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THE RELATIONSHIP OF SERUM LYSOZYME TO LEUKOCYTES AND OTHER CONSTITUTIONAL FACTORS

The primary objective of this study was to explore the relationship between leukocyte turnover and serum lysozyme. Polymorphonuclear leukocytes and monocytes contain relatively high concentrations of this enzyme,[1,2] and it seems possible that the major portion of the lysozyme found in plasma is derived from the eventual breakdown of these cells. Thus, variations in rates of leukocyte production and destruction would alter serum lysozyme concentration. In order to evaluate these relationships, however, it was necessary to determine the influence of age, sex and certain other constitutional factors on serum lysozyme. Of special interest in this study was whether or not serum lysozyme levels might reflect alterations in leukocyte or other body tissue turnover in subjects previously exposed to excessive amounts of ionizing radiation.

Previous investigations with the enzyme, lysozyme, suggest its suitability for studies of this type. Lymphocytes and erythrocytes contain little or none[1,2] while certain other body cells contain moderate to relatively large concentrations.[3-7] Following *in vitro* physical disruption of leukocytes some of the enzyme is released,[2,7-11] but much of it probably remains firmly within granules and other cellular components.[12] Additional experimental evidence has shown that *in vivo* lysis of granulocytes will elevate serum lysozyme.[13]

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METHOD OF STUDY

During the months of April, May, and June of 1961 serum lysozyme determinations were performed for 670 regularly-scheduled Japanese subjects participating in the Adult Health Study in Hiroshima, Japan. This continuing clinical investigation of possible late effects attributable to ionizing radiation from the atomic bomb is a joint undertaking of the Japanese National Institute of Health (NIH) and the Atomic Bomb Casualty Commission (ABCC).

TABLE 1. ADULT HEALTH STUDY SAMPLE, BY SEX AND DISTANCE FROM HYPOCENTER

	Hiroshima		
Distance in meters	*Male*	*Female*	*Total*
0-2,000	2,824	4,231	6,855
with symptoms	1,312	2,116	3,428
without symptoms	1,312	2,115	3,427
3,000-3,499	1,312	2,119	3,431
Not in city at time of bomb	1,313	2,120	3,433
TOTAL:	5,249	8,470	13,719

The sample for the ABCC-NIH Adult Health Study is composed of four age-sex balanced components:

Group 1: within 2,000 meters of the hypocenter; reported acute radiation symptoms.

Group 2: within 2,000 meters of the hypocenter; reported no acute radiation symptoms.

Group 3: 3,000 to 3,499 meters from the hypocenter.

Group 4: beyond 10,000 meters from the hypocenter or not in the city at the time of the bomb.

The Hiroshima sample totals slightly more than 13,000 persons, and the Nagasaki sample consists of slightly more than 6,000 persons who receive detailed clinical examinations at approximately two-year intervals (Table 1). The entire sample is divided equally into twenty-four examination groups, designated A through X. Each month, one of these alphabetically-designated groups is scheduled for routine clinical examination at ABCC.

Ten milliliters of venous blood was removed from each person in the lysozyme study subsample of the Adult Health Study. Serum lysozyme values were determined on 1 ml. aliquots of undiluted serum according to the method of Smolelis and Hartsell.[14] The remaining blood was anticoagulated with EDTA for routine hematologic and serologic studies.

Serum lysozyme levels were related to total leukocyte count, absolute granulocyte and monocyte counts, age, sex, month of examination, medical diagnosis, and radiation-exposure status.

RESULTS

Serum lysozyme values were not strongly related to the total leukocyte count or to the absolute granulocyte count, although the latter relationship was more impressive than the former. The two correlation coefficients were

TABLE 2. SERUM LYSOZYME VALUES, BY WHITE BLOOD COUNT AND ABSOLUTE GRANULOCYTE COUNT

White blood count and absolute granulocyte count	*Lysozyme value*							
	Total		<1.40		1.40-2.19		2.20+	
	No.	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Total	670	100.0	129	19.3	396	59.1	145	21.6
WBC < 6,000	292	100.0	64	21.9	176	60.3	52	17.8
6,000-7,999	253	100.0	43	17.0	155	61.3	55	21.7
8,000+	125	100.0	22	17.6	65	52.0	38	30.4
AGC < 3,000	197	100.0	52	26.4	111	56.3	34	17.3
3,000-4,999	341	100.0	58	17.0	215	63.1	68	19.9
5,000+	132	100.0	19	14.4	70	53.0	43	32.6

Lysozyme x WBC, $X^2 = 9.67$, d.f. $= 4$, $P < .05$.
Lysozyme x AGC, $X^2 = 19.2$, d.f. $= 4$, $P < .001$.

