Lysozyme Turnover in Man

NIELS EBBE HANSEN, HANS KARLE, VAGN ANDERSEN, and KLAUS ØLGAARD

From the Division of Hematology, Department of Medicine A, Department of Medicine TA, and Division of Nephrology, Department of Medicine P, Rigshospitalet, University Hospital of Copenhagen, Blegdamsvej, DK 2100, Denmark

ABSTRACT Lysozyme turnover studies with ^{125}I-labeled human lysozyme were carried out on 22 patients, viz. nine control patients, seven nephrological patients with varying degrees of renal insufficiency, including three bilaterally nephrectomized patients, and six hematological patients with disturbed turnover of the neutrophilic granulocytes.

It was found that plasma lysozyme has a rapid turnover with a fractional catabolic rate of 76%/hr of the plasma content. Lysozyme catabolism varied with the endogenous creatinine clearance; in addition however, extrarenal sites of catabolism were demonstrated since lysozyme could be broken down in the anephric patients, although only at a rate amounting to about 15% of the rate found in persons with intact kidneys. In the uremic patients the increased plasma lysozyme concentration was due to decreased rates of catabolism; in the hematological patients the increased plasma lysozyme level was due to increased rates of synthesis which supports the hypothesis that plasma lysozyme mainly stems from disintegrating neutrophilic granulocytes. Furthermore, it was shown that in the non-hematological patients examined, the rate of synthesis varied with the endogenous creatinine clearance.

INTRODUCTION

Lysozyme (muramidase) exists, among the cells of the blood, exclusively in neutrophilic granulocytes and monocytes (1–2), whereas in the tissues the greatest concentrations are found in the bone marrow, lungs, intestines, spleen, and kidneys (3–6). Plasma lysozyme is believed to stem mainly from disintegrating neutrophilic granulocytes (7–9), and the high concentrations in bone marrow, lung, spleen, and intestine have been suggested to be due to the presence and probable breakdown of neutrophilic granulocytes in these organs (6). Previous turnover studies in the rat have demonstrated that the high lysozyme concentration in the kidney reflects the catabolism of lysozyme in that organ (6); lysozyme is believed to be filtered in the glomeruli, and reabsorbed in the proximal tubules, where probably also the breakdown of the lysozyme molecule takes place. This provides the basis for the application of lysozyme determinations in blood and urine as diagnostic tools in hematology and nephrology, where lysozyme measurements have been of value in the assessment of states with disturbed granulocyte turnover and in patients with proximal tubular defects.

The aim of the present study was to obtain quantitative data concerning plasma lysozyme turnover in normal man using ^{125}I-labeled human lysozyme, and, in addition to study lysozyme turnover in patients with two groups of diseases known to increase plasma lysozyme concentration, viz. hematological disorders with disturbed turnover of neutrophilic granulocytes and renal disorders with reduced glomerular filtration rate. Furthermore, turnover studies were carried out on three bilaterally nephrectomized patients in order to investigate the importance of possible extrarenal factors in lysozyme catabolism.

METHODS

Patients. 22 patients were studied. Relevant clinical data on these patients are summarized in Table I. Nine patients constitute the control group, fulfilling the following criteria: normal hemoglobin concentration, leukocyte, differential, and thrombocyte counts, serum creatinine below 1.3 mg/100 ml and endogenous creatinine clearance above 65 ml/min, normal urinary sediment, and normal concentrations of lysozyme in plasma and urine. Seven patients had renal disease with reduced glomerular filtration rate, including three bilaterally nephrectomized patients; six patients had hematological diseases with increased lysozyme concentration. Before the study, the purpose and the procedures were explained to the patients and their consent obtained.

