SERUM AND URINARY LYSOZYME (MURAMIDASE) IN MONOCYTIC AND MONOMYELOCYTIC LEUKEMIA*

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Plates 91 and 92

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By electrophoretic analysis, large quantities of an exceptionally basic, cationic protein (CP) have been found in the urine of ten consecutive cases of monocytic and monomyelocytic leukemia.[1] The protein CP has been isolated and characterized as a relatively low molecular weight constituent with the enzymatic properties of lysozyme (muramidase).[2] Increased levels of CP-lysozyme have been demonstrated in the serum of monocytic leukemics by immunochemical and enzymatic techniques but not by electrophoresis. Evidence has been obtained that CP-lysozyme is the normal enzyme which is elaborated in excess in the monocytic leukemics. The relatively small molecular size of CP-lysozyme is apparently responsible for its urinary excretion.

Materials and Methods

Clinical Material.—The ten patients with monocytic and monomyelocytic (Naegeli-type) leukemia (six males, four females) ranged in age from 46 to 75 years. The clinical and hematologic patterns were consistent with a diagnosis of subacute or chronic monocytic or monomyelocytic leukemia. The total peripheral leukocyte counts ranged from 5450 to 200,000 with 30 to 82% monocytic forms. Representative peripheral blood and bone marrow smears of two cases are shown in Fig. 1. Case 10 had a peripheral leukocyte count of 117,000 per mm^3 with 82% monocytic forms and was considered to have monocytic leukemia; case 6 had a peripheral leukocyte count of 68,500 with 40% monocytes and 42% polymorphonuclear leukocytes, and was considered to have monomyelocytic leukemia. Preliminary electron microscope studies have been made in cooperation with Dr. Henry Azar, and an electron photomicrograph of the predominant cell type in the bone marrow of case 3 is shown in Fig.

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[1] A report of these studies (1) was presented at the annual meeting of the American Society for Clinical Investigation and the American Federation for Clinical Research, Atlantic City, New Jersey, 1 May 1966.

[2] Muramidase is the designation recommended for lysozyme(s) by the Commission on Enzymes of the International Union of Biochemistry (C.C. 3.2.1. 17). However, since the term "lysozyme" continues to be more generally employed, it will be used in this text.

2. Particularly noteworthy are the numerous lysosomal granules, the large Golgi zone and the scattered endoplasmic reticulum.

Six of the ten cases had evidence of renal and electrolyte abnormalities, particularly hypokalemia and hyperkaluria. The mechanisms of these abnormalities and their possible relationship to the excretion of CP are under investigation, and detailed clinical, hematologic, and cytologic studies will be published subsequently.

Blood and urine samples from 20 normal subjects and 250 patients with a wide variety of disease states, including other forms of leukemia, infectious mononucleosis, multiple myeloma and other plasma cell dyscrasias, renal diseases, tuberculosis and other chronic infections, sarcoidosis, carcinoma, and Hodgkin's disease were also studied. Fresh serum, separated from the clot within 2 hr of collection or EDTA (Versene), plasma samples were used. Heparinized plasma is unsatisfactory because of the formation of complexes between heparin and lysozyme.

Normal tears were obtained from D. O., age 10, provoked by her two older siblings.

Protein Quantitation.—24-hr urinary collections were obtained and no preservative was added. Aliquots of whole urine were concentrated 40- to 50-fold by dialysis against 25% polyvinylpyrollidone (PVP). Total urinary protein excretion was determined by precipitation with 5% trichloroacetic acid and Kjeldahl analysis.

Standard procedures were employed for the electrophoretic (cellulose acetate, Spinco) analyses and for the starch gel and acrylamide gel electrophoretic studies. Ultracentrifugal analyses were performed with the Spinco Model E ultracentrifuge, and sedimentation constants calculated to infinite dilution ($S_{20,w}$).

Isolation of CP.—The urinary proteins, including CP, were initially precipitated from 24-hr collections by 60% saturation with ammonium sulfate. The precipitates were suspended in water and dialyzed against phosphate-buffered saline, pH 7.2, until free of ammonium ion. Saline insoluble material was discarded and the saline solution of protein dialyzed against 0.1 M NaOH:glycine buffer, pH 9.5. Chromatographic separation of CP from other urinary proteins was achieved by DEAE (diethylaminoethylcellulose) with the 0.1 M Na glycinate buffer, by using either column or batch separation techniques. Under these conditions, CP was recovered in the void volume, and the other urinary proteins were retained by the DEAE.

