Serum and Urinary Proteins, Lysozyme (Muramidase), and Renal Dysfunction in Mono- and Myelomonocytic Leukemia

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ABSTRACT Serum levels, urinary excretion, and clearances of several proteins of different molecular weights were studied in 18 patients with mono- and myelomonocytic leukemia. Nine patients had normal renal function (group A) and nine had impaired renal function with azotemia (group B). The majority of patients in both groups had increased concentration of immunoglobulins, particularly IgG, IgA, and IgM; IgD level was normal. Serum transferrin and α_2-macroglobulin were frequently reduced while the level of ceruloplasmin was often increased, especially in patients with azotemia. The activity of lysozyme in the serum was high in all patients, but was considerably higher in group B.

Proteinuria was found in most patients but was more prominent in group B. Almost invariably albumin constituted less than 25% of the total protein excreted. Qualitative analysis of various urinary proteins by immunochemical techniques and clearance studies suggested the presence of glomerular as well as tubular dysfunction. Determination of urinary lysozyme frequently showed no direct correlation between the serum level of the enzyme and its concentration in the urine or its clearance by the kidney. In addition to glomerular filtration, impaired tubular reabsorption may account for the high level of lysozyme in the urine. It is postulated that the very high level of lysozyme in the glomerular filtrate and possibly hypergammaglobulinemia may play a role in the induction of tubular damage. Renal impairment has been correlated with histological changes in the kidneys. From a comparative study of various leukemias, it seems that the combined glomerular-tubular dysfunction is a manifestation unique to mono- and myelomonocytic leukemia.

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INTRODUCTION

Hypogammaglobulinemia and susceptibility to infection have been well documented in various leukemias (1–3). However, a few authors have reported an increase rather than a deficiency of serum gammaglobulins in monocytic and myelomonocytic leukemia (4–8). Surprisingly, the literature contains little or no information concerning proteinuria in leukemia patients (9) and, with the exception of well documented uric acid nephropathy (10, 11), the problem of renal injury has been studied from a histologic rather than a functional point of view (12). The latter has recently assumed importance in view of the demonstration of increased levels of serum and urinary lysozyme (LZM) in mono- and myelomonocytic leukemia (7, 13). In addition to lysozymuria, other proteins have also been found in the urine of these patients. Some of them have had electrolyte disturbances with hypokalemia and hypomagnesemia (7, 14, 15). Osserman and Azar have postulated that the abnormally high concentration of LZM in the renal cortex results in injury to the proximal tubular cells, thus contributing to the tubular dysfunction (16).

The present report concerns our observations on various serum and urinary proteins in 18 patients with mono- and myelomonocytic leukemia. It was found that a majority of the patients had hypergammaglobulinemia. Renal clearance studies of several proteins of different molecular weights, as well as immunochemical analyses of various urinary proteins suggested that these patients probably had both glomerular and tubular dysfunction. The determination of urinary LZM frequently showed no significant correlation between the serum level of the enzyme and the degree of lysozymuria, or its clearance by the kidney. In addition to glomerular filtration, it is possible that impaired tubular reabsorption and perhaps other mechanisms might be responsible for the high level of LZM in the urine. An attempt has

also been made to correlate the histological changes in kidneys with the degree of functional impairment.

METHODS

18 patients with monocytic and myelomonocytic leukemia were divided into two goups (A and B) on the basis of their renal function. Group A consisted of nine patients having normal renal function. In this group, blood urea nitrogen (BUN), serum creatinine, and creatinine clearance were within the normal range. (Normal values were: BUN, 5–25 mg/100 ml; serum creatinine, male 0.9–1.4 mg/100 ml, female 0.8–1.2 mg/100 ml; creatinine clearance, 70–130 ml/min.) Group B consisted of nine patients having renal impairment with azotemia. A group of 20 healthy subjects and 250 patients with various diseases with or without renal failure served as controls. Data pertaining to this part of the study were reported elsewhere (17).

Samples of serum and urine were examined either immediately or stored at −20°C for varying periods of time. When necessary, samples of urine were concentrated as much as 500-fold, by dialysis in boiled seamless cellulose membranes against 25% polyvinylpyrrolidone or by negative pressure dialysis. Total protein was estimated by the biuret method (18). Microzone cellulose acetate electrophoresis was performed using 0.075 M barbital buffer, pH 8.6, and the membranes scanned in a Beckman's Analytrol. Immunoelectrophoresis was performed according to the technique of Grabar and Williams (19) with minor modifications. Antisera against whole human serum, IgG, IgA, IgM, transferrin, ceruloplasmin, and Fc and Fab fragments were obtained from Hyland Laboratories, Los Angeles, Calif. Rabbit anti-IgD antiserum (R133A) was kindly provided by the NCI Immunoglobulin Reference Center, Springfield, Va., courtesy of Dr. J. Fahey. This antiserum readily detected a level of 0.3 mg/ml of IgD. Anti-Bence Jones type kappa and lambda antisera and anti-human lysozyme antiserum were prepared in our laboratory by immunizing rabbits with purified Bence Jones proteins from urines of myeloma patients or with human LZM. Purified LZM was generously provided by Dr. E. F. Osserman, New York, and used in conjunction with our own material isolated from the urine of a patient with monocytic leukemia using the technique of Alderton, Ward, and Fevold (20). The purity of the isolated LZM was verified by electro- and immunoelectrophoresis and by analytical ultracentrifugation. Immunoquantitation of IgG, IgA, IgM, transferrin, ceruloplasmin, and α_2-macroglobulin was done using standard immunoplates obtained from Hyland Laboratories, and that of IgD using immunoplates of Kallestad Laboratories Inc., Minneapolis, Minn. The minimal concentration of IgD detectable by these plates was 0.02 mg/ml. Lysozyme quantitation was performed by the lysoplate technique as described by Osserman and Lawlor (7). Lysozyme activity in the samples tested was compared to that of pure human lysozyme, microgram equivalent per milliliter of buffer. Prepared standards of pure human lysozyme containing 5, 25, 100, 250, and 500 μg/ml were applied to each lysoplate and a semilog curve plotted. Analyses of urinary amino acids were kindly performed by Dr. S. H. Jackson, Hospital for Sick Children, Toronto, using one-dimensional thin-layer chromatography (21). Clearances of urinary proteins, creatinine, and uric acid were calculated on the basis of 24-hr collections of urine using the formula $(U_c \times U_v)/(S_c \times 1440) = \text{ml/min}$. The clearances of eight proteins were determined; viz. lysozyme, mol wt 14,000–15,000 (7); transferrin, mol wt 73,000–76,000 (22); ceruloplasmin, mol wt 160,000; IgG, mol wt 160,000; IgA and IgD, mol wt 160,000 or higher; α_2-macroglobulin, mol wt 840,000; and IgM, mol wt 900,000. The selectivity of protein excretion was examined by plotting the clearance values of IgG and α_2-macroglobulin expressed as a percentage of transferrin clearance accepted as 100%, against the respective molecular weights on double-log paper. The slope of the plotted line would be an expression of the selectivity and values exceeding a slope of 67° were accepted as highly selective (23, 24). The estimation of BUN, uric acid, electrolytes, and creatinine was performed in the same sera and urines in which the proteins were determined. White blood cells were usually counted on the same day that the serum and urines were collected. In three patients, however, there was a lapse of 24–48 hr between these determinations. Autopsy examination was performed in six patients with special attention directed to histological changes in the kidneys. The kidneys of seven additional patients with monocytic or myelomonocytic leukemia (not included in the present study) were also examined.