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TABLE I

Clinical Data on Patients Examined

Patient	Age	Sex	Diagnosis	Neutrophils	Monocytes	Plasma creatinine	Creatinine clearance	Proteinuria
	yr			*per μl blood*	*per μl blood*	*mg/100 ml*	*ml/min*	*g/day*
Controls								
SBS	50	m	ulcer of the stomach	8500	180	1.0	77	0
KFL	57	m	bronchial asthma, chronic pancreatitis	3700	450	0.6	92	0
KHKS	57	m	steatosis of the liver	2800	380	0.9	115	0
SH	61	m	cerebral thrombosis	4400	230	1.2	68	0
HAP	70	m	coronary sclerosis	3800	300	1.2	66	0
KBL	43	m	ankylosing spondylitis	3500	450	0.6	141	0
IC	26	f	obesity (bilat. oophorectomized)	4200	160	0.7	147	0
LFJ	53	f	psychoneurosis	3900	310	1.2	78	0
JDN	39	f	psychoneurosis (bilat. oophorectomized)	6700	740	0.9	88	0
Nephrological patients								
GSR	65	m	hypertrophy of the prostate, chronic pyelonephritis	6900	430	5.4	10.3	0
VKS	70	m	chronic glomerulonephritis	3500	420	7.0	7.7	6
KN	47	m	arterial hypertension, chronic pyelonephritis	5800	470	2.5	43	0
HSM	54	m	chronic interstitial nephropathy, arterial hypertension	3900	500	1.7	56	0
GVN	42	f	bilat. nephrectomized (polycystic kidneys)	4300	330	4.4, 11.4	0	—
PHM	55	m	bilat. nephrectomized (chronic glomerulonephritis)	5300	590	6.7, 14.6	0	—
ACP	18	f	bilat. nephrectomized (chronic glomerulonephritis)	4600	620	3.1, 11.2	0	—
Hematological patients								
KKI	26	f	chronic myelocytic leukemia	2600	120	1.2	55	0
KOB	75	m	chronic myelocytic leukemia	21,300	260	1.3	50	0
KJ	60	f	chronic myelocytic leukemia	55,800	200	0.9	60	0
KS	24	f	acute myeloid leukemia	1900	50	0.8	86	0
KHR	53	m	myelofibrosis	41,900	18,200	1.2	80	0
PA	65	f	chronic myelocytic leukemia	144,400	0	0.9	81	0

Enzymatic activity. Purified human lysozyme was kindly provided by Professor E. F. Osserman, Columbia University, New York. This lysozyme was isolated from the urine of patients with monocytic and myelomonocytic leukemia by bentonite adsorption and elution with 5% aqueous pyridine (10) and has been shown to be identical with normal human plasma lysozyme (11). The enzyme was crystallizable (10), and no impurities or signs of denaturation in this preparation could be demonstrated by electrophoresis, immunoelectrophoresis, acrylamide gel analysis, or ultracentrifugation (11); in fact, the lysozyme preparation may be considered more than 99% pure.[1]

The study was conducted over a 3 month period; during that time the labeled protein was kept in a phosphate buffer

[1] Osserman, E. F. Personal communication.

(pH 7.5) at a concentration of 6.2 mg/ml at 4°C. At the end of the study period the lytic properties of the enzyme were as before the study; also, the fractional catabolic rates of the patients with normal renal function did not vary with the time of the study, thus militating against denaturation during the storing of the labeled protein.

Lysozyme activity in plasma (with EDTA as anticoagulant) and in urine was measured by the lysoplate method of Osserman and Lawlor (11) with human lysozyme as a reference standard. Measurements were carried out in duplicate.

Labeling procedure. The human lysozyme was labeled with ^{125}I at New England Nuclear Corp., Boston, Mass. by a modified chloramine-T method as described by McConahey and Dixon (12). Although the chloramine-T method as described by Greenwood, Hunter, and Glover (13) causes considerable denaturation of proteins, the present modification which uses only 1/1000 of the amount of chloramine-T applied by Greenwood et al. has been shown by in vivo tests not to denature proteins (12). The specific activity was approximately 0.1 mCi/mg. After iodination the enzymatic activity was normal when examined on lysoplates, and with a modified quantitative immunoelectrophoretical technique the precipitation lines were identical for labeled and unlabeled enzyme. Furthermore, as an in vivo test for denaturation of the labeled protein ^{125}I-labeled lysozyme was injected i.v. into four rats which were killed 15, 30, 60, and 120 min, respectively, after injection and several organs examined for radioactivity precipitable by trichloracetic acid as previously described (6). The concentrations in the organs examined are shown in Table II. In case of denaturation accumulation of labeled protein would be expected especially in the liver and spleen. As shown, this was not the case, since radioactive protein in these organs was at approximately the same concentration as in the lungs, intestines, and cardiac muscle. The concentration in the skeletal muscle was lower and the concentration in kidney tissue higher as demonstrated earlier for rat lysozyme (6).

Experimental procedure. Approximately 100 μCi lysozyme-^{125}I was injected i.v.; the nephrectomized patients were given approximately 30 μCi. The amount of injected lysozyme was determined by weighing the syringe before and after injection. Blood samples were drawn at intervals over 5-day periods as indicated in the figures; the study period for the nephrectomized patients was the time between two hemodialyses, i.e., 3–4 days. During the time of study urine was collected over 24-hr periods. Plasma and urine were precipitated twice with a double volume of 10% trichloroacetic acid. The radioactivity of the plasma precipitate and urinary precipitate and supernatant was measured in a gamma well counter (Selektronik, Hørsholm, Denmark).