More recently we have used the procedure of Alderton, Ward, and Fevold (2) for the isolation of lysozyme using bentonite adsorption and elution with 5% aqueous pyridine adjusted to pH 5.0 with sulfuric acid.

Peptide Analyses.—Tryptic peptide maps were made with the cooperation of Dr. Frank Tischendorf by the procedure of Katz, Dreyer, and Anfinsen (3) with minor modifications. DEAE-isolated CP samples were oxidized with performic acid and precipitated with 10% trichloroacetic acid. The precipitates were washed with trichloroacetic acid, absolute ethanol, and ether. The oxidized CP samples were incubated for 4 hr with trypsin (Worthington Biochemical Corporation, Freehold, New Jersey, 2 × crystallized trypsin SF, 1 mg per 100 mg protein) in 0.2 M NH_4HCO_3 buffer, pH 8.2, with phenol red as an indicator. Aliquots of 2 to 3 mg were applied to Whatman 3 MM paper, and the peptides separated first by ascending chromatography for 22 hr in butanol:acetic acid:water (4:1:5). This was followed by electrophoresis for 1 hr at 2000 v in pyridine:acetic acid:water (1:10:289) at pH 3.8. After drying, the peptides were stained with a ninhydrin spray.

Amino Acid Analyses.—Amino acid analyses were performed by Dr. Robert E. Canfield of the Department of Medicine of the College of Physicians and Surgeons by the method of Spackman, Stein, and Moore (4). Three DEAE-isolated CP samples were hydrolyzed by 6 N HCl for 24 hr at 110° C, and the amino acid analyses carried out on a Spinco Model 120 amino acid analyzer. Tryptophane is destroyed by acid hydrolysis and is not included in these analyses.

Immunologic Analyses.—Antiserum to the DEAE-isolated CP of case 1 (Anti-CP (1)) was

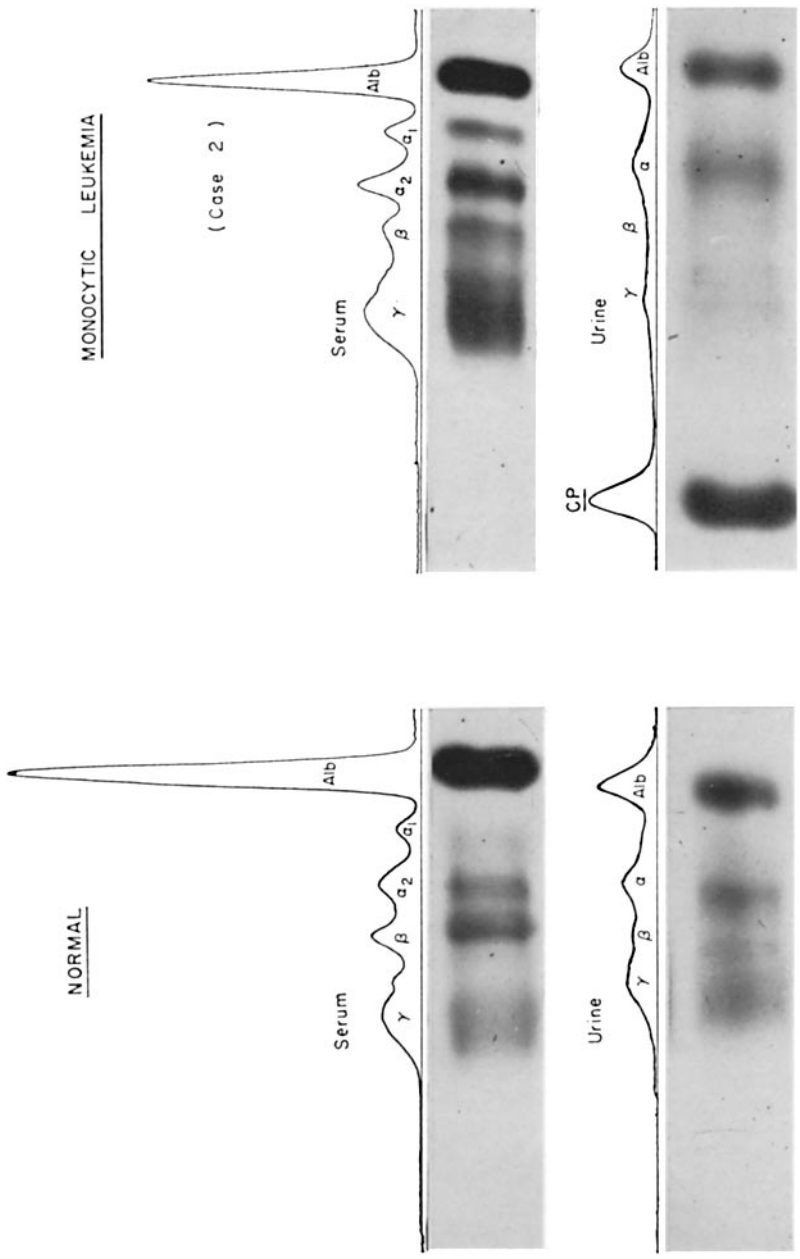
prepared in rabbits, employing complete Freund adjuvants. Precipitin analyses were performed by conventional Ouchterlony and immunoelectrophoretic techniques using highly purified agar (Agarose, Fisher Scientific Company, Pittsburgh, Pennsylvania), at a concentration of 1%, in Veronal buffer, pH 8.6, ionic strength, $\mu = 0.025$ or 0.075. At the lower ionic strength, CP precipitated nonspecifically in the gel.