RESULTS

There were no significant differences in age or sex between groups A and B (Tables I and IV). The duration of the disease in group A varied from 2 wk to 48 months with a mean of 11 months. In group B, the duration ranged from 3 wk to 24 months, on the average, 5.1 months. Patients without renal failure (group A) often had fewer leukemic cells in the peripheral blood than patients with azotemia (group B). In nine patients, monoblasts were observed in addition to the abnormal monocytes and these cells were included in the total count of monocytes.

Group A (Tables I and II)

Serum. By definition, patients included in this group had normal BUN, serum creatinine, and creatinine clearance. Blood urea nitrogen varied between 7 and 23 mg/100 ml and serum creatinine ranged from 0.8 to 1.4 mg/100 ml. Creatinine clearances varied from 71 to 104 ml/min. The serum uric acid was normal in all but one patient in whom a value of 12 mg/100 ml was recorded. The serum potassium was normal in all but two patients (Nos. 4 and 5) in whom values of 3.0 and 3.2 mEq/liter were recorded. In two patients (Nos. 5 and 6) the serum calcium was 7.6 mg/100 ml while the serum phosphorus was normal. No other disorders of electrolytes were found. One of the patients with hypocalcemia (No. 5) also had a low serum albumin of 2.9 g/100 ml.

The total serum protein varied from 4.67 to 9.6 g/100 ml (mean 6.84 ±1.33 g/100 ml). In two patients, the level exceeded the normal range and in two others was below normal (normal values, Table III). The gamma globulin concentration ranged from 0.84 to 3.5 g/100 ml (mean 1.7 ±0.73) with hypergammaglobulinemia in four patients. None had hypogammaglobulinemia. Im-

TABLE I

Hematologic and Metabolic Data in Patients

No.	Patient	Sex	Age	No. of monocytes	No. of polymorphonuclears	Serum K*	Serum Ca*	Serum P_*
			yr	*mm³*	*mm³*	*mEq/liter*		*mg/100 ml*
1	A. G.	M	85	1054	42.780	4.7	8.2	3.4
2	T. P.	M	48	1044 (14)‡	696	3.8	8.4	2.9
3	P. K.	F	50	670 (67)	268	3.9	9.1	4.4
4	M. N.	F	56	2870 (21)	12.095	3.0	9.1	3.8
5	E. B.	F	69	6900	21.000	3.2	7.6	2.6
6	H. B.	M	54	17.850	38.556	3.9	7.6	3.1
7	B. W.	F	60	858	5148	3.8	8.0	2.9
8	L. B.	F	70	615	2796	4.0	9.2	4.4
9	O. B.	M	49	2256 (19)	0	3.7	8.6	4.2
Mean			60.1	3790	13.704	3.8	8.4	3.5
SD			11.7	5313	15.806	0.46	0.59	0.66

C_{cr} = creatine clearance; $C_{u.a.}$ = uric acid clearance.

* Normal values: K, 3.6–5.0 mEq/liter; P, 2.5–4.8 mg/100 ml; BUN, 5–25 mg/100 ml; creatinine clearance, 70–130 ml/min; urinary uric acid, 250–750 mg/24 hr; Ca, 8.5–10.5 mg/100 ml; Mg 1.6–3.2 mg/100 ml; creatinine, 0.8–1.4 mg/100 ml; serum uric acid, 2.5–7.0 mg/100 ml; uric acid clearance, 5.0–12.5 ml/min.

‡ Per cent of monoblasts.

munoquantitation (See Table II) showed IgG concentrations ranging from 550 to 2400 mg/100 ml. Levels exceeding 1600 mg/100 ml were observed in four patients. IgA concentrations varied from 100 to 2360 mg/100 ml and exceeded 350 mg/100 ml in three of the same four patients. IgM concentrations ranged from 35 to 322 mg/100 ml and was clearly elevated in only one patient. Among individual cases, one patient had hypergammaglobulinemia involving all three immunoglobulins, and two patients had an increase in both IgG and IgA. In all patients, the level of IgD was either low or normal. The Sia test was positive in one patient who also had a high concentration of IgM. No cryoglobulins or pyroglobulins were found. Serum transferrin was decreased as compared to the normal controls, and varied from 48 to 224 mg/100 ml (mean 121 ±44 mg/100 ml) being 150 mg/100 ml or less in seven patients. Ceruloplasmin varied between 13.4 and 62 mg/100 ml (mean 31.8 ±17.7), and was less than 25 mg/100 ml in three patients. α_2-Macroglobulin ranged from 148 to 710 mg/100 ml. The level was below 200 mg/100 ml in the sera of five patients. Serum LZM varied from 5 to 84 μg/ml with a mean of 31.3 ±16.6 μg/ml. This range included a number of determinations obtained while patients were in remission. The LZM level was consistently increased before therapy varying from 24 to 84 μg/ml with a mean of 41 μg/ml. No direct correlation was observed between the level of serum LZM and the number of the circulating leukemic cells. In five instances, the C'3 level was recorded in the range of 116–208 mg/100 ml with a mean of 156 mg/100 ml. None of the patients suffered from bacterial or other infections during the period of study. Six patients were treated with various antileukemic agents while three patients received steroids.