During the period of study daily measurements of plasma and urinary lysozyme concentrations and of 24-hr endogenous creatinine clearance were carried out. These parameters, as well as the body weight remained constant in all patients with intact kidneys. In the nephrectomized patients plasma creatinine concentration rose during the study period, whereas the other parameters, including plasma lysozyme concentration, remained constant. None of the patients had fever during the study period.

The thyroid gland was blocked with 52 mg potassium iodine given daily for a 7 day period, the nephrectomized patients for a 14 day period.

The plasma volume was determined with Evans blue (14).

Methods of calculation. In all the patient examined turnover data were calculated on the basis of the plasma disappearance curve using the "peel-off" technique described by Nosslin (15). The plasma curves were construed by taking the injected dose divided by the plasma volume as 100% activity, and plotting the activity on semilogarithmic paper. The individual plasma disappearance curves were divided in a series of exponential functions, each expressed by intercept (c_i) and slope (b_i). From this the area under the curve (A) was calculated by integration ($A = \Sigma\, c_i/b_i$). *Fractional catabolic rate* (FCR)[2] was calculated as 1/A, (strictly speaking, what is determined in this way is the fractional *disappearance* rate; however, in studies such as the present, where urinary lysozyme excretion and, presumably, also excretion by other routes is low, the difference between the two terms is negligible. For this reason we have used the conventional terminology). *Rate of synthesis* was calculated as plasma volume × plasma lysozyme concentration × FCR, and expressed as rate of synthesis per kilogram per hour.

In addition to this method of calculation, turnover data were calculated also by the "metabolic clearance method" (16). This method which is based upon the clearance concept is independent of the peel-off technique since it employs only the final slope of the plasma disappearance curve plus the radioiodine excreted in the urine. In this calculation the results of the first 1 or 2 days are disregarded thereby eliminating from the calculation possibly denatured protein which is quickly catabolized.

By this method the FCR (k_c) was calculated as:

$$k_c = \frac{\sum (X_U/X_P)}{N \times 24} \text{ (per hour)},$$

where X_U = daily excretion of radioiodine in the urine, expressed as a fraction of the administered dose; X_P = fraction of the administered dose of radioiodine remaining in the intravascular pool; and N = number of days.

The fractional catabolic rate thus derived (k_c) was then corrected by using the following formula:

$$k_{c'} = k_c\left(1-\frac{\beta}{k_u}\right) \text{ (per hour)},$$

where $k_{c'}$ = the "true" fractional catabolic rate (see below); β = the constant of the final slope of the plasma disappearance curve; and $k_u = 0.0625$ (per hour).

TABLE II

Distribution of Protein-Bound Radioactivity in Organs of the Rat, after i.v. Injection of ^{125}I-Labeled Human Lysozyme

Tissue	Rat killed after			
	15 min	30 min	60 min	120 min
	% injected dose/g tissue			
Kidney	5.1	8.3	18.6	11.9
Muscle	0.03	0.02	0.05	0.04
Intestine	0.17	0.60	0.28	0.15
Heart	0.09	0.10	0.16	0.31
Spleen	0.20	0.31	0.23	0.14
Lung	0.42	0.14	0.25	0.26
Liver	0.21	0.16	0.25	0.26

[2] *Abbreviations used in this paper:* FCR, fractional catabolic rate.

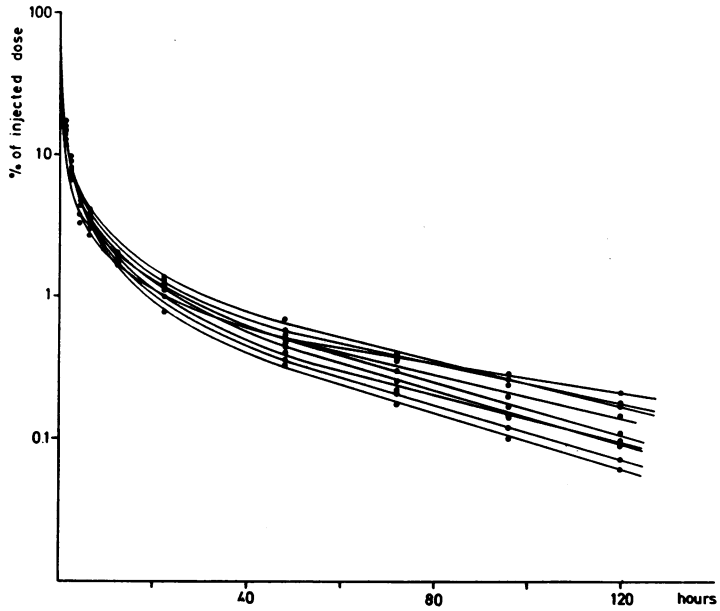

FIGURE 1 Plasma disappearance curves from the nine control patients.