TABLE 3. AVERAGE LYSOZYME VALUES, BY SEX

Sex	*Number tested*	*Average lysozyme value* $\overline{X} \pm$ *S.E.*	*F**		
			Value	*Degrees of freedom*	*Probability*
All subjects	670	1.83±0.02			
Male	247	1.91±0.04	10.2	1; 668	< .01
Female	423	1.78±0.02			

* F value refers to the result of the analysis of variance test.

+ .039 and + .079, only the latter being significantly different from zero at the 5 per cent significance level. When the data were arranged in categorical fashion, however, the relationships were seen more clearly (Table 2). Chi square tests returned significant values, barely so for the total leukocyte count but highly significant for the absolute granulocyte count. Comparisons between the 50 highest and lowest absolute monocyte counts and corresponding serum lysozyme values showed no significant relationship.

Serum lysozyme values varied significantly with sex, age, and month of examination (Tables 3, 4, and 5). Significantly higher values were obtained for males in comparison to females.

Serum lysozyme varied directly with age in a consistent, steady, stepwise fashion for each of the age decades measured. Differences through the middle years of life, however, were small. As measured by the correlation

TABLE 4. AVERAGE LYSOZYME VALUES, BY AGE AT EXAMINATION

Age at examination	*Number tested*	*Average lysozyme value* $\bar{X} \pm$ *S.E.*	*F*		
			Value	*Degrees of freedom*	*Probability*
15-19	15	1.45±0.10			
20-29	65	1.70±0.04			
30-39	188	1.75±0.03			
40-49	116	1.75±0.06	7.46	5; 626	<.001
50-59	145	1.87±0.04			
60-69	103	2.03±0.06			
70+					

TABLE 5. AVERAGE LYSOZYME VALUES, BY TIME OF TEST

Month of test	*Number tested*	*Average lysozyme value* $\bar{X} \pm$ *S.E.*	*F*		
			Value	*Degrees of freedom*	*Probability*
April 1961	119	2.06±0.05			
May 1961	379	1.85±0.03	26.7	2; 667	<.001
June 1961	172	1.62±0.03			

ratio, month of examination was as influential on serum lysozyme as was age, and even more influential than sex.

The data relating serum lysozyme values to distance from the hypocenter at the time of the bombing are shown in Table 6. There is no suggestion from this material that level of serum lysozyme is related to distance from the hypocenter or symptoms of acute radiation injury following the bombing.

Relationships between serum lysozyme and medical diagnoses are more difficult to evaluate. The subjects under study come to the ABCC clinic not because they are ill, but for a routine physical examination at a prearranged time. A few arrive with acute illnesses of moderate severity and some have

chronic illnesses, but the majority are completely well or have only trivial medical problems. In fact, of the 670 subjects studied here, 237, or 35 per cent, had no disorder diagnosed; while the remaining 433 subjects had 744 diagnoses assigned to them. For analysis in relation to serum lysozyme values, certain diagnoses were grouped following the International Classification of Diseases.[15] The distributions of lysozyme values by diagnoses are shown in Table 7. For patients with multiple diagnoses, each diagnosis

TABLE 6. AVERAGE LYSOZYME VALUES, BY DISTANCE FROM HYPOCENTER

Distance	*Number tested*	*Average lysozyme value* $\overline{X} \pm S.E.$	*F*		
			Value	*Degrees of freedom*	*Probability*
All subjects	670	1.83±0.02	—	—	—
Group 1 < 2,000 with radiation symptoms	170	1.87±0.04			
Group 2 < 2,000 without symptoms	159	1.79±0.04	0.74	3; 666	> .50
Group 3 3,000-3,499	198	1.81±0.04			
Group 4 Not in city ATB	143	1.85±0.06			
<1,400 meters	189	1.80±0.03			
1,400-1,999 meters	140	1.87±0.04	0.58	3; 666	> .50
3,000-3,499	198	1.81±0.04			
Not in city ATB	143	1.85±0.06			

with its corresponding serum lysozyme value is considered separately.