Urine. The total urinary protein varied from 31 to 1720 mg/24 hr with an average of 524 mg/24 hr, and exceeded 300 mg in five instances. Cellulose acetate electrophoresis of the urinary proteins revealed variable patterns, frequently with low contents of albumin and prominent α_2- and β-proteins. In two patients, lysozyme constituted the major component excreted in the urine, the electrophoretic pattern resembling Fig. 1 *a*. In seven others, the electrophoretic patterns were similar to those shown in Fig. 1 *b* and *c*. Albumin constituted 1–32% of the total urinary protein, with an average of

with Normal Renal Function (Group A)

Serum Mg*	BUN*	Creatinine*	Uric acid*	Urine, uric acid*	C_{cr}	$C_{u.a.}$	Treatment
mg/100 ml				*mg/24 hr*	*ml/min*	*ml/min*	
1.80	21	1.3	6.7	744	82	8.06	—
1.72	12	0.8	4.3	780	104	12.6	Vincristine 6 MP
1.75	11	1.1	5.8	544	75	6.57	Prednisone 6 MP
1.75	7	0.7	6.4	396	78	4.29	Allopurinol 6 MP
1.70	23	0.6	3.3	266	89	12.1	Prednisone
1.90	11	1.0	5.6	798	92	9.9	—
1.70	9	0.8	3.4	596	71	12.16	Amethopterin 6 MP
1.74	16	1.4	3.4	328	73	6.69	Prednisone 6 MP, MTX
1.73	17	1.0	12.0	1009	89	6.48	Vincristine 6 MP Allopurinol
1.75	14	0.97	5.7	606	83.7	8.77	
0.06	5.1	0.25	2.6	232	10.1	2.85	

12.8 ±5.7%. The amount of IgG excreted ranged from 6.8 to 364 mg/24 hr. Clearances of IgG varied greatly but were approximately 7 times higher than in healthy controls. Excretion of IgA varied from 1.1 to 111.0 mg/24 hr. In one instance, the amount of IgA excreted exceeded that of IgG in the urine. In the remainder, the ratios of IgG to IgA varied from 2:1 to 30:1. Clearances of IgA also showed great variations, and were approximately 4 times higher than in healthy individuals. IgD and IgM were detected in only one urine each. Fc and F'c fragments of IgG were detected in the urines of five patients. This observation bore no relationship to the storage time of the urine since these fragments were observed in three completely fresh urines. In one instance transitory excretion of Bence Jones type kappa protein was noted.

Transferrin was excreted in amounts varying from 0 to 33.6 mg/24 hr and comprised up to 37% of the total urinary protein with a mean of 1.34 %. The average clearance of transferrin was approximately twice as high as in healthy persons although a great variability in individual clearances was observed.

Ceruloplasmin varied from 0.78 to 8.65 mg/24 hr, and accounted for 0.09–9.6% of the total urinary protein with a mean of 0.43%. The mean clearance was similar to that in normal controls.

α_2-Macroglobulin was excreted in the urines of four patients in amounts varying from 0.87 to 13.54 mg/24 hr. This protein was not found in the urines of nine healthy persons. The C'3 level was tested in five urines and detected in one only in the amount of 8.5 mg/24 hr.

Lysozyme excretion varied greatly from 0.9 mg to 1320 mg/24 hr (mean 142.5). This range included values obtained during remissions. Pretreatment values were always higher with an average excretion of 239 mg/24 hr. (The maximum excretion of LZM in the urines of healthy persons never exceeded 2.9 mg/24 hr.) Lysozyme constituted from 1 to 47.2% of the total urinary protein, with an average of 9.4%. Renal clearances of LZM varied greatly, being in the normal range in 12 instances and increased in 18 others. A correlation coefficient (r) of a linear function was calculated for the serum and urinary LZM levels and also for the serum LZM and Clzm. In the first instance, the correla-

TABLE II

Protein Studies in Patients with

No.	Serum		Urine		Lysozyme			Transferrin			Ceruloplasmin		
	Total protein	Gamma globulin	Total protein	Albumin	Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance
	g/100 ml		*g/24 hr*	*% of total protein*	*μg/ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*
1	5.60	1.52	0.440	9	38	15.4	0.28	91	9.02	0.007	13.4	3.52	0.018
2	6.78	1.07	0.095	32	27.4	0.95	0.024	152	1.25	0.0006	26.2	0.78	0.002
3	6.50	1.65	0.192	15	34	7.15	0.146	48	1.70	0.0024	62.0	1.36	0.0015
4	6.47	1.43	0.052	13	38.5	15.4	0.28	150	2.53	0.0018	41.0	1.35	0.0023
5	9.60	3.50	0.031	14	36.0	20.2	0.39	224	33.6	0.010	57.0	8.65	0.011
6	8.15	2.18	0.570	8	43	269	4.34	116	1.89	0.0011	41.0	2.30	0.004
					84	1320	21.3						
7	4.67	0.84	1.140	7	24	65	1.89	124	4.21	0.0024	56.0	2.17	0.0027
					13.2	16.6	0.87						
8	6.73	1.42	1.720	16	20.3–	0.9–	0.03–	123	1.27	0.0007	17.0	1.47	0.006
					34.0*	45.7	0.93						
9	7.05	1.66	0.48	1	5.0–	1.46–	0.06–	61–149	0–7.95	0–0.0049	14.8–	0.80–	0.0028–
					53.0‡	693	11.47				19.6	2.60	0.012
Mean	6.84	1.70	0.524	12.8	31.3	142.5	2.53	121	5.61	0.00279	31.8	2.43	0.0066
SD	1.33	0.73	0.535	5.7	16.6	277.1	4.51	44	8.87	0.00293	17.7	2.02	0.0049

All these were included in the calculation of the mean value and standard deviation.

* 7 sets of estimations.

‡ 14 sets of estimations.

tion was significant at the level of $P < 0.02$ and in the second, at $P < 0.025$ (Figs. 2 and 3).

An analysis of the relationship between the level of serum LZM and the amount of urinary LZM revealed three patterns. In 21 instances, both values were proportionally high. Four estimations showed increased serum LZM and normal level of the enzyme in the urine. In these patients, the serum LZM concentration varied from 21.3 to 34 μg/ml and the urinary LZM ranged from 0.48 to 1.97 μg/ml. In five instances, serum LZM was dysproportionally low as compared to the urinary LZM. The serum values varied from 6.5 to 28.7 μg/ml while the urinary LZM values ranged from 4.1 to 242 μg/ml. In 16 instances, urinary LZM was high although serum LZM level was increased less than three times as compared to maximum normal value. Here the serum LZM varied from 6.5 to 43 μg/ml with a mean of 29.2 μg/ml, whereas urinary LZM ranged from 4.1 to 242 μg/ml with a mean of 74 μg/ml.

Investigation of selectivity of protein excretion showed that ratios of C_{IgG}/C_{Tf} varied from 5.4 to 772 and were below 100 in only five instances. In four patients, the ratios of $C\alpha\text{-}2M/C_{Tf}$ varied from 3.5 to 89. Five patients showed no α_2-macroglobulin in the urine. None of the individual cases showed high selectivity pattern.

Uric acid excretion was normal in all but three patients, one with hyperuricemia and two with normal serum uric acid level.

The kidneys of three patients (Nos. 2, 4, and 5) were available for histological study. All three showed a moderate number of protein casts in the collecting tubules. In two, there were small interstitial leukemic infiltrates and in one, occasional hyalinization of the glomeruli. In one patient (No. 5) there were many hyaline droplets in the proximal tubular cells. This patient had a moderately high level of urinary LZM (20 μg/ml) and a very high level of serum gammaglobulins (3.5 g/100 ml). Hypokalemia and hypocalcemia were additional findings. None of the three patients had excessive uricosuria or hyperuricemia and none suffered from intercurrent infection.