This correction is made because the radioiodine liberated by breakdown of the labeled protein is not excreted immediately but is distributed within an iodine pool equal to one-third of the body weight and subsequently excreted with a rate constant (k_u) of about 0.0625/hr (17).

This method is applicable to patients with normal renal function and was applied to those control and hematological patients in whom urinary collection was sufficient (11 patients, see Tables III and V).

The renal lysozyme filtration rate expressed as per cent of the glomerular filtration rate (as measured with the endogenous creatinine clearance method) was calculated for the patients of the control group using the following formula: (plasma volume × FCR′/creatinine clearance) × 100, where FCR′ = the FCR per minute (calculated with the peel-off technique) of the control patients minus the FCR per minute found in the nephrectomized patients (discussed below). The creatinine clearance is expressed as milliliters per min.

TABLE III

Lysozyme Turnover Data: Control Patients

Patient	Plasma lysozyme	Urine lysozyme	Fractional catabolic rate	Rate of synthesis	Lysozyme glomerular filtration rate
	μg/ml	*μg/day*	*fraction/hr*	*μg/kg per hr*	*% of creatinine clearance*
SBS	9.3	340	0.866 (0.462)	344	47.0
KFL	8.0	120	0.800 (0.338)	372	37.1
KHKS	8.5	1170	0.676 (0.671)	234	29.3
SH	9.0	400	0.613 (0.524)	228	36.6
HAP	11.3	2000	0.714 (0.559)	338	40.0
KBL	7.4	0	0.877 (0.469)	381	30.6
IC	8.3	0	0.820	197	23.0
LJF	8.6	710	0.781	274	36.9
JDN	6.0	660	0.694	219	32.6
Mean±SD	8.5±1.4	610±650	0.760±0.090	287±72	34.8±6.9

Figures in parenthesis indicate FCR as calculated by the metabolic clearance method (see Methods).

RESULTS

In all the patients examined, a two-phase plasma disappearance curve with a "final" straight slope was obtained. Fig. 1 shows the plasma disappearance curves from the nine control patients. The turnover data calculated on the basis of these plasma disappearance curves are summarized in Table III. This table also shows the FCR calculated with the metabolic clearance method and the renal lysozyme filtration rate as per cent of the endogenous creatinine clearance.

Fig. 2 shows the plasma disappearance curves from the three nephrectomized patients. Fig. 3 is a magnification of the composite plasma curves from the nephrectomized and control patients during the first 200 min after injection. This clearly demonstrates the importance of the kidneys in the removal of plasma lysozyme also during the initial phase after injection, since a difference between the two curves is discernible already at the first sampling after 5 min.

Figs. 4 and 5 demonstrate the plasma disappearance curves from the patients with renal insufficiency and from the patients with hematological disorders. Turnover data from these curves are shown in Tables IV and V. It appears that the FCR varies with the glo-