Only a few diagnostic groups demonstrated relationships to serum lysozyme values. The only statistically significant different distribution of values in comparison to the over-all occurred with diabetes mellitus (chi square of 12.75, and $p < .01$). For tuberculosis and diseases of the respiratory system the differences were suggestive, but not significant (p between .05 and .10).

In an attempt to explain the increased serum lysozyme values found in patients with diabetes mellitus and tuberculosis, the data were examined from the standpoint of peculiarities in distribution of persons with these diagnoses. It was found that, for each of the two diagnoses the increases in average lysozyme values were not entirtely explicable on the basis of unusual

TABLE 7. LYSOZYME VALUE, BY DIAGNOSIS

ISC code no.	*Diagnosis*	*Number with this Dx.*	*Lysozyme value*					
			<1.40		1.40-2.19		2.20+	
			No.	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
	Total	981	165	16.8	571	58.2	245	25.0
y—	No illness diagnosed	237	46	19.4	151	63.7	40	16.9
00–01	Tuberculosis, all forms	37	5	13.5	17	46.0	15	40.5
02	Syphilis and its sequelae	35	4	11.4	20	57.2	11	31.4
03–13	Other infectious and parasitic diseases	56	9	16.1	35	62.5	12	21.4
14-23	Neoplasms, malignant and benign	17	4	23.5	10	58.8	3	17.7
24	Allergic disorders	6	1	16.7	4	66.6	1	16.7
25	Diseases of thyroid gland	14	3	21.4	7	50.0	4	28.6
26	Diabetes mellitus	26	1	3.8	11	42.3	14	53.9
27	Diseases of other endocrine glands	1	1	100.0	–	–	–	–
28	Avitaminoses and other metabolic diseases	45	7	15.6	29	64.4	9	20.0
29	Diseases of blood and blood-forming organs	69	15	21.7	32	46.4	22	31.9
30–32	Mental, psychoneurotic and personality disorders	4	1	25.0	3	75.0	–	–
33–39	Diseases of the nervous system and sense organs	95	18	18.9	54	56.9	23	24.2
40–46	Diseases of the circulatory system	159	21	13.2	90	56.6	48	30.2
47–52	Diseases of the respiratory system	45	3	6.7	26	57.8	16	35.5
53–58	Diseases of the digestive system	28	7	25.0	18	64.3	3	10.7
59–63	Diseases of the genito-urinary system	17	4	23.5	9	53.0	4	23.5
64-68	Complications of pregnancy, childbirth and puerperium	1	–	–	1	100.0	–	–
69-71	Diseases of the skin and cellular tissue	10	1	10.0	8	80.0	1	10.0
72-74	Diseases of the bones and organs of movement	24	2	8.3	16	66.7	6	25.0
75	Congenital malformations	7	2	28.6	4	57.1	1	14.3
78-79	Symptoms, senility and ill-defined conditions	40	7	17.5	23	57.5	10	25.0
80-99	Accidents, poisonings and violence	8	3	37.5	3	37.5	2	25.0

distributions by sex, age, or month of examination. After correction for all three factors simultaneously, persons with these diagnoses still had elevated serum lysozyme values, but the elevation was reduced to about one half of the uncorrected difference, and was no longer statistically significant.

The clinical records of the 26 patients with diabetes mellitus were examined for evidence of infection. It was found that of the 14 with clearly elevated lysozyme values, 11 had suggestive or definite evidence of some type of inflammatory process. Three had active pulmonary tuberculosis and for these patients the serum lysozyme values were 2.37 unit, 3.43 unit, and 4.14 unit. Of the 12 remaining diabetic patients with normal or low lysozyme values, none had overt evidence of infection, but two had moderately increased sedimentation rates.