Group B (Tables IV and V)

Serum. In this group, the blood urea nitrogen varied between 8 and 150 mg/100 ml. However, in every patient the level exceeded 30 mg/100 ml during 2 wk immediately preceding or following the date of serum and urinary collections. The serum creatinine was in-

Normal Renal Function (Group A)

IgG			IgA			IgD			IgM			α_2-Macroglobulin			Comments
Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance	
mg/100 ml	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	
2150	121	0.0039	420	18.7	0.0031	2<	0	—	50	0	0	151	13.54	0.0062	Fc and F'c fragments in the urine
1380	7.2	0.00036	186	3.60	0.0013	9.2	0	—	110	0	0	260	0	0	—
1260	20.4	0.0011	175	6.80	0.0027	5.1	0	—	100	0	0	355	4.42	0.00086	Fc and F'c fragments in the urine
1430	24.8	0.0012	340	3.85	0.00079	2<	0	—	60	0	0	197	0	0	—
2400	20.2	0.00054	2360	26.26	0.00070	6.5	0	—	322	0	0	175	0.87	0.00035	Sia test +; Fc and F'c fragments in the urine
1730	212	0.0085	430	7.33	0.0012	2<	0	—	53	0	0	148	0	0	Fc and F'c fragments in the urine
550	34.2	0.0052	100	2.51	0.0020	2<	5.2	—	35	5.7	0.013	710	3.99	0.00039	—
960	6.8	0.00078	125	1.13	0.0010	2<	0	—	140	0	0	160	0	0	Fc and F'c fragments in the urine
1730	364	0.0099	260	111.0	0.0020	2<	0	—	137	0	0	265	0	0	Transitory excretion of Bence Jones type kappa globulin in the urine
1510	90.1	0.0035	488	20.13	0.0016	—	—	—	112	—	—	269	—	—	
538	116.3	0.0034	671	33.08	0.0008	—	—	—	83	—	—	169	—	—	

creased in three patients and creatinine clearances were lower than normal in seven. The serum uric acid varied from 3.0 and 17.9 mg/100 ml and exceeded 7.5 mg/100 ml in three patients. Serum potassium was repeatedly low in three patients (Nos. 1, 4, and 7) varying between 2.2 and 3.4 mEq/liter. In two of these, the

TABLE III

Protein Studies in Healthy Individuals

Protein	No. of cases	Serum			Urine			Clearance		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
		mg/100 ml			*mg/24 hr*			*ml/min*		
Lysozyme*	20	6.6–13.8	9.69	1.80	7.6–2900	1570	800	0.005–0.33	0.116	0.071
Transferrin	9	160–276	219	37	2.07–7.31	3.61	1.88	0.00072–0.0023	0.0012	0.0006
Ceruloplasmin	9	23.5–41.0	30.2	5.1	0.69–4.33	2.68	1.26	0.0013–0.012	0.0064	0.0034
IgG	10	980–1530	1278	227	12.6–112	53.62	40.7	0.00019–0.00061	0.00046	0.00014
IgA	10	204–322	259	45	0–3.36	2.3	1.25	0–0.0011	0.00045	0.00037
α_2-Macroglobulin	9	—	372‡	—	0§	0	0	0	0	0
	9	—	355							
IgM	10	63–165	93	38	0	0	0	0	0	0

Serum total protein, normal range 6.3–8.0 g/100 ml; serum gamma globulin, normal range 0.7–1.6 g/100 ml; urinary total protein, normal range 50–187 mg/24 hr; IgD, normal range 0.3–40 mg/100 ml (57).

* Serum level in μg/ml, daily excretion in μg/24 hr.

‡ Pooled serum samples of nine women and nine men.

§ Nine individual estimations.

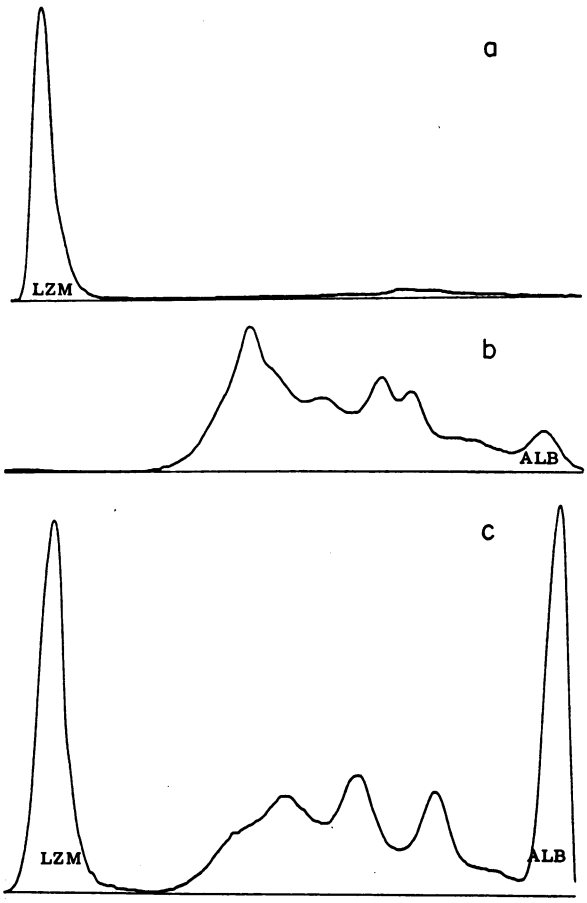

FIGURE 1 Representative electrophoretic patterns of concentrated urine of patients with mono- and myelomonocytic leukemia. (*a*) Lysozyme is the major protein excreted. Patient 8, group B: BUN, 48 mg/100 ml; K, 2.9 mEq/liter; proteinuria 1.26 g/24 hr; lysozymuria 1.19 g/24 hr. (*b*) Predominant excretion of α- and β-proteins. Patient 6, group B: BUN, 150 mg/100 ml; K, 3.4 mEq/liter; proteinuria 6.24 g/24 hr; lysozymuria 18 mg/24 hr. (*c*) Albumin and globulins, and lysozyme excreted in large quantities. Patient 4, group B: BUN, 77 mg/100 ml; K, 3.3 mEq/liter; proteinuria 3.26 g/24 hr; lysozymuria 298 mg/24 hr.

calcium was also low (7.4 and 7.7 mg/100 ml) with a low serum phosphorus and magnesium in one patient. The latter patient had hypoalbuminemia of 2.8 g/100 ml and suffered from chronic pyelonephritis in addition to leukemia. In two other patients (Nos. 6 and 8) a single estimation also showed hypokalemia.

The total serum protein varied from 5.6 to 8.5 g/100 ml (mean 7.14 ±0.84/100 ml). It was in excess of normal in one patient, and below the normal range in two. Gamma globulin ranged from 0.90 to 3.67 g/100 ml (mean 2.26 ±0.84 g/100 ml). The level exceeded the upper normal limit in seven patients. None were subnormal.

