THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 49, No. 3

Printed in U.S.A.

SERUM AND URINARY LYSOZYME IN PATIENTS WITH MASTOCYTOSIS (URTICARIA PIGMENTOSA), SYSTEMIC SCLERODERMA AND PSORIASIS*

SOICHIRO SASAKI, M.D. AND GUSTAV ASBOE-HANSEN, M.D.

In 1922 Sir Alexander Fleming (1) reported the presence of a bacteriolytic principle in tears, egg white and other animal fluids which he called lysozyme.

The lytic action on susceptible organisms was found to be due to the hydrolysis of a mucoid component of the cell wall. This component was identified as a high polymer mucopolysaccharide which was hydrolysed at a rate comparable to the speed of bacterial lysis (2).

It seems that little clinical investigation of lysozyme has been made, though it has been studied thoroughly bacteriologically (3) and biochemically (4, 5, 6). Taniguchi and Sasaki (7) were the first to report on serum lysozyme activity in skin diseases.

In the present study an attempt was made to measure serum and urinary lysozyme activities in normal individuals and patients with mastocytosis (urticaria pigmentosa), systemic scleroderma and psoriasis vulgaris.

MATERIALS AND METHODS

The blood and urine specimens were obtained from 43 normal individuals, 11 patients with urticaria pigmentosa, 16 with systemic scleroderma and 34 with psoriasis vulgaris. The supernatants of blood and urine centrifuged at 2,500 g for five minutes were used for the assay.

The enzymic activities were assayed by the modified method of Litwack (8) and Fogelson *et al* (9) as follows: 2.0 ml of buffer solution, 0.4 ml of the sample, and 1.6 ml of the substrate were incubated at 37° C for five minutes. The buffer was M/15 Sorensen phosphate buffer pH 6.2 containing 0.071 N NaCl (10). The substrate solution was prepared by suspending 10 mg lyophilized Micrococcus lysodeikticus[1] in 100 ml of the buffer solution. The absorption was measured at 645 mμ on a Beckman DB spectrophotometer. The values observed were corrected for nonspecific absorption by subtracting the optical density of a color blank containing 0.4 ml of the sample and 3.6 ml of the buffer solution. Standard curves were produced with egg white lysozyme (Sigma Chemical Co., Mo., U.S.A.).

RESULTS

The serum lysozyme activity in 23 normal males was found to be 6.5 ± 0.2 μg/ml, and 6.4 ± 0.2 μg/ml in 20 normal females (Table 1). There was no significant difference between them.

Serum and urinary lysozyme activities of the patients with urticaria pigmentosa, systemic scleroderma and psoriasis vulgaris are listed in Tables 2, 3 and 4. Serum lysozyme values were 4.5 ± 0.3 μg/ml in urticaria pigmentosa, 5.4 ± 0.3 μg/ml in systemic scleroderma, and 6.4 ± 1.2 μg/ml in psoriasis vulgaris. The mean values of urinary lysozyme were 0.3 μg/ml in urticaria pigmentosa, 0.4 μg/ml in systemic scleroderma and 0.8 μg/ml in psoriasis vulgaris.

Figure 1 illustrates the distribution of serum lysozyme values of normal individuals and of patients with the three different skin diseases.

DISCUSSION

Lysozyme is an enzyme which is widely distributed in plants and animals, especially in the latter. It exists in body fluids *e.g.* tears, saliva, nasal secretion, gastric juice, milk, blood plasma and leukocytes (1, 11). The enzyme is a basic protein of low molecular weight (around 15,000). Its lytic activity against certain gram-negative and gram-positive bacteria is suggestive of a role of the lysozyme in the defence against bacterial invasion (3). A possible source of urinary lysozyme are peritubular macrophages. However, the possibility exists that urinary lysozyme represents an overflow from the plasma or evidence of glomerular damage. Lysozyme is known as a mucopolysaccharidase which acts on $\beta(1 \rightarrow 4)$ N-acetylglucosaminides (12).

The fact that mast cells synthesize acid mucopolysaccharides, and that scleroderma is

Received for publication March 21, 1967.
* From the University of Copenhagen, Department of Dermatology, Rigshospital, Copenhagen, Denmark.
[1] Micrococcus lysodeikticus was generously supplied by Eisai Co. Ltd., Tokyo, Japan.