Enzymatic Studies.—The initial assays of CP samples for acid phosphatase, cathepsin, β-glucuronidase, and lysozyme activity were kindly performed by Dr. Zanvil Cohn of the Rockefeller University, employing procedures previously detailed (5). β-glycerolphosphate (pH 5.0) was used as substrate for acid phosphatase; phenophthalein glucuronide (pH 4.8) for β-glucuronidase; denatured hemoglobin (pH 3.8) for cathepsin; and suspensions of washed *Micrococcus lysodeikticus* organisms in phosphate buffer, pH 7.0 for lysozyme. Ribonuclease assays were kindly performed by Dr. Herbert Rosenkrantz of the Department of Microbiology of the College of Physicians and Surgeons, using radiophosphorus (P^{32})-labeled chick embryo RNA as substrate.

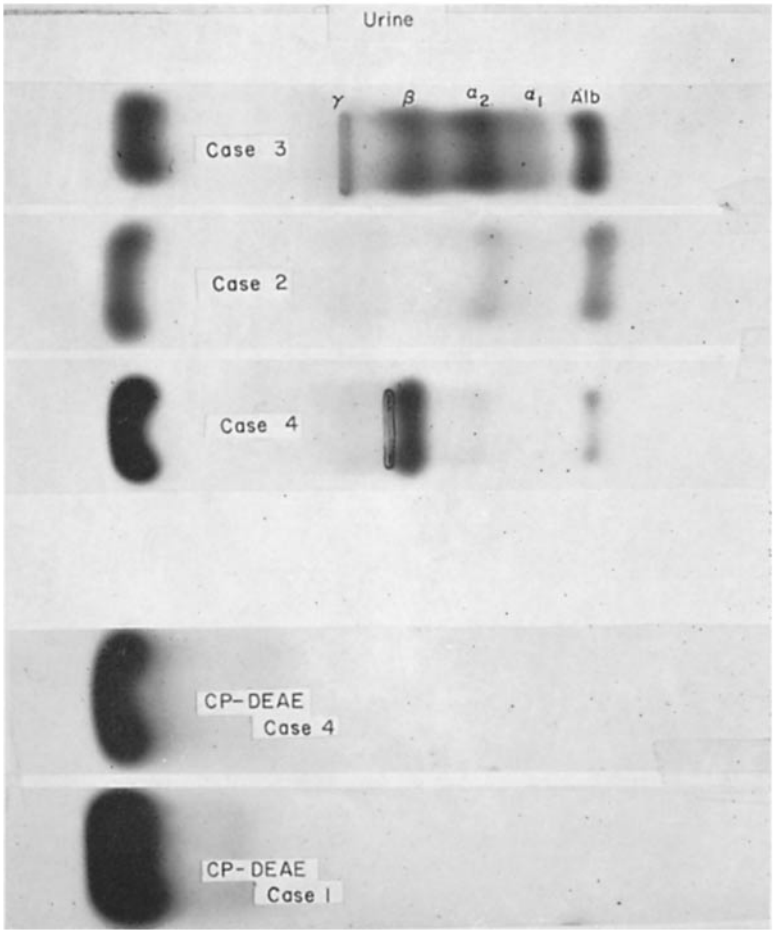
In this laboratory, lysozyme assays were initially performed by the methods of Smolelis and Hartsell (6) and Litwack (7), with 3 ml aliquots of a suspension of 25 mg of heat-killed *M. lysodeikticus* (Worthington Biochemical Corporation, Freehold, New Jersey) in 100 ml of M/15 phosphate buffer, pH 6.3 with 0.1% NaCl and 0.3 ml of standard or unknown solutions. Twice-crystallized egg white lysozyme (Muramidase, LY 644A, Worthington Biochemical Corporation) was used as the reference standard in the initial studies, and serial dilution of DEAE or bentonite-isolated CP in later studies. Lysozyme activity was measured in a Bausch and Lomb Spectronic-20 spectrophotometer as change in per cent transmission at 645 mμ for intervals of 30 to 90 sec or 3 to 9 min following addition of the sample to the bacterial suspension, with adequate mixing. These procedures (6, 7) were found to be valid for only a limited range of concentration and necessitated adjustment of the concentration of samples to within this narrow range.