Immunoquantitation of IgG (see Table V) showed values from 930 to 3200 mg/100 ml, the level exceeding 1600 mg/100 ml in eight patients. IgA levels varied from 106 to 1870 mg/100 ml and exceeded 350 mg/100 ml in seven patients. IgD was normal or low. IgM ranged from 36 to 685 mg/100 ml and exceeded 200 mg/100 ml in seven patients. Seven patients showed an increase in all three immunoglobulins, and one an increase in IgG and IgA only. The Sia test was positive in four patients who also had high levels of macroglobulin. One patient had cryoglobulin in the serum and Bence Jones proteinuria.

Transferrin levels varied from 89 to 289 mg/100 ml with values below 150 mg/100 ml in four patients. Three patients suffered from bacterial infection during the study (Nos. 1, 7, and 9), requiring treatment with antibiotics. The gammaglobulins were reduced in one of these and increased in two. Transferrin was 173, 176, and 263 mg/100 ml, respectively.

Ceruloplasmin ranged from 22.4 to 100 mg/100 ml and was less than 25 mg/100 ml in one serum only. α_2-Macroglobulin varied from 130 to 370 mg/100 ml. In four patients, the level was low (200 mg/100 ml or less). Serum LZM varied from 28.2 to 150 μg/ml with a mean of 85 ±36.8 μg/ml. C'3 was estimated in three cases and varied from 100 to 254 mg/100 ml.

Urine. The total protein excreted in the urine ranged from 0.83 to 6.64 g/24 hr (mean 3.1 ±2.04 g).

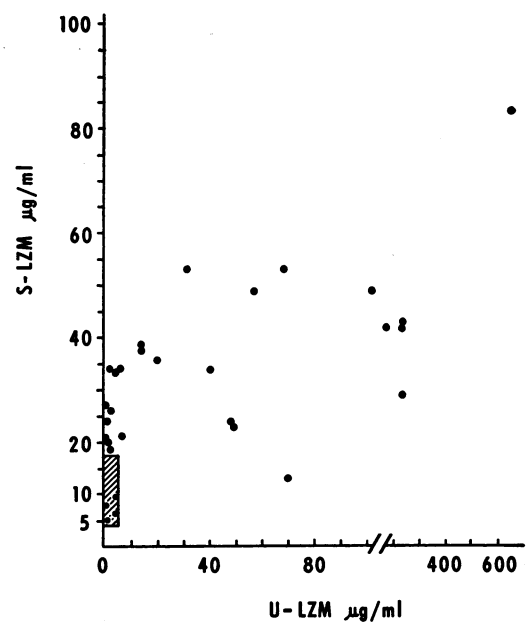

FIGURE 2 Group A. Relationship between serum lysozyme (S-LZM) and urinary lysozyme (U-LZM), 30 estimations. $r^2 = 0.4597$. Correlation significant at the level $P < 0.02$. Shaded area indicates normal range.

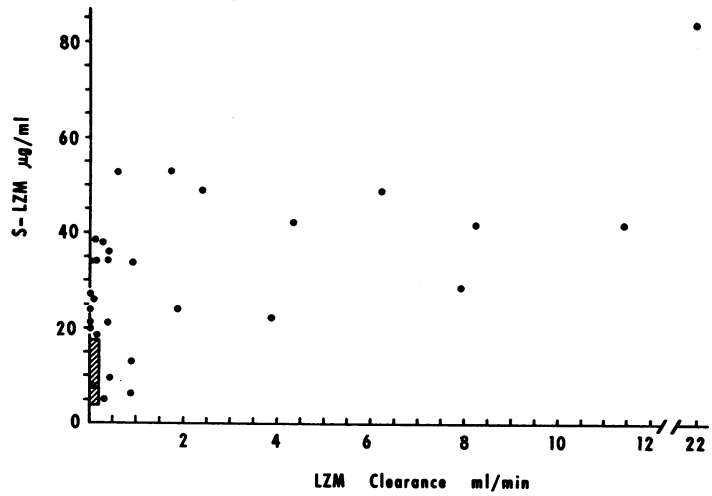

FIGURE 3 Group A. Relationship between serum lysozyme (S-LZM) and its clearance by the kidneys, 30 estimations. $r^2 = 0.42230$. Correlation significant at the level $P < 0.025$. Shaded area indicates normal range.

Cellulose acetate electrophoresis of the urinary proteins revealed lysozyme as the predominant constituent in three patients with a pattern resembling Fig. 1 *a*. In the remainder, the patterns were similar to those in Fig. 1 *b* and *c*. Albumin, expressed as a percentage of the total protein excreted in the urine, ranged from 3 to 33% (mean 18 ±9.9%) being less than 25% in seven patients. IgG ranged from 6.8 to 445 mg/24 hr and IgA from 3.75 to 228 mg/24 hr. A great variability was noted in the renal clearances of both IgG and IgA. IgD was found in five instances ranging from 2.5 to 146 mg in 24 hr. IgM was found in urines of five patients, varying from 0.9 to 257 mg/24 hr. Fc and F'c fragments of IgG were found in urines of seven patients. The Bence Jones heat test was positive in the urine of one patient.

Transferrin levels ranged from 5.25 to 52.5 mg/24 hr, and accounted for 0.08 to 4.4% of the total protein (mean 0.6%). Ceruloplasmin ranged from 0.59 to 50.4 mg/24 hr with a great variability in the renal clearances and urinary concentrations. Ceruloplasmin accounted for 0.015–0.62% of the urinary protein (mean 0.16%). α_2-Macroglobulin was found in urines of eight patients varying from 3.15 to 38.84 mg/24 hr (mean 15.06 mg/24 hr). C'3 was determined in three urines and varied from 0.176 to 0.648 mg/100 ml with a total excretion of 0.29 to 9.7 mg/24 hr.

Urinary LZM values ranged from 14 to 1480 µg/ml. The total amount in individual urines was high, reaching values up to 2 g/24 hr. Lysozyme constituted from 0.3 to 94% of the total protein excreted (mean 23%). In all but one instance these were high levels of serum and urinary LZM. One patient had a relatively low serum LZM (28.2 µg/ml) and high urinary LZM (14.0 µg/ml). Since the number of circulating monocytes and the levels of serum LZM were greater in patients of group B, a correlation of the levels of serum LZM to urinary LZM was determined to check whether the number of circulating monocytes was the only factor responsible for the increased excretion of LZM. The correlation coefficient (r) of a linear function for serum level of LZM and urinary LZM was significant at $P < 0.05$ and for serum LZM and C_{LZM} there was no statistically significant relationship (see Figs. 4 and 5). The calculation of C_{IgG}/C_{Tf} and $C\alpha_2\text{-M}/C_{Tf}$ ratios showed variations from 8.4 to 2136 for the former and from 0 to 1905 for the latter. In one instance only was the so-called high selectivity pattern observed.