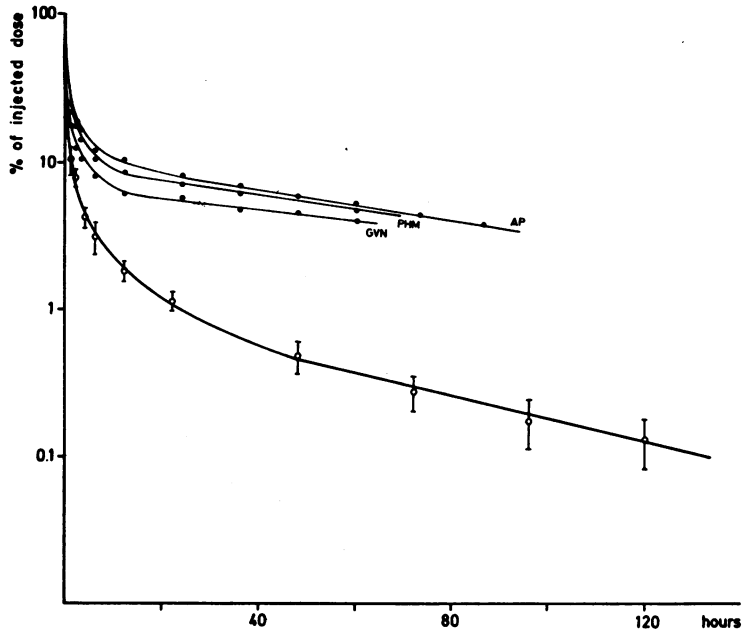

FIGURE 2 Plasma disappearance curves from three nephrectomized patients. The composite plasma curve from the control patients is shown for comparison. Vertical bars indicate SD.

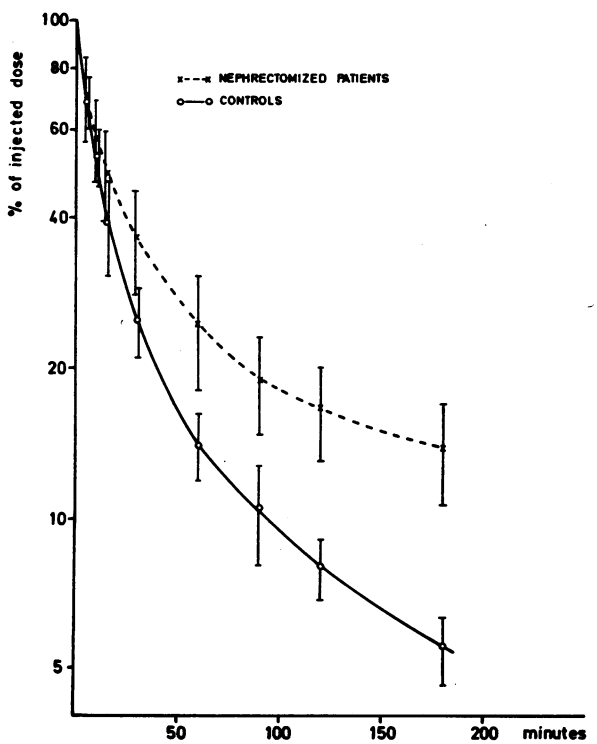

FIGURE 3 Composite plasma disappearance curves from nephrectomized and control patients during the first 200 min after injection. Vertical bars indicate SD.

merular filtration rate as measured with the endogenous creatinine clearance in both groups of patients. Computed for all 22 patients examined, the relation between creatinine clearance and FCR was statistically highly significant ($r = 0.90$; P, less than 0.001).

The rate of synthesis was significantly lower in the seven uremic patients than in the control group (P, less than 0.005). Within the uremic group of patients, again the anephric patients had significantly lower rates of synthesis than the other uremic patients (P, less than 0.01). Fig. 6 shows the relation between creatinine clearance and plasma lysozyme concentration. Besides the 22 turnover patients 20 additional patients were included in this graph. It is seen that while apparently a curvilinear relationship exists between these two parameters the hematological patients had higher plasma lysozyme levels than would be expected on the basis of their creatinine clearance values. This agrees well with the finding of this study that the rate of synthesis in the hematological patients was higher than in the remaining patients. The relation between log creatinine clearance and plasma lysozyme concentration (hematological patients excluded) was statistically highly significant ($r = 0.86$; P, less than 0.001).

DISCUSSION

The present study has shown that plasma lysozyme has a rapid turnover rate, comparable to that of immuno-