Lyso-Plate Assay Method.—Because of difficulties encountered with conventional lysozyme assay methods (6, 7), an agar plate (lyso-plate) technique was developed which has yielded more satisfactory results over a much wider range of concentrations. Heat-killed *M. lysodeikticus* organisms, suspended uniformly (Vibro-Mixer, A. G. F. Chemie Apparatgebau, Zürich, Switzerland) in a small volume of phosphate buffer are added to molten (60–70°C) 1% agar (Agarose, Fisher) in M/15 phosphate buffer, pH 6.3 to a final concentration of 50 mg of organisms in 100 ml of buffered agar and poured into Petri dishes to a depth of 4 mm. After the agar solidifies, sample wells, 2 mm in diameter, are cut with a thin-walled brass tube, machined to a bevelled cutting edge. Sixteen sample wells, in four rows of four, 15 mm apart, can be accommodated in a Petri dish, 85 mm in diameter. The sample wells have a volume of approximately 25 μl and are easily and reproducibly filled by capillary tubes. Standard dilutions of purified CP-lysozyme (5, 25, 100, and 500 μg/ml) are run with each group of test samples. After being filled, the plates are left at room temperature (24–26°C) for 12 to 18 hr, during which period zones of clearing develop in the initially translucent gel as the result of bacterial lysis. The diameters of the cleared zones are proportional to the log of concentration of lysozyme. The diameters can be measured directly with an enlarger-viewer (Universal projector, National Instrument Laboratories, Rockville, Maryland), or the plates can be photographed, using the plate itself as a "negative" in a condenser-type enlarger, and the diameters measured from the enlarged photographs. A semilogarithmic plot is made of the diameters given by the standard solutions, and this is used for quantitating (μg/ml) CP-lysozyme in the test samples.

The time of development of the lyso-plates is not critical and can be varied over several hours. If the diameters are read directly rather than by photography, it is important that the standard and test sample measurements be made in as short an interval as possible. It is



TEXT-FIG. 1. Electrophoretic analyses (cellulose acetate, Spinco system; Veronal buffer, pH 8.6). Left, normal serum and normal urine (concentrated 50 times). Right, monocytic leukemia (case 2) serum and urine (concentrated 40 times). CP identifies the abnormal cationic protein in the monocytic leukemia urine.



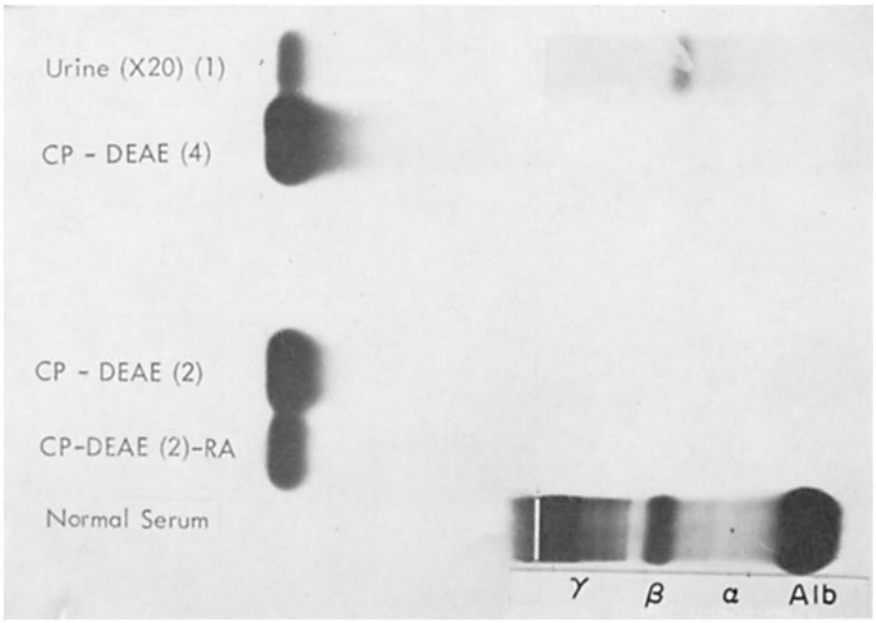
TEXT-FIG. 2. Monocytic leukemia. Electrophoretic analyses (cellulose acetate, pH 8.6). Upper, urinary proteins (concentrated 50 times) from three cases of monocytic leukemia showing the identity of the CP components. Lower, DEAE-isolated CP's from cases 4 and 1.