Uric acid excretion was normal in all but two patients, both with hyperuricemia.

The kidneys of three patients were examined histologically (Nos. 6, 7, and 8). In two patients (Nos. 6 and 8) there were protein casts in the tubules and small interstitial leukemic infiltrates. In patient 6, red blood cells were also present in the tubules and some glomeruli showed epithelial crescents. In this instance, LZM excretion was only moderate, but proteinuria was prominent and there was hypergammaglobulinemia. In patient 8, lysozymuria was prominent and there was moderate proteinuria. Both had hypokalemia. No uric acid abnormalities were observed in either. In one patient (No. 7) there was severe tubular degeneration with hyaline droplets in the proximal tubular cells. Many casts were found in the tubules. There were also some inflammatory changes compatible with chronic pyelonephritis. This patient had markedly elevated serum

TABLE IV

Hematologic and Metabolic Data in Patients

No.	Patient	Sex	Age	No. of monocytes	No. of polymorphonuclears	Serum K	Serum Ca	Serum P
			yr	*mm³*	*mm³*	*mEq/liter*		*mg/100 ml*
1*	L. L.	F	53	96,480 (44)‡	21,960	2.2	7.7	4.5
2	T. N.	M	76	570	1,920	6.5	8.8	4.1
3	A. L.	M	41	6,400 (20)	8,800	4.0	9.5	3.6
4	H. R.	M	72	72,880	38,440	3.3	8.6	3.9
5	A. G.	M	85	610	3,100	4.4	8.7	3.4
6	L. G.	F	69	4,700	8,580	3.4	8.1	2.5
7‖	G. M.	F	80	5,880 (2)	1,680	2.2	7.4	1.1
8	D. T.	M	58	3,740 (7)	2,450	2.9	8.9	4.0
9*	T. T.	M	68	17,780 (3)	4,680	3.7	8.6	3.7
Mean			66.9	23,227	10,179	3.6	8.6	3.4
SD			13.1	33,653	12,361	1.2	0.6	0.97

* Patients 1 and 9 had bronchopneumonia.
‡ Per cent of monoblasts.
§ In a few days BUN increased to 32 mg/100 ml.
‖ Patient 7 had chronic pyelonephritis.

gammaglobulins, pronounced lysozymuria, persistent hypokalemia, low serum calcium, phosphorus, and magnesium. No hyperuricemia was recorded.

In addition to the kidneys of six patients from the present series, renal tissue was also available from seven other patients with mono- or myelomonocytic leukemia. All seven had azotemia with hyperuricemia in three. Proteinuria was recorded in three patients. Significant histological findings included protein casts in the tubules and focal leukemic infiltrates of variable degree in six cases. Two patients also showed severe hyaline droplet degeneration of the proximal tubular cells. The serum LZM was high in both patients (42 and 113 μg/ml). The kidneys of one patient with hyperuricemia of 14.2 mg/100 ml contained crystals in the collecting tubules. Occasional hyalinization of the glomeruli was observed in three instances and thickening of the basement membranes in one patient.

DISCUSSION

Hypogammaglobulinemia is recognized as an important feature in leukemia, especially in acute and chronic lymphocytic types (1, 3, 25–27). In contrast, studies in patients with mono- and myelomonocytic leukemia have revealed significant hypergammaglobulinemia, affecting particularly IgG and IgA (5, 7, 15).

In our series, serum IgG and IgA were increased in 12 patients and IgM was increased in 8 patients. In general, the increase was greater in patients with renal failure. There was no increase in IgD. Only 2 of 18 patients had acute intercurrent infection, while one had chronic pyelonephritis. Hypergammaglobulinemia was evident in two of the three patients while one had hypogammaglobulinemia. The remaining 15 patients showed no evidence of increased susceptibility to infections. It would appear, therefore, that the increased levels of gammaglobulins were not attributable to chronic infection. Thus far, no suitable explanation for the hypergammaglobulinemia has been forthcoming. The observation that some patients may have homogeneous M-components in the serum (5–7, 28, 29) while a few may excrete Bence Jones globulin in the urine may be significant, giving rise to speculations concerning the possible relationship of monocytic to plasmacytic dyscrasia (5, 29). In this regard, it should be noted that there was no correlation between the number of circu-

with Impaired Renal Function (Group B)

Serum Mg	BUN	Creatinine	Uric acid	Urine, uric acid	C_{cr}	$C_{u.a.}$	Treatment
mg/100 ml				*mg/24 hr*	*ml/min*	*ml/min*	
2.25	8§	2.1	17.9	803	33	3.24	Vincristine 6 MP
1.42	39	2.2	7.1	530	51	5.25	Chlorambucil Allopurinol
1.50	35	1.0	4.0	360	41	6.25	Vincristine 6 MP
1.30	77	1.4	3.0	483	39	10.82	Vincristine 6 MP
1.42	36	2.0	7.8	333	31	2.96	—
1.86	150	0.7	6.2	520	82	5.82	Prednisone, 6 MP Allopurinol
0.86	28	1.2	5.4	584	42	7.50	Myleran
1.88	48	1.4	7.1	705	77	7.21	Vincristine 6 MP
1.87	27	1.3	7.9	995	65	8.74	Prednisone 6 MP
1.60	49.8	1.48	7.38	590	51.2	6.42	—
0.41	39.5	0.49	4.04	200	17.9	2.36	—

lating monocytes and the level of immunoglobulins in our patients, and no plasmacytosis of the bone marrow.

No reports are available concerning the serum levels of transferrin, ceruloplasmin, α_2-macroglobulin, or other proteins in monocytic leukemia. The present study revealed low serum transferrin in 11 patients, in the absence of evidence of hemolysis. Ceruloplasmin was either normal or mildly elevated. α_2-Macroglobulin was decreased in nine patients. No correlation was found between the low level of transferrin and the low α_2-macroglobulin, the former being more frequent in patients with normal renal function and the latter in patients with azotemia.

Finch, Gnabasic, and Rogoway (30) and Jolles, Sternberg, and Mathé (13) were the first to report an increased serum level of lysozyme (muramidase) in monoblastic and myelomonocytic leukemia. Correlations have been shown between the level of lysozyme and the number of circulating monocytes and granulocytes (7, 31–33). The marked variability observed in this correlation has been attributed to differences in the rate of production, tissue binding, or renal handling of the enzyme (7). However, it is also possible that the total body pool of lysozyme-producing cells and the rate of destruction of the enzyme may play a role. In our series, the serum lysozyme was elevated in all patients during the active phase of the disease, being higher in the presence of azotemia. Renal failure (34, 35) would offer an explanation for the more significant elevation of serum LZM in group B, if it were not for the fact that several of these patients also had much more pronounced peripheral monocytosis than those without azotemia. Therefore, the possibility remains that enhanced production of lysozyme may account for some of this difference.