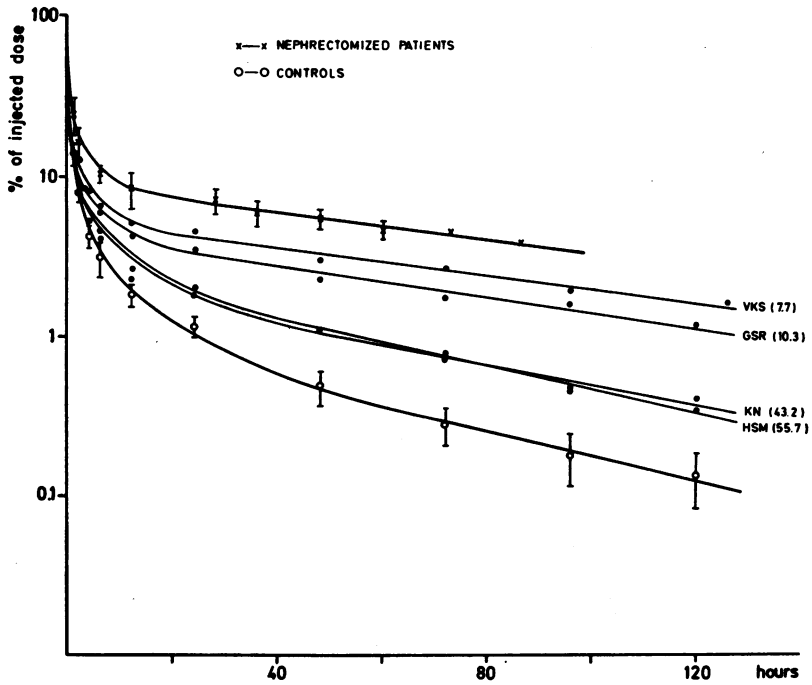

FIGURE 4 Plasma disappearance curves from patients with renal insufficiency. The figures in parenthesis indicate the endogenous creatinine clearance (ml/min). The composite plasma disappearance curves from the nephrectomized and control patients are shown for comparison. Vertical bars indicate SD.

globulin light chains which are of slightly higher molecular weight (18). It has also been demonstrated that the FCR varies with the glomerular filtration rate, but that, at least in uremia, the kidneys are not solely responsible for lysozyme degradation, since lysozyme was catabolized in the anephric patients. Furthermore, it

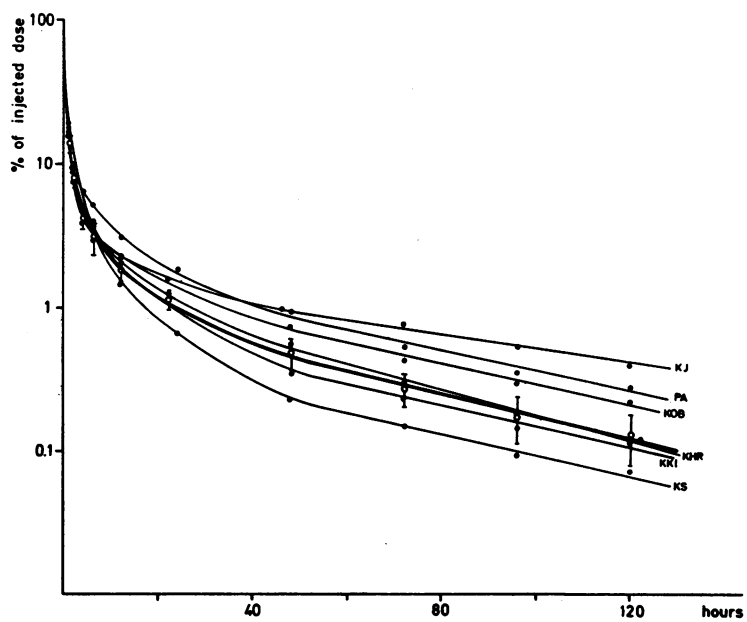

FIGURE 5 Plasma disappearance curves from the six hematological patients. For comparison the composite curve from the control patients is shown. The vertical bars indicate SD.

TABLE IV

Lysozyme Turnover Data: Nephrological Patients

Patient	Plasma lysozyme	Urine lysozyme	Fractional catabolic rate	Rate of synthesis
	μg/ml	*μg/day*	*fraction/hr*	*μg/kg per hr*
GSR	25.4	4810	0.182	219
VKS	28.8	45,480	0.105	207
KN	10.7	400	0.429	186
HSM	12.1	1210	0.430	247
Nephrectomized patients				
GVN	25.0, 24.0	—	0.119	145
PHM	22.0, 22.0	—	0.106	131
ACP	31.0, 30.0	—	0.102	143
Mean±SD (nephrectomized patients)			0.109±0.009	140±8

was shown that the increased plasma lysozyme concentration found in severe uremia is due to diminished glomerular filtration whereas the high plasma levels found in certain hematological disorders with disturbed neutrophil turnover is due to increased production of plasma lysozyme probably through release of enzyme from disintegrating cells.

The importance of the kidney for the degradation of plasma lysozyme has been shown in both experimental and clinical studies (4, 6, 19–25) although the quantitative relationship between lysozyme turnover and kidney function has not been documented previously.