also important that the sample wells be initially filled in as short an interval as possible. The lyso-plates can be prepared in batches and stored at 9–10°C for 2 wk without deterioration, if wrapped in plastic to prevent evaporation.

RESULTS

Electrophoresis.—Text-fig. 1 illustrates electrophoretic patterns of the proteins of normal serum and urine (concentrated 50-fold) and the serum and urine (similarly concentrated) of one of the cases of monocytic leukemia. In the urine pattern of the monocytic leukemic, CP is readily identified as an elec-

trophoretically homogeneous component migrating to a position far beyond the cathodal boundary of the gamma globulins. The serum pattern of the monocytic leukemic shows the nonspecific changes of decreased albumin and increased alpha-2 and gamma globulins, but does not show the CP component. However, as will be noted subsequently, abnormally elevated concentrations of CP-lysozyme were demonstrated in the serum of monocytic leukemics by immunologic and enzymatic methods.

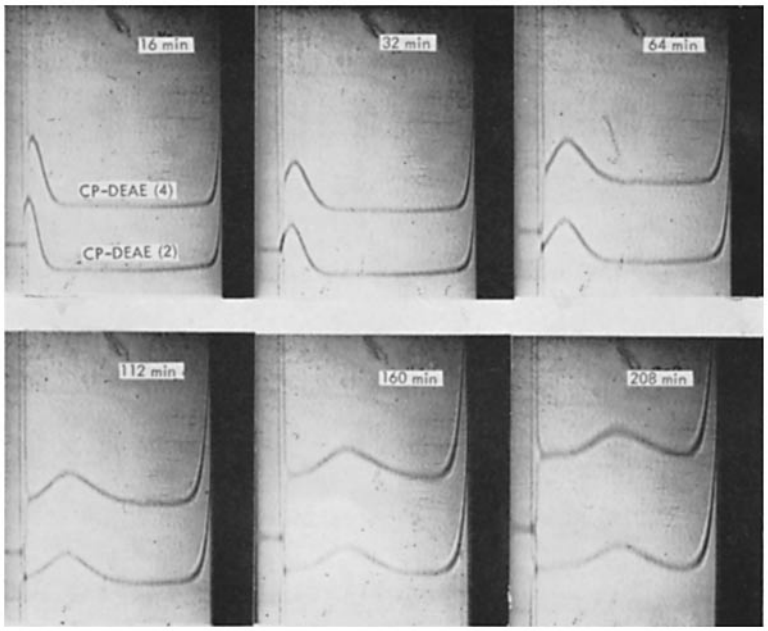


TEXT-FIG. 3. Acrylamide gel electrophoresis (Veronal, pH 8.5). From the top, monocytic leukemia, case 1, whole urine (concentrated 20 times): DEAE-isolated CP's from cases 4 and 2; DEAE-isolated CP (2) following reduction and alkylation (RA) with cysteine amine (2-mercapto-ethylamine) and iodoacetamide. Bottom, normal serum for mobility reference.