TABLE 1

Serum lysozyme values in normal subjects

Males				Females			
No.	Name	Age	Lysozyme values (μg/ml)	No.	Name	Age	Lysozyme values (μg/ml)
1	B. A.	22	5.8	1	R. B.	30	5.8
2	F. J.	33	7.4	2	G. R.	47	7.5
3	Y. F.	23	5.8	3	G. A.	16	5.8
4	S. S.	26	5.4	4	A. L.	18	6.0
5	E. R.	27	6.9	5	Y. L.	25	5.5
6	L. B.	46	6.9	6	B. N.	20	6.3
7	B. T.	27	8.5	7	G. Ø.	34	5.8
8	Y. T.	24	5.3	8	C. V.	20	5.7
9	J. O.	23	7.0	9	H. P.	19	6.5
10	A. R.	25	5.9	10	B. S.	18	8.0
11	J. H.	21	6.5	11	E. S.	19	7.7
12	T. H.	22	6.3	12	M. A.	18	7.5
13	E. J.	25	8.0	13	A. V.	19	8.0
14	F. V.	34	8.0	14	P. C.		5.7
15	K. L.	19	7.8	15	M. J.	20	5.1
16	V. V.	24	6.2	16	R. I.	18	7.4
17	Y. A.	29	6.8	17	A. L.	18	5.7
18	P. L.	20	5.8	18	L. W.	23	5.2
19	C. C.	21	5.4	19	B. F.	20	6.1
20	A. R.	21	6.9	20	M. B.	22	7.6
21	F. S.	22	7.8				
22	A. C.	25	5.0				
23	H. O.	22	5.0				
Results		6.5 ± 0.2*				6.4 ± 0.2*	
Range of values		5.0–8.5				5.1–8.0	

* Results are expressed as the mean ± the standard error of the mean.

a mesenchymosis, the development of which may depend on changes in mucopolysaccharide metabolism (13) led to the studies of lysozyme in mastocytosis and scleroderma. In contradistinction, the connective-tissue involvement in psoriasis is relatively scarce. The mastocytosis cases were of the macular type showing urtication as a response to mechanical trauma. The cases of scleroderma were of the acrosclerosis type. The severity and extensiveness of the sclerodermic changes were very similar from case to case. The psoriasis lesions were nummular.

The average normal value for serum lysozyme activity obtained in the present study is in

TABLE 2

Serum and urinary lysozyme values in mastocytosis (urticaria pigmentosa)

No.	Name	Age	Sex	Serum lysozyme values (μg/ml)	Urinary lysozyme values (μg/ml)
1	A. L.	16	F	4.1	0
2	W. S.	22	M	2.8	0
3	T. E.	6	M	2.7	0
4	S. H.	34	M	5.2	1.3
5	M. S.	4	M	5.2	0
6	H. M.	1	M	4.9	0
7	P. A.	21	M	5.1	0
8	A. F.	6	F	4.1	0
9	G. J.	6	F	4.5	0
10	I. H.	20	F	5.3	1.0
11	B. H.	22	F	5.2	1.2
Results				4.5 ± 0.3*	0.3
Range of values				2.7–5.3	0–1.3

* Results are expressed as the mean ± the standard error of the mean.

TABLE 3

Serum and urinary lysozyme values in systemic scleroderma

No.	Name	Age	Sex	Serum lysozyme values (μg/ml)	Urinary lysozyme values (μg/ml)
1	S. E.	57	M	5.3	0
2	B. R.	55	F	5.5	0
3	E. A.	51	F	5.0	0
4	D. M.	47	F	4.0	0
5	I. L.	35	F	4.3	0
6	I. J.	26	M	3.8	0
7	L. S.	42	M	3.8	0
8	B. L.	47	F	5.6	0.6
9	K. B.	64	F	5.5	0
10	K. J.	57	F	6.6	0
11	K. K.	66	F	6.4	0.5
12	S. L.	42	M	5.5	0.8
13	K. K.	51	F	7.5	1.3
14	K. K.	55	F	8.1	0.9
15	M. J.	11	M	3.5	0
16	B. H.	17	F	6.5	1.5
Results				5.4 ± 0.3*	0.4
Range of values				3.5–8.1	0–1.5

* Results are expressed as the mean ± the standard error of the mean.

TABLE 4

Serum and urinary lysozyme values in psoriasis vulgaris

No.	Name	Age	Sex	Serum lysozyme values (μg/ml)	Urinary lysozyme values (μg/ml)
1	J. M.	37	M	4.3	0
2	B. H.	31	F	3.4	1.0
3	C. P.	28	F	5.9	1.2
4	M. W.	12	F	5.8	1.8
5	S. G.	28	M	5.1	0.8
6	B. H.	58	F	5.3	0.6
7	F. S.	18	F	5.6	0.6
8	J. A.	72	M	5.6	0.9
9	H. J.	69	M	3.0	0.9
10	J. S.	17	M	7.0	0.9
11	G. G.	44	F	3.4	0
12	F. P.	76	M	10.3	3.8
13	T. L.	24	M	9.4	3.5
14	I. K.	41	F	8.4	1.0
15	E. J.	45	F	7.5	0.5
16	M. N.	56	M	4.3	0
17	E. T.	52	F	5.1	0
18	L. B.	44	M	5.4	0
19	J. F.	64	F	5.6	0
20	C. D.	57	M	8.2	0
21	L. B.	7	F	5.2	0
22	P. B.	7	F	6.8	
23	K. H.	51	F	7.5	0.8
24	O. T.	20	M	7.6	0.3
25	D. J.	83	F	7.5	0.9
26	N. L.	62	M	6.8	0
27	K. E.	54	M	7.6	0.9
28	A. V.	21	F	7.3	0.6
29	J. P.	53	M	10.0	0.8
30	E. M.	36	F	7.5	0
31	E. M.	48	F	6.7	0.8
32	I. H.	28	F	4.5	0.5
33	E. B.	19	F	6.9	0
34	A. H.	15	F	7.2	2.1
Results				6.4 ± 1.2*	0.8 ± 0.2*
Range of values				3.0–10.3	0–3.8

* Results are expressed as the mean ± the standard error of the mean.

agreement with that published by Prockop and Davidson (14) *i.e.* 6.4 μg/ml.

Changes of the mucopolysaccharide content in serum and urine of the patients suffering from mastocytosis (urticaria pigmentosa) have been demonstrated by Asboe-Hansen and Clausen (15, 16, 17). The urticaria pigmentosa patients of this study showed significantly lowered serum lysozyme values ($P < 0.001$). The scleroderma patients showed comparatively low serum values, the difference from normal being statistically significant ($P < 0.01$). On the other hand, the serum lysozyme level in psoriatic patients did not differ significantly from the level of normal individuals ($P > 0.05$), and the range varied widely. On the basis of the obtained results, no conclusions about any relationship between serum lysozyme activity and the pathology of psoriasis vulgaris can be drawn. It was also apparent that no correlation between the extensiveness of the diseases and the lysozyme values could be demonstrated.

From the data of lysozyme assays of urine samples, it is evident that the urines contained little lysozyme. Fleming (1), Prockop and Davidson (14), Wilson and Hadley (18) and Burghartz and Boosfeld (19) reported that urinary lysozyme activity in normal subjects was nil or very low. The low lysozyme content of the urine renders the assay somewhat unreliable. The values obtained for urinary lysozyme in urticaria pigmentosa, systemic scleroderma and psoriasis

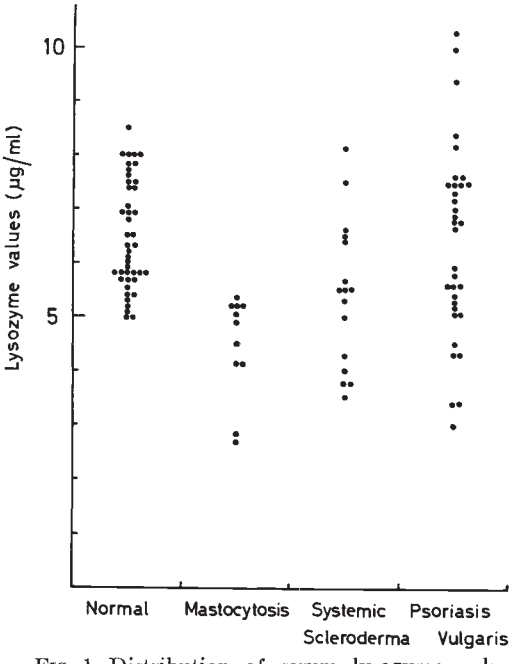

FIG. 1. Distribution of serum lysozyme values in normal subjects and patients with mastocytosis (urticaria pigmentosa), systemic scleroderma and psoriasis vulgaris.

vulgaris show a considerable variation making interpretation difficult. Both the patients and the controls of this study had normal renal function.

The present study suggests a correlation between changes in mucopolysaccharide metabolism and serum lysozyme activity.

SUMMARY

Serum and urinary lysozyme activities were measured in normal subjects and in patients with mastocytosis (urticaria pigmentosa), systemic scleroderma and psoriasis vulgaris. In serum, a significant decrease was observed in urticaria pigmentosa, a moderate decrease in systemic scleroderma, while no significant changes were seen in psoriasis vulgaris. The determination of urinary lysozyme demonstrated no reliable changes.

The study suggests a correlation between changes in mucopolysaccharide metabolism and serum lysozyme activity.

REFERENCES

1. Fleming, A.: On a remarkable bacteriolytic element found in tissues and secretions. Proc. Roy. Soc. (London) Ser. B, *93:* 306, 1922.
2. Meyer, K., Palmer, J. W., Thompson, R. and Khorazo, D.: On the mechanism of lysozyme action. J. Biol. Chem., *113:* 479, 1936.
3. Thompson, R.: Lysozyme and its relation to the antibacterial properties of various tissues and secretions. Arch. Path., *30:* 1096, 1940.
4. Suu, V-T., Congdon, C. C. and Kretchmar, A. L.: Increase in lysozyme activity in kidneys of irradiation chimeras. Proc. Soc. Exper. Biol. & Med., *113:* 481, 1963.
5. Stevens, C. O., Henderson, L. E. and Tolbert, B. M.: Radiation chemistry of proteins, II. Enzymatic activity and deuterium exchange properties of lysozyme and α-chymotrypsin. Arch. Biochem. Biophys., *107:* 367, 1964.
6. Osawa, T.: Lysozyme substrates, synthesis of p-nitrophenyl 2-acetamid-4-0-(2-acetamid-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranoside, and its β-D-(1 → 6) isomer. Carbohydrate Res., *1:* 435, 1966.
7. Taniguchi, T. and Sasaki, S.: Reported at the 65th General Meeting of Jap. Dermat. Soc., Kyoto, 1966, Skin Res. (Osaka), In press.
8. Litwack, G.: Photometric determination of lysozyme activity. Proc. Soc. Exper. Biol. & Med., *89:* 401, 1955.
9. Fogelson, S. J., Ross, A. and Lobstein, O. E.: A method for quantitative determination of lysozyme activity in blood. Am. J. Dig. Dis., *21:* 327, 1954.
10. Lobstein, O. E. and Fogelson, S. J.: The effect of chloride concentration in the determination of lysozyme activity. Am. J. Dig. Dis., *18:* 298, 1951.
11. Jolles, P.: In The Enzymes, vol. 4, p. 341. Ed. Boyer, P. D., Lardy, H. and Myrbäck, K. New York, N.Y. Academic Press, 1960.
12. Salton, M. R. J. and Ghuysen, J. M.: The structure of di- and tetra-saccharides released from cell walls by lysozyme and streptomyces F_1 enzyme and the β(1 → 4) N-acetylhexosaminidase activity of these enzymes. Biochim. Biophys. Acta, *36:* 552, 1959.
13. Asboe-Hansen, G.: Dermatologic aspects of mast cell activity. Dermatologica, *128:* 51, 1964.
14. Prockop, D. J. and Davidson, W. D.: A study of urinary and serum lysozyme in patients with renal disease. New Engl. J. Med., *270:* 269, 1964.
15. Asboe-Hansen, G.: Bullous diffuse mastocytosis with urinary hyaluronate excretion. Treatment with cyproheptadine. Acta dermat.-venereol., *42:* 211, 1962.
16. Asboe-Hansen, G. and Clausen, J.: Urinary excretion of acid mucopolysaccharides in twelve cases of mastocytosis (urticaria pigmentosa). J. Invest. Derm., *43:* 81, 1964.
17. Clausen, J. and Asboe-Hansen, G.: Urinary acid mucopolysaccharides in mastocytosis. A new technique for quantitative estimation. Clin. Chim. Acta, *13:* 475, 1966.
18. Wilson, A. T. and Hadley, W. P.: Urinary lysozyme. II. Lysozymuria in healthy children and in children with miscellaneous diseases (A sex difference). J. Pediat., *36:* 45, 1950.
19. Burghartz, N. and Boosfeld, E.: Bericht über Lysozymuntersuchungen im Urin. Klin. Wchnschr., *32:* 182, 1954.