Osserman and Lawlor and others have noted hypokalemia and hyperkaluria in a number of patients with mono- and myelomonocytic leukemia (7, 14, 15, 36). Excessive excretion rates of potassium, reduced excretion of titratable acid, and glycosuria were also noted in some patients (15). In our series, seven patients had hypokalemia, three of whom also had a low serum calcium. Two of the latter also had a low magnesium and one had hypophosphatemia. In several of these patients, attempts were made to correct the low level of serum potassium by administration of 40–80 mEq of potas-

TABLE V

Protein Studies in Patients with

No.	Serum		Urine		Lysozyme			Transferrin			Ceruloplasmin			IgG		
	Total protein	Gamma globulin	Total protein	Albumin	Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance
	g/100 ml		*g/24 h*	*% of total protein*	*μg/ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*
1	7.10	2.23	2.21	22	114	595	3.62	263	24.8	0.0066	100	2.21	0.0015	2440	293	0.0056
2	7.75	2.26	0.83	30	64	187	2.03	213	13.5	0.0044	72	2.60	0.0025	3200	40	0.00087
3	7.70	2.11	1.20	14	58	533	6.38	289	52.5	0.013	82.5	1.43	0.0012	1660	132	0.0055
4	8.50	3.41	3.26	21	150	298	1.38	147	11.97	0.0057	26.5	2.58	0.0067	3000	6.83	0.00048
5	6.54	1.91	2.15	33	52	75.5	1.01	96	22.7	0.016	33	0.59	0.0012	1910	27.1	0.00098
6	6.25	2.52	6.24	7	28.2	18.2	0.45	89	10.73	0.008	35.5	1.76	0.0034	2810	69.6	0.0017
7	7.63	3.67	6.64	23	90	1488	11.48	176	5.52	0.0022	22.4	0.96	0.003	3010	445	0.047
8	7.18	1.31	1.26	8	58	1185	14.19	173	5.25	0.0021	53	5.64	0.0074	1860	70.1	0.0026
9	5.60	0.90	4.13	3	125	1940	10.77	173	25.1	0.010	67	25.70	0.027	930	15.44	0.0011
					111*	1914	11.97	133	17.4	0.009	75	50.40	0.047			
Mean	7.14	2.26	3.10	18	85	823.4	6.33	175	18.95	0.0077	56.7	9.39	0.011	2313	122	0.0073
SD	0.84	0.84	2.04	9.9	36.8	709.5	5.03	62	13.16	0.0043	25.2	15.41	0.010	724	141	0.014

* Second set of estimations done 1 wk after the first.

sium daily without success. Two patients with hypokalemia showed droplet degeneration in the proximal tubular cells and one also had chronic pyelonephritis. There was no correlation between the electrolyte abnormalities and the level of serum or urinary LZM. Increased serum uric acid levels and hyperuricosuria were noted in one patient of group A and in three of group B. Hyperuricosuria without hyperuricemia was observed in two others. No correlation was found between uricosuria, the level of electrolytes, proteinuria, or lysozyme excretion.

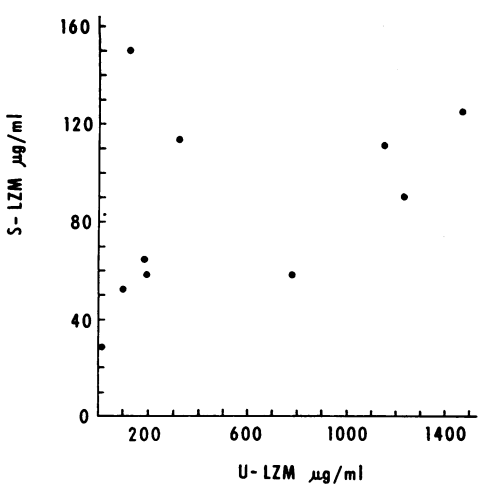

FIGURE 4 Group B. Relationship between serum lysozyme (S-LZM) and urinary lysozyme (U-LZM), 10 estimations. $r^2 = 0.1665$. Correlation significant at the level $P < 0.05$.

There are no detailed studies on proteinuria in leukemia, although patients with lymphatic leukemia have been found to excrete more protein in the urine than normal persons, particularly in regard to gamma globulins (9). In a series of mono- and myelomonocytic leukemia, proteinuria varying from 1.4 to 6.6 g/24 hr has been recorded, of which lysozyme has constituted up to 40%. Other components have included albumin and some alpha, beta, and gamma globulins (7). While our own control group of healthy subjects aged 21–62 yr showed proteinuria varying from 59 to 187 mg/24 hr with a mean of 103 mg, in most of our patients the total urinary protein consistently exceeded 300 mg/24 hr, being higher in patients with impaired renal function. Albumin generally constituted less than 25% of the total protein. Variable amounts of alpha, beta, and gamma globulins were found in all urines, with lyso-

Impaired Renal Function (Group B)

IgA			IgD			IgM			α_2-Macroglobulin			Comments
Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance	Serum	Urine	Clearance	
mg/100 ml	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	*mg/100 ml*	*mg/24 hr*	*ml/min*	
570	24.6	0.003	16.0	17.6	0.00076	202	0	0	200	3.22	0.0011	Fc and F'c fragments in the urine
1870	11.0	0.00041	6.5	0	—	275	0	0	263	0	0	Sia test (+)
490	15.1	0.0021	10.2	2.5	0.00017	291	0	0	260	20.71	0.0055	Fc and F'c fragments in the urine
400	14.0	0.0024	2<	3.2	—	510	0.9	0.00037	147	10.57	0.0050	Sia test (+); Fc and F'c fragments in the urine
370	39.96	0.0075	2<	0	—	216	17.5	0.0056	370	37.89	0.0071	Fc and F'c fragments in the urine
338	10.4	0.0021	6.5	0	—	685	1.17	0.00012	234	6.76	0.0020	Sia test (+); Fc and F'c fragments in the urine
1250	228	0.013	6.5	0	—	258	0	0	197	3.15	0.0011	Cryoglobulinemia, Sia test (+); Fc, and F'c fragments in the urine, Bence Jones heat test (+)
393	3.75	0.00066	2<	37.8	—	78	31.8	0.028	250	14.40	0.040	Fc and F'c fragments in the urine
106	103	0.067	2<	146.2	—	36	257.4	0.50	130	38.84	0.0210	
643	30.43	0.011	—	—	—	283	—	—	228	15.06	0.0092	
525	30.10	0.020	—	—	—	191	—	—	71	12.82	0.012	

zyme as the predominant constituent in five instances. In one urine, a significant amount of prealbumin and in another a band of postgamma protein, with slightly slower electrophoretic mobility than lysozyme, were also observed. In several patients, the electrophoretic patterns resembled those reported in tubular disorders, such as cadmium poisoning, adult Fanconi syndrome, and others in which excretion of low molecular weight proteins of alpha, beta, and even gamma mobility have exceeded that of albumin. Lysozymuria has frequently been observed in this type of proteinuria (37–43).