Our finding that lysozyme can be degraded by extrarenal mechanisms is a counterpart in the human to the demonstration of Balazs and Roepke (20) in the rat. These workers found linearly rising plasma lysozyme concentrations immediately after bilateral nephrectomy but after 24 hr plasma levels were lower than would

TABLE V

Lysozyme Turnover Data: Hematological Patients

Patient	Plasma lysozyme	Urine lysozyme	Fractional catabolic rate	Rate of synthesis
	μg/ml	*μg/day*	*fraction/hr*	*(μg/kg per hr)*
KKI	28.1	12,330	0.602 (1.020)	832
KOB	17.4	520	0.535 (0.442)	451
KJ	16.6	1260	0.515 (0.896)	504
KS	17.1	370	0.702	781
KHR	26.2	3630	0.615 (0.711)	886
PA	31.9	2110	0.521 (0.548)	945

Figures in parenthesis indicate FCR as calculated by the metabolic clearance method (see Methods).

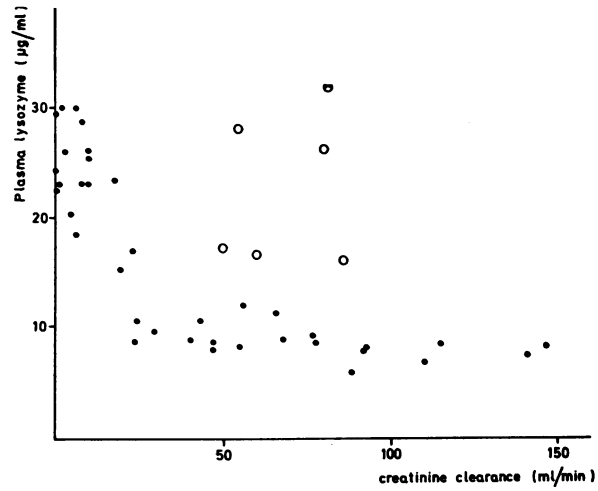

FIGURE 6 The relation between endogenous creatinine clearance and plasma lysozyme concentration. Hematological patients are indicated as O.

be expected on the basis of the initial values. In addition to the demonstration in this work that labeled enzyme is catabolized in anephric man, extrarenal sites of catabolism were suggested by the finding that plasma lysozyme concentration remained constant in the nephrectomized patient during the study period; this would be possible only if extrarenal sites existed.

Three possible interpretations should be considered: (*a*) extrarenal factors always contribute to the breakdown of lysozyme with a FCR of about 15% of the total FCR found in persons with intact kidney function, (*b*) extrarenal lysozyme catabolism is a function of the uremic state, or (*c*) with rising plasma lysozyme levels (no matter whether due to increased production or decreased destruction) new extrarenal pathways of catabolism are opened. The first explanation appears the most likely. A priori, it seems probable that lysozyme is subjected to the same (admittedly unknown) mechanisms of random destruction as other proteins (e.g. albumin). Besides, it is difficult to comprehend how the uremic state could induce new pathways for destruction of lysozyme since it has been shown that at least the albumin turnover rate is decreased or normal in uremic patients (26). Also, if lysozyme breakdown in the nephrectomized patient should be dependent upon the uremic state it might be expected that lysozyme catabolism was accelerated during the period of study between two hemodialyses when uremia increased. The lysozyme turnover rate was, however, constant. The possibility that rising levels of plasma lysozyme should induce new pathways of destruction is not compatible with the demonstration that hematological patients with plasma lysozyme concentrations as high as those found

in patients with severe uremia had FCRs as expected from their creatinine clearance values. Thus, although no conclusive evidence can be presented, we consider it most likely that extrarenal sites for the destruction of lysozyme exist even in normal conditions.

As no human lysozyme turnover studies have been published previously it is difficult to evaluate the quantitative turnover data reported in this study. However, in a study of four patients with the adult Fanconi syndrome, where the function of the proximal tubules is severely impaired and consequently a large part of the filtered lysozyme is excreted in the urine, Harrison, Lunt, Scott and Blainey (22) found a lysozyme clearance of 28% of that of creatinine clearance (range 18–49%). This figure would probably give a minimum estimate of lysozyme filtration since it would assume that no filtered lysozyme is reabsorbed in the proximal tubules. In the present study lysozyme filtration by the kidneys as percent of the endogenous creatinine clearance was 34.8% (SD 6.9), in the control group of patients. In the dog, Harrison and Barnes measured the lysozyme filtration to be 38% of that of creatinine clearance (23).

