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.

Received September 18, 1963. P.S.E.B.M., 1964, v115.

## Thyroid Function and Kidney Muramidase.* (28865)

GIULIO C. PERRI, MARGRIT FAULK AND WILLIAM L. MONEY
(Introduced by C. C. Stock)

*Divisions of Experimental Chemotherapy and Clinical Investigation, Sloan-Kettering Institute for Cancer Research and Sloan-Kettering Division of Cornell University Medical College, New York City*

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.

* Supported in part by grants from NCI and from Am. Cancer Soc.

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

TABLE I. Effect of Change in Thyroid Activity on Kidney Muramidase.

| Treatment | Muramidase activity, mg/g | $\frac{\text{Thyroid}}{\text{Body}}$ wt × 100 | $I^{131}$ uptake, % |
|---|---|---|---|
| Controls | .384 ± .072* | 7.3 ± .37 | 10.4 ± 1.58 |
| Thiouracil | .397 ± .075 | 12.3 ± .73 | 37.4 ± 4.52 |
| (T4) | .502 ± .065 | 6.6 ± .33 | .5 ± .03 |
| T4 + thio | .700 ± .240 | 6.2 ± .42 | .8 ± .28 |
| EWM (50 mg) | 1.439 ± .314 | 8.3 ± .36 | 5.5 ± 1.14 |
| Thiouracil + EWM | 1.574 ± .891 | 13.7 ± .70 | 19.8 ± 3.49 |
| (T4) + EWM | 1.388 ± .600 | 6.2 ± .27 | .4 ± .06 |
| T4 & thio + EWM | 1.224 ± .409 | 6.9 ± .39 | 2.2 ± 1.14 |
| BCG | 2.106 ± .844 | 8.5 ± .49 | 8.0 ± .72 |
| Thiouracil + BCG | 1.693 ± .746 | 13.1 ± .78 | 22.6 ± 3.29 |
| T4 + BCG | 1.357 ± .330 | 6.5 ± .51 | .5 ± .11 |
| T4 & thio + BCG | 1.544 ± .245 | 6.7 ± .46 | 1.2 ± .47 |

EWM was injected daily for 20 days I.P. Each group represents 10 animals.
* Mean ± S.D.

Sprague-Dawley strain (Charles River) were maintained on Purina Laboratory Chow. Approximately 200 animals were used. Experiments were repeated at least twice, and typical results are presented. Egg white muramidase (EWM)† was dissolved in phosphate buffer pH 7.3 1/15 M and administered i.p.

Tuberculobacillus Calmette-Guerin (B.C.G.) (Phipp strain) from a 14-day-old culture in Dubos Medium‡ was injected i.v. into each animal as a saline suspension of 10 mg (wet weight).

Zymosan§ was injected i.p. in a single dose of 100 mg per animal. Thiouracil was given in drinking water in a concentration of 0.1%, and treatment was stopped 24 hours before $I^{131}$ administration. Five micrograms of L-thyroxine‖ (T4) were injected daily s.c. Thyroid stimulating hormone (TSH)¶ was given s.c. in a dose of 0.5 USP unit/day to the younger animals and 2.5 USP units/day to hypophysectomized rats.

$I^{131}$ (0.1 μc i.p.)** and $I^{131}$ T4 (0.1 μc i.v.)** were administered 24 hours and 45 minutes, respectively, prior to sacrifice. Radioactivity was determined in blood and tissues in a well type scintillation counter using standard procedures.

Animals were sacrificed under nembutal narcosis and tissues rapidly excised, blotted and weighed. Muramidase activity was determined on kidney, spleen and lung extracts (3) by a modification of the Litwack method (17). Results are expressed as activity in mg equivalents of EWM per gram of wet tissue.

*Results.* Administration of thiouracil to normal animals did not significantly change the level of muramidase activity in the kidney (Table I). The slight increase in kidney muramidase activity in normal animals given 5 μg of T4 daily, or thiouracil and T4 for the same period, was not statistically significant. However, both thiouracil and thyroxine induced the expected changes in weight of the thyroid and in uptake of radioiodine.