Text-fig. 2 shows electrophoretic patterns of the concentrated urinary proteins from three cases of monocytic leukemia, and two of the DEAE-isolated CP's. The identity of the electrophoretic mobilities of the CP's is evident. The pattern of the DEAE-isolated CP of case 1 shows a minor component on the anodal side of the main CP peak which was identified immunoelectrophoretically as a small quantity of low molecular weight gamma-u. As illustrated in Text-figs. 1 and 2, the urine protein patterns of all cases of monocytic leukemia demonstrated significant quantities of albumin, alpha, beta, and gamma globulins, in addition to CP. The 24-hr urinary protein excretion in these cases

ranged from 1.4 to 6.6 g, of which CP constituted 16 to 40% (0.6 to 2.6 g/day). The prominent beta globulin constituent seen in the case 4 urinary pattern in Text-fig. 2 was immunologically identified as transferrin.

Text-fig. 3 shows acrylamide gel electrophoretic analyses (Veronal buffer, pH 8.5) of whole urine and DEAE-isolated CP samples prior to and following reduction and alkylation with 0.02 M cysteine amine (2 mercapto-ethylamine) and iodoacetamide. In acrylamide gel, there was no evidence of heterogeneity of either the native or reduced CP, as indicated by the absence of banding, and



TEXT-FIG. 4. Ultracentrifugal analyses of CP-DEAE (2) and CP-DEAE (4). Aqueous solutions, 6.7 mg/ml Spinco, Model E, 59,780 RPM. Direction of sedimentation, right to left.

the CP samples from three different cases again showed identical electrophoretic mobilities.

Ultracentrifugation.—Ultracentrifugal analyses of two DEAE-isolated CP samples are shown in Text-fig. 4. The sedimentation patterns of the two CP's are identical with symmetrical boundaries and there is no evidence of heterogeneity. The sedimentation constant ($S_{20,w}$) of both, calculated to infinite dilution, was 1.8S, corresponding to a molecular weight of the order of 14,000 to 15,000. Ultracentrifugal analyses of two other CP samples yielded $S_{20,w}$ values of 1.8 and 2.0S. The variability is attributed to methodologic error.

Tryptic Peptide Maps.—Peptide maps of the tryptic digests of two DEAE-

isolated CP's are shown in Text-fig. 5. The over-all similarity of these maps is evident. A total of 19 ninhydrin-positive peptides was identified in CP-DEAE (2) and 18 in CP-DEAE (4). The single peptide (P_2) not detected in CP-DEAE (4) is only faintly visible in the map of CP-DEAE (2), and its absence from CP-DEAE (4) may be due to methodologic limitations. The number of tryptic

TABLE I

Amino Acid Analyses of Three CP-Lysozyme Samples

Amino acid	Moles of amino acid per 100 moles			Amino acid residues (estimated*)	Amino acid residues per mole	
	CP (1)	CP (2)	CP (4)	CP-LZM	Human saliva LZM‡	Egg white LZM§
Lysine	3.22	4.81	4.17	5	5–6	6
Histidine	0.72	1.38	0.82	1	1	1
Arginine	10.61	9.24	10.69	12	11 ± 1	11
Aspartic acid	14.47	13.22	14.55	17	18 ± 1	21
Threonine	4.18	4.89	4.48	5	6 ± 1	7
Serine	4.72	5.43	5.04	6	6 ± 1	10
Glutamic acid	7.93	8.74	8.32	10	9 ± 1	5
Proline	2.20	3.82	2.50	3	2	2
Glycine	9.22	10.36	8.39	11	12 ± 1	12
Alanine	11.77	9.61	11.02	13	12 ± 1	12
Half-cystine	5.35	4.17	4.64	6	6	8
Valine	7.08	6.32	6.61	8	7	6
Methionine	1.63	1.50	1.81	2	1–2	2
Isoleucine	4.06	3.62	4.16	5	4–5	6
Leucine	6.35	6.41	5.94	8	8	8
Tyrosine	4.72	4.25	4.87	6	5	3
Phenylalanine	1.79	2.15	1.91	2	2	3
Tryptophane				(5)	5 ± 1	6
Total				(125)	122 ± 5	129