Although comparisons between turnover data from the different groups of patients of this study may be quite informative, correct turnover data in absolute terms are dependent on the use of undenatured protein, when calculations are based upon analysis of the plasma disappearance curve only. The initial, extremely rapid disappearance of labeled lysozyme from the circulation might be taken to suggest partial denaturation since it it well known that the RES avidly takes up denatured protein. Although denaturation could not be demonstrated by the in vitro and in vivo methods described above, and although apparently our data seem to fit in well with previous data, we felt obliged to apply another method of calculation in addition to the peel-off technique which is based solely on analysis of the plasma disappearance curve. The metabolic clearance method is based on the clearance concept and is independent of the peel-off technique since the metabolic clearance is determined from the ratio urine/plasma of radioiodine, disregarding the first 1 or 2 days, as described in Methods. Thus, if the initial rapid disappearance of labeled lysozyme were due to denaturation the radioiodine liberated in that process would expectedly be excreted within the first 1 or 2 days and consequently not influence the FCR obtained with the clearance method. Consequently, if the initial rapid disappearance of labeled lysozyme were due to denaturation the clearance method would yield lower FCRs than the peel-off technique. Taking together all patients examined with the two methods (Tables III and V) there was no difference between FCRs obtained (mean FCR with the peel-off technique: 0.667, and with the metabolic clearance method: 0.604; P, more than 0.1), and even if the control patients as a group showed somewhat lower values with the metabolic clearance method (Table III), the close agreement of the results obtained with the two methods is an argument against denaturation as the reason for the initial rapid decline in plasma activity.

It remains to be investigated how lysozyme is distributed in the human organism. If the Nosslin equation for the calculation of the volume of distribution (15) is applied on the present data the volume of distribution in the control group of patients amounts to about 100% of the body weight. It must be emphasized, however, that this volume of distribution is a theoretical concept, and that very large figures may result if the protein in question is concentrated somewhere in the organism. For lysozyme, such a site of concentration may be the kidneys even if other sites cannot be excluded. Our earlier studies of lysozyme turnover in the rat thus showed considerable concentrations of protein-bound radioactivity in the kidneys also at a time when radioactivity in the plasma had fallen to very low levels (6). The present findings in our anephric patients may suggest a similar role for the kidneys in the human: thus, if the volume of distribution is calculated for the anephric patients a reduction by 50% as compared to the normal group of patients is found. A slow release of labeled lysozyme from such sites of concentration back into plasma would account for the not negligible activities found in plasma several days after the injection of labeled lysozyme. This mechanism would also explain the biological half-life of 21 hr which is found for the normal group if the Nosslin equation for the calculation of this figure (15) is applied on the present material.

Evidence has been presented that plasma lysozyme mainly stems from disintegrating neutrophilic granulocytes (7–9). Well in line with this is the demonstration of the present study that the rate of synthesis in the hematological patients was higher than for the remaining patients, and that the high plasma lysozyme concentration was due to increased production rate whereas the fractional catabolic rate was normal. As further evidence that plasma lysozyme stems from disintegrating neutrophils, the mean content per cell of lysozyme as calculated from the present turnover data may be compared to figures from the literature where intracellular lysozyme concentrations have been reported at 1–6.3 μg/1 million leukocytes and 7.2 μg/1 million neutrophils (27–30). In a preliminary study we have, in 13 normal persons, found an intracellular lysozyme content of 7.6±2.1 (SD) μg/1 million neutro-

phils.[3] Using the mean figure for lysozyme synthesis in the control group of patients (287 μg/kg per hr) and figures from the literature for the neutrophil turnover rate (mean 68×10^6 cells/kg per hr, with 95% limits at $21–142 \times 10^6$) (31) a mean content of 4 μg/1 million neutrophils is arrived at (range 2.0–13.7). Figures calculated from this study are thus of the same order of magnitude as the experimentally determined figures of others and ourselves. It should be emphasized that it is a basic assumption for such a calculation that all disintegrating neutrophils liberate their lysozyme content to the plasma.

Somewhat surprisingly, this study demonstrated that the rate of synthesis varied with the creatinine clearance. If it is accepted that plasma lysozyme stems from disintegrating neutrophils this would mean that in uremia either the lysozyme content of the neutrophilic granulocytes is diminished or that the neutrophil turnover rate (number of cells disintegrating per unit time) is decreased. Preliminary studies on the intracellular lysozyme content in neutrophilic granulocytes have shown that neutrophils from severely uremic patients contain significantly lower lysozyme concentrations than normals (mean lysozyme concentration in seven uremic patients 5.2±1.0 (SD) μg/1 million neutrophils, and in 13 normals 7.6±2.1 (SD) μg/1 million neutrophils; *P*, less than 0.025).[4]

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