Administration of 50 mg/day of EWM to normal animals markedly increased the kidney muramidase level. However, administration of EWM to animals receiving thiouracil or T4 alone, or together, did not significantly change the kidney muramidase activity. EWM produced a questionable difference in thyroid weight and significantly depressed thyroid $I^{131}$ uptake in the EWM control animals and in those receiving thiouracil.

Enhancing endogenous muramidase production by BCG infection produced changes in the kidney activity of muramidase similar to those found in animals treated with EWM. BCG produced a minimal weight change in

† Nutritional Biochemicals Corp. Lot 2734.
‡ We thank Dr. Lloyd Old who kindly supplied the B.C.G. culture.
§ Travenal Laboratory.
‖ Baxter Laboratories.
¶ Parke Davis Co. Lot 099802.
** Abbott Laboratories.

TABLE II. Thyroid Function and Kidney Muramidase in Hypophysectomized Rats.

| Treatment | Muramidase activity, mg/g | $\frac{\text{Thyroid}}{\text{Body}}$ wt × 100 | $I^{131}$ uptake, % |
|---|---|---|---|
| Controls | .777 ± .163 | 4.7 ± .32 | .4 ± .006 |
| Thyroxine | .500 ± .162 | 5.5 ± .53 | .4 ± .07 |
| TSH | .706 ± .233 | 8.1 ± .57 | 4.1 ± 2.20 |
| Zymosan | 1.392 ± .338 | 9.4 ± .69 | .6 ± .09 |

TABLE III. Radioactivity in the Kidney and Blood of Normal Rats After Injection of $I^{131}$ T4 and Muramidase.

| Treatment | | Muramidase, mg/g or mg/ml | $I^{131}$, cpm/g | $\frac{\text{Kidney}}{\text{Blood}}$ radio $I^{131}$ |
|---|---|---|---|---|
| $I^{131}$ | Kidney | .380 | 3,458 | .52 |
| | Blood | | 6,555 | |
| $I^{131}$ + EWM (25 mg) I.V. | Kidney | 1.082 | 2,525 | .52 |
| | Blood | | 5,180 | |
| $I^{131}$ T4 | Kidney | .557 | 21,587 | 9.00 |
| | Blood | .031 | 19,977 | |
| $I^{131}$ T4 + EWM (50 mg) I.V. | Kidney | 2.151 | 26,386 | 8.00 |
| | Blood | 2.089 | 24,053 | |

the thyroid gland and depressed thyroid $I^{131}$ uptake of thiouracil treated animals.

Hypophysectomized rats (Table II) compared with unoperated controls (Table I) showed significantly increased muramidase activity. Thyroxine and TSH alone did not significantly change the kidney muramidase activity. Administration of zymosan, however, was followed by an increase in kidney muramidase activity of the same magnitude as that observed in normal animals(18). TSH resulted in marked changes in the thyroid, as shown by increases in weight of the gland and $I^{131}$ uptake.

In an attempt to determine whether EWM formed an *in vivo* thyroxine-muramidase complex which localized in the kidney, normal animals were given 50 mg EWM (i.v.) and after 5 minutes 0.1 $\mu$c $I^{131}$ T4, and killed 45 minutes later. Controls were given EWM and $I^{131}$ (as NaI) in the same manner. Table III shows that the kidney-blood ratios of $I^{131}$ were essentially identical, whether EWM was given or not. In these animals, as in normals(7), administration of exogenous muramidase resulted in a significant accumulation of the enzyme in the kidney.

*Discussion.* It is difficult to explain the apparent depression of thyroidal $I^{131}$ uptake in animals given 50 mg of EWM or in those treated with BCG (Table I) because in other groups of animals such an effect was not obtained consistently.

Our results demonstrate that the functional state of the thyroid gland does not influence the activity of muramidase in the kidney of rats. In fact, endogenous or exogenous increases of either thyroid hormone or muramidase, or both, did not produce statistically significant changes in kidney muramidase activity. Moreover, there is no evidence for an *in vivo* mechanism which forms a thyroxine-muramidase complex, or that such a complex accumulates in the kidney. Direct evidence of this comes from the study of $I^{131}$ accumulation in the kidneys of animals treated with muramidase and labeled thyroxine. Even when blood muramidase was increased 670-fold there was no change in the kidney/blood ratio of $I^{131}$ ratio after administration of labeled thyroxine. However, the kidney/blood ratio of muramidase was increased 173-fold (Table I).

Although hormones markedly influence protein synthesis and the absence of the thyroid or pituitary results in decreased metabolic activity, our data and those of Litwack(15)

show that muramidase activity in the kidney increases in hypophysectomized rats. Presumably, any effects of the endocrine system on muramidase levels in the kidney represent an indirect effect on kidney excretion rather than on enzyme production.

We previously found(7) that the accumulation of muramidase in the kidney is a direct reflection of the blood level of the enzyme. Furthermore, the production of the enzyme is related to the functional state of the reticuloendothelial system (RES)(18).

The close relationship between muramidase activity and the RES functional state, suggests that the spleen, as part of the producing system, should reflect possible hormonal control better than the kidney, which mainly accumulates the muramidase. We have found (unpublished data) that neither hypophysectomy nor administration of thyroid hormone alters the splenic level of muramidase. This supports the concept that neither the pituitary nor thyroid gland is involved directly in production of muramidase. In fact, in hypophysectomized rats, administration of zymosan results, as in normal animals, in a significant increase in kidney muramidase activity (Table II). Thus muramidase producing mechanisms are probably intact in hypophysectomized animals and respond normally to the proper stimuli.

The present observations differ from those previously reported(15,16). These differences may be explained by the dose of thyroid hormone administered; the dose used by us was essentially physiological and did not induce marked loss of body weight.

Although adequate evidence has been given for *in vitro* inhibition of muramidase by thyroid hormone, our data do not support the concept that such a mechanism is operating *in vivo*. This is in accord with recent evidence that muramidase does not behave like a thyroxine binding protein(14).

1. Perri, G. C., Faulk, M., Mellors, J., Stock, C. C., *Nature,* 1962, v193, 649.

2. Vu-Thi-Suu., Congon, C. C., Kretchmar, A. L., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 481.

3. Perri, G. C., Anigstein, R., *Cancer Res.,* 1960, v20, 1054.

4. Florey, H., *Brit. J. Exp. Path.,* 1930, v11, 251.

5. Wolff, L. K., *Z. f. Immunitatsfors.,* 1927, v51, 88.

6. Wilson, A. T., *J. Pediat.,* 1950, v36, 39.

7. Perri, G. C., Faulk, M., Shapiro, E., Money, W. L., in press.

8. Klotz, I. M., Walker, F. M., *Arch. Biochem.,* 1948, v18, 319.

9. Steiner, R. F., *Arch. Biochem. Biophys.,* 1953, v46, 291.

10. Kaiser, E., *Nature,* 1953, v171, 607.

11. Skarnes, R. C., Watson, D. W., *J. Bact.,* 1955, v1, 110.

12. Kerby, G. P., Eady, G. S., PROC. SOC. EXP. BIOL. AND MED., 1953, v88, 111.

13. Litwack, G., Reimer, S. M., *Proc. 1st Intern. Symp. Flemings Lysozyme, Milan, Italy,* 1959, p167.

14. Litwack, G., *Biochem. Biophys. Acta,* 1963, v67, 501.

15. ———, PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 764.

16. ———, *J. Biol. Chem.,* 1958, v231, 175.

17. ———, PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 401.

18. Cappuccino, J. G., Stuart, W., Faulk, M., Perri, G. C., in press.

Received September 19, 1963. P.S.E.B.M., 1964, v115.