, H. K., Hungerford, G. F., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 441.

2. Hungerford, G. F., Karson, E. F., *Blood,* 1960, v16, 1642.

3. Belanger, L. F., Van Erkel, G. A., Jakerow, A., *Science,* 1957, v126, 29.

4. Panzenhagen, H., Speirs, R. S., *Blood,* 1953, v8, 536.

5. Speirs, R. S., Meyer, R. K., *Endocrinology,* 1949, v45, 403.

6. Tufts, E. V., Greenberg, D. M., *J. Biol. Chem.,* 1937,-38, v122, 693.

7. Riley, J. F., *The Mast Cells,* E. and S. Livingstone, Ltd., 1959, Edinburgh and London.

8. Maitland, H. B., Kohn, R., MacDonald, A. D., *J. Hyg.* (Camb.), 1955, v53, 196.

9. Parrot, J., Laborde, C., *Ciba Foundation Symposium, Histamine,* Boston, Little, Brown and Co., 1956, p52.

10. Kohn, R., Millichap, J. G., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 623.

11. Vaughn, J., *Blood,* 1953, v8, 1.

12. Gordon, A. S., *Ann. N. Y. Acad. Sci.,* 1955, v59, 907.

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Thyroid Function and Kidney Muramidase.* (28865)

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Animal experiments have shown that both endogenous and exogenous muramidases accumulate in the kidney(1,2,3). Muramidase activity is not normally found in urine(4,5, 6) but appears there when the blood muramidase is elevated. The increase in kidney muramidase and its appearance in urine have been interpreted to be associated with the excretory function of the kidney rather than an indication of synthesis of the enzyme by this organ(7). There is, however, no information regarding the form in which the enzyme is stored by kidney tissue.

Muramidases are basic proteins and bind with many acidic compounds in tissues to form more or less stable complexes(8,9,10). For example, muramidase combines with heparin *in vitro* and *in vivo* to form a reciprocally inactive complex(11,12). Muramidase also combines *in vitro* with thyroxine (13,14) to form an inactive complex. However, no confirmation of this type of inactivation has been obtained *in vivo*. Kidney muramidase activity in rats fed thyroid is decreased(15,16), suggesting an enhanced formation of an inactive muramidase-thyroid complex and increased storage of it in the kidney at the expense of the free enzyme. The demonstration of such a complex *in vivo* would be important physiologically. Biochemically it would indicate a new function of muramidase in mammalian tissue. Therefore, the possible relationship between the functional state of the thyroid and kidney accumulation of muramidase was investigated in this study.

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Materials and methods. Normal and hypophysectomized rats, male and female of the