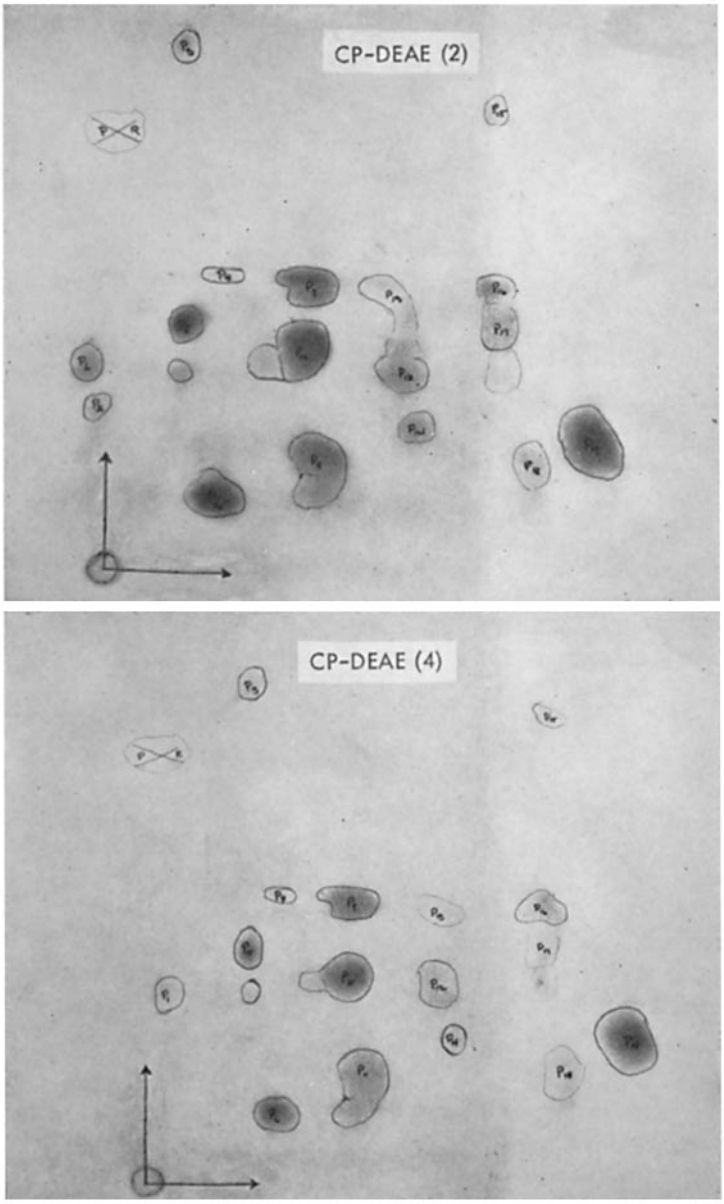
* Calculated on the basis of 125 residues, including an estimated 5 tryptophane residues.

‡ Data of Jollès *et al.* (8) estimated from analyses of human salivary, milk, spleen, and leukocytic lysozymes.

§ Data of Canfield (9).

peptides is consistent with the sum of lysine and arginine residues (5 + 12) found by amino acid analyses.

Amino Acid Analyses.—Amino acid analyses of acid hydrolysates of three DEAE-isolated CP samples are shown in Table I. The results of the individual analyses are expressed as moles of amino acid per 100 moles. Tryptophane is not estimated because of its destruction by acid hydrolysis, but a peak corresponding to the position of tryptophane was present in the chromatograms of all three samples, indicating its presence in CP. In general, the analyses of the three