Excretion of IgG and IgA, as well as their clearances, were increased as compared to healthy individuals. IgD and IgM were excreted in the urines of six patients, especially in patients with azotemia. Fc and F'c fragments were observed in urines from 12 patients while the Bence Jones heat test was positive in two. One patient excreted Bence Jones type kappa globulin for a period of 2 months. While excessive excretion of transferrin was noted in most patients, ceruloplasmin and α_2-macroglobulin were especially excreted in urines of patients with azotemia.

Increased urinary lysozyme has been reported in various renal disorders particularly in patients with azotemia and proteinuria and in those with proximal tubular dysfunction (34, 35, 39, 40, 44, 45). More recently, Osser-

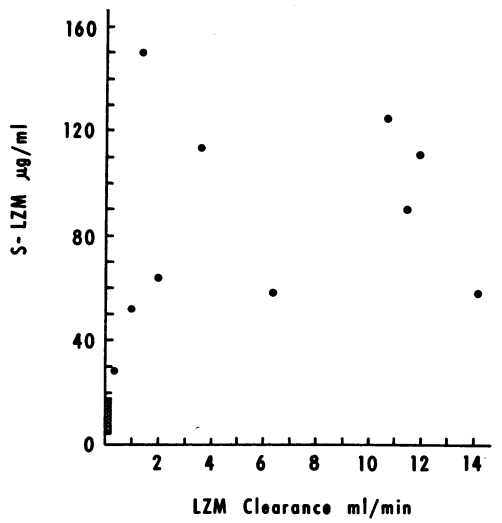

FIGURE 5 Group B. Relationship between serum lysozyme (S-LZM) and its clearance by the kidneys, 10 estimations. $r^2 = 0.0598$. No statistically significant correlation. Shaded area indicates normal range.

man and Lawlor and other authors have observed increased lysozymuria in patients with monocytic and monomyelocytic leukemia (7, 31, 46). In our series, the excretion of lysozyme was increased approximately 90-fold in group A and 520-fold in group B. In patients with azotemia, no correlation was found between the serum level of lysozyme and its renal clearance. It has been reported that the clearance of LZM is not so great as might be anticipated from its molecular weight (44, 47). Since LZM is probably partly bound to the serum proteins (47), its actual glomerular filtration may be lower than that which might be expected for a substance of similar molecular weight and of comparable serum concentration. It is also possible that the tubular reabsorption of LZM, which normally exceeds 99% of the glomerular-filtered enzyme (48), is impaired by the toxic effect of LZM itself. In our patients, in the majority of instances, the serum and urinary lysozyme levels were proportionately high. However, on four occasions, the level of urinary lysozyme was normal despite an elevated lysozyme in the serum. In these patients, although lysozyme may have been filtered excessively through the glomeruli, apparently it was efficiently reabsorbed by the tubular cells. In five other instances, a high concentration of urinary lysozyme was noted in the presence of a normal or only slightly elevated serum level of the enzyme and normal renal function. Since lysozyme is continuously elaborated by the leukemic cells and glomerular filtration was normal in these instances, one might postulate impaired reabsorption and(or) active tubular secretion of the enzyme or its leakage from the damaged tubular cells.

From the above data, it is evident that the patients with mono- and myelomonocytic leukemia almost invariably develop prominent proteinuria and that azotemia develops in approximately 50% of cases. Among 12 additional patients with this disease recently studied and not included in the present series, nine had proteinuria and six had azotemia. In contrast to this, a comparative study of 37 patients with other types of leukemia revealed proteinuria only occasionally and azotemia very rarely. When present, both were usually related to other complicating renal diseases such as pyelonephritis (49). Any explanation for the renal damage observed therefore must take into account at least two major differences between monocytic leukemia and other types of leukemia, namely the prominent hypergammaglobulinemia and the increase in lysozyme production with subsequent lysozymuria.

It has been reported that hypergammaglobulinemia may contribute to the development of renal tubular acidosis (50). Hypergammaglobulinemia and also M-components have been observed in patients with adult Fanconi syndrome (43, 51). None of our patients had tubular acidosis, aminoaciduria, or glycosuria but all these abnormalities have been reported in a few cases of monocytic leukemia (15). The fact that immunoglobulin levels were frequently much higher in the patients comprising our group B than in those with normal renal function, may be of some importance. Nevertheless, in the absence of renal tubular acidosis, one would question the role of hypergammaglobulinemia as the most important factor in the production of renal tubular injury.

Recent evidence suggests that high concentrations of lysozyme may damage the proximal tubular cells. Normally, lysozyme is reabsorbed in the proximal convoluted tubule and its concentrations in the cortex may be 10–25 times greater than in the medulla (52). Exogenous lysozyme injected into animals concentrates in the proximal tubular cells in the form of droplets which may be detected by light microscopy (53) or by immunofluorescent techniques (54). Renal injury in rats treated with tubular poisons results in greater urinary lysozyme excretion than in those treated with antiglomerular antiserum (34). Osserman has suggested that humans and rats with monocytic leukemia, as well as rats injected with exogenous lysozyme, develop "lysozyme nephropathy" (55). It has been shown that the increased concentration of the enzyme in the kidney may be associated with cytoplasmic droplet degeneration of the proximal tubular cells and distortion of the mitochondria and nuclei (16, 36). Similarly, in autopsy material available to us, droplets of homogenous eosinophilic substances have been found in the proximal tubular cells in 4 of 12 cases studied. The higher levels of serum lysozyme in patients comprising our group B may therefore be of considerable significance in contributing to renal tubular injury. It is possible that the low glomerular filtration rate in these patients may lead to the retention of LZM in the serum and subsequently to the higher concentration of the enzyme in the glomerular filtrate. This leads to the inability of the proximal tubular cells to reabsorb all the enzyme filtered. Spillover of LZM into the urine and possible damage to the tubular cells by LZM itself may consequently appear. It seems improbable that occasional intercurrent infections or hyperuricemia, which are usually treated promptly, contribute to the renal damage.

The analyses of the proteins excreted in the urine would appear to suggest the tubular as well as glomerular dysfunction. In many instances, the electrophoretic patterns of the urinary proteins including a low albumin and an excessive excretion of α-, β-, and γ-globulins, resembled those seen in various tubular disorders. On the other hand, the presence of high molecular weight proteins in the urine suggests that glomerular damage also occurred. The pathogenesis of azotemia and renal

failure which were found in 50% of the patients with mono- and myelomonocytic leukemia has not yet been elucidated. Recent observations on the glomerular damage in myeloma (56) or on azotemia found in patients with adult Fanconi syndrome (43), may imply that, in conditions involving mainly tubular cells, glomerular damage may also occur.

From the comparative study of various leukemias, this particular glomerular-tubular dysfunction appears to be a manifestation unique to mono- and myelomonocytic leukemia.

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