With respect to respiratory illness, more detailed study showed that 9 of the 45 had acute respiratory disease and 36 had chronic disturbances. Of the 9 with acute respiratory infections, 7 had serum lysozyme values in excess of 2.20 units, and of the 36 with chronic respiratory disorders only 9 had serum lysozyme values in excess of 2.20 units.

The data showing variation in serum lysozyme values by month of examination were examined in several ways. Although the relative number of patients with diabetes mellitus, active pulmonary tuberculosis and acute respiratory disease was greater in the studies completed in April compared to those for June, the numbers were small and did not appear influential. Furthermore, the mean lysozyme values for patients with these associated disorders declined progressively with month of examination, suggesting that the processes were quite independent of one another. Other tests showed that the time trend was not the result of variations in distribution of subjects examined in relationship to sex, age, or absolute level of granulocytes.

DISCUSSION

Total leukocyte count and serum lysozyme were, at best, weakly related. A much stronger relationship was established between absolute granulocyte level and serum lysozyme. This later relationship was studied for other possible influences and it was determined that it could not be explained on the basis of age, sex, month of examination, or type of medical illness. If the total granulocyte counts in the individuals studied was a reasonable reflection of the size of the total leukocyte mass it then becomes possible to relate serum lysozyme levels to granulocyte turnover.

In this study it was not possible to adequately examine the relationship between circulating monocytes and serum lysozyme. Those subjects with the

highest and lowest absolute monocyte counts, however, did not show significant variation. It seems likely that the variation in total monocytes in healthy subjects is too small to significantly influence serum enzyme levels.

The results of this investigation give indirect support to the belief that lymphocytes contain little lysozyme. No enzyme activity was detected by two of the authors (S.C.F. and J.P.L.) following *in vitro* lysis of platelets removed from two patients with thrombocytosis.

The kinetics of leukopoiesis are poorly understood, but there now is much evidence that with certain abnormalities, the number of leukocytes in circulation is not necessarily an accurate reflection of either total leukocyte mass or rate of leukocyte production and destruction. A low peripheral white count may be the result of decreased leukocyte survival, a shift of leukocytes from circulating to noncirculating sites, diminished leukopoiesis, or combinations of any of these. An elevated leukocyte count may be the result of prolongation of leukocyte survival, a shift in leukocytes from noncirculating sites to active circulation, increased leukopoiesis, or various combinations of these.

If a valid relationship exists between *in vivo* granulocyte destruction and serum lysozyme, then those subjects with elevated granulocyte counts and increased serum lysozyme levels have increased granulocyte turnover. Similarly, those patients with low absolute granulocyte counts and reduced serum lysozyme levels have decreased granulocyte turnover. This study does not prove these relationships but the data are consistent with the hypothesis. It is quite likely that the patients with high leukocyte counts and high lysozyme levels not only have increased granulocyte turnover but also increased fixed tissue breakdown associated with infection to contribute to the elevated lysozyme content of the serum. More convincing is the fact that low absolute granulocyte counts were associated with low lysozyme levels. If these low granulocyte counts were the result of diminished leukopoiesis and a reduced rate of granulocyte destruction, then there is good support to the thesis that the serum receives its major contribution of lysozyme from destroyed granulocytes.

A reasonable test of this hypothesis would be to determine serum lysozyme levels in patients with disorders associated with diminished leukopoiesis such as aplastic anemia and agranulocytosis. Patients with leukemoid reactions, granulocytic leukemia and monocytic leukemia might give information at the other end of the spectrum.

The direct relationship between age and serum lysozyme cannot be explained at the present time, but the data suggest that increased lysozyme levels may be part of the normal aging process. The data for serum lysozyme

levels by age were examined for other possible influences. It was determined that sex, diagnosis, and month of examination were not responsible for the relationships. The absolute granulocyte count varied inversely with age, but the relationship was weak. It was, however, stronger in females than in males. Although the prevalence of diabetes mellitus and tuberculosis increased with advancing age, this did not account for the relationship betwen age and serum lysozyme.

The most intriguing possibilities to explain the high serum lysozyme levels in the aged are that intracellular enzyme accumulations are increased or that there is increased catabolism of tissues containing lysozyme. Increased accumulation of hydrolytic enzymes, appearing in association with "age pigment," has been described in the cytoplasm of "aged" cells.[16] It was postulated in these studies that the increased quantity of intracellular hydrolytic enzyme in the aging cell results in eventual death of the cell by a process of autodigestion. Cathepsin was one of the enzymes described, but it seems not improbable that lysozyme also could be similarly involved. Other mechanisms to be explored are diminished renal function with advancing age and increase in the rate of granulocyte destruction. Irrespective of cause, however, the relationship between serum lysozyme level and age may provide an additional tool for study of the aging process.

No correlation between serum lysozyme level and distance from hypocenter or radiation symptoms was found. It has been speculated that a late effect of heavy exposure to ionizing radiation might be diminished granulopoiesis. This should be associated with decreased granulocyte destruction and low serum lysozyme. Neither reduced granulocyte counts nor low serum lysozyme characterized the heavily exposed A-bomb survivors. Prevoius investigations within the Adult Health Study population also have failed to show a significant relationship between previous irradiation and absolute granulocyte levels.[17] If lysozyme is related to the aging process, the question arises as to whether there are differences in the age-lysozyme relationship among survivors compared to those not in the city at the time of the bomb. No differences were observed in this small series, but perhaps these relationships should be tested on a much larger scale.

The increased serum lysozyme levels in diabetic subjects is difficult to explain. The diabetes in this group of patients was mild with persistent moderate hyperglycemia and glycosuria without acidosis. Most of the patients did not take insulin, but our observations have shown no evidence of a relationship between hyperglycemia and elevation of the serum lysozyme. Degenerative processes may have been accelerated, but it is more likely that changes of this type would have been insignificant. Bacterial

infection probably was the single most important factor, even though peripheral leukocyte counts were not elevated. At least in part, the higher serum lysozyme values were attributed to the greater than average age for this group in comparison to the controls.

Local tissue inflammation with increased fixed tissue and granulocyte destruction probably was responsible for the small, nonsignificant elevations in serum lysozyme levels in the groups with respiratory diseases and tuberculosis. Increased lysozyme levels have been noted previously in the plasma of patients with tuberculosis.[18] At the present time we are studying a large group of patients who are in different stages of active pulmonary tuberculosis. The modest relationship between increased serum lysozyme and tuberculosis in the present study may have been due to the fact that most of the diagnoses were in patients with minimal or inactive disease. In general, this group of patients tended to have slightly higher granulocyte counts.

No explanation is offered for the moderate sex variation in serum lysozyme values. It was shown not to be due to peculiarities in respect to age, disease, month of examination, or absolute granulocyte count.

The most difficult relationship to explain is that between serum lysozyme values and month of examination. Tests for unusual distribution of patients during each month in respect to age, sex, type of disease, and absolute granulocyte count were not rewarding. The laboratory techniques did not change during this period, and consistent results have been repeatedly obtained. Although these relationships should be explored further, the monthly change in mean values did not interfere with other portions of the study. A possible explanation for this phenomenon is that in the early months clinically unrecognizable respiratory disease was responsible.

SUMMARY

1. Serum lysozyme levels were determined in 670 consecutive outpatients seen for regularly scheduled clinic examinations as part of the Adult Health Study in Hiroshima, Japan.
2. Serum lysozyme levels were found to vary significantly with the absolute peripheral granulocyte count, age, sex, and month of study.
3. A high level of correlation was noted between serum lysozyme and diabetes mellitus. This may have been due to a combination of greater than average age in patients with diabetes and underlying inflammatory disease.
4. A suggestive relationship was established between serum lysozyme levels, respiratory diseases and tuberculosis. These changes are believed

to reflect active inflammation with excessive destruction of granulocytes and parenchymal tissues in those patients with the more acute processes.

5. No relationship was found between serum lysozyme and previous exposure to ionizing radiation.
6. These studies indicate that the serum lysozyme level may be useful in the study of the kinetics of leukopoiesis, the aging process, and in the detection of subtle inflammatory processes.

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