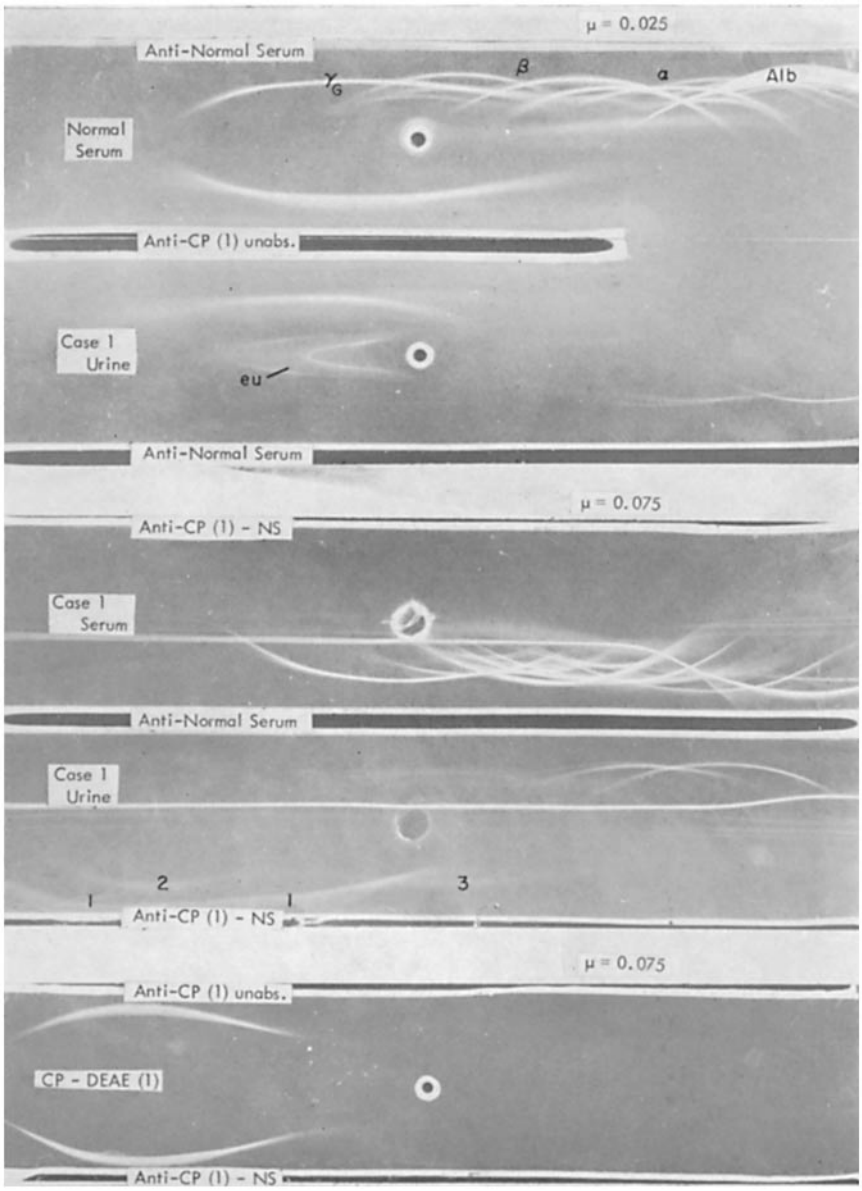
TEXT-FIG. 5. Peptide maps of tryptic digests of two DEAE-isolated CP's. Points of application of samples are at the lower left corners. Ascending chromatography in butanol:acetic acid for 24 hr in the vertical axis was followed by electrophoresis (2,000 v, 1 hr; pyridine:acetic acid pH 3.6) along the horizontal axis. Ninhydrin stain. *PR* identifies the phenol red marker.

samples are in agreement and show no differences which cannot be accounted for by methodologic error. The ratios of basic to acidic amino acid residues for the three samples were 0.65, 0.70, and 0.68, respectively.

Also tabulated in Table I are the estimated minimal number of amino acid residues in CP calculated from the averaged values of the three analyses on the basis of one histidine and two methionines with the further assumption of five tryptophanes. The tryptophane estimate is based on the data of Jollès and coworkers (8) for several human lysozymes. These data and the data of Canfield (9) for egg white lysozyme are also shown in Table I. A close correspondence is apparent between the CP analyses and the values obtained for other human lysozymes by Jollès et al. (8). When compared with egg white lysozyme, these proteins show several significant differences, particularly the higher glutamic and tyrosine, and lower serine and aspartic contents.

Immunologic Analyses.—Immunoelectrophoretic and Ouchterlony analyses of CP samples are shown in Text-figs. 6 and 7. As seen in the top patterns in Text-fig. 6, the rabbit antiserum to CP (1), prior to absorption with normal serum (Anti-CP (1) unabsorbed) contained antibodies to gamma-G globulin, probably as a result of the presence of a small amount of low molecular weight gamma-u in the sample of DEAE-isolated CP (1) used for immunization. The anti–gamma globulin activity was removed by absorption with normal serum. When the immunoelectrophoretic analyses were performed in agar with a low ionic concentration ($\mu = 0.025$), CP migrated only slightly in the gel and precipitated in the form of two chevrons on the cathodal side of the antigen well. This precipitation is probably due to interaction of the basic CP with acidic groupings in the agar matrix, in combination with a lesser solubility of CP at low salt concentrations. When the immunoelectrophoretic analyses were performed with a salt concentration of 0.075 (middle and lower patterns, Text-fig. 6), CP migrated to the expected postgamma, cathodal position and was precipitated by the Anti-CP(1) antiserum. The unabsorbed Anti-CP (1) also precipitated two unidentified constituents in the whole urine of case 1, one of which migrated to the same cathodal region as CP. Neither of these constituents was detectable in the DEAE-isolated CP (1) sample used for immunization, by either the absorbed or the unabsorbed Anti-CP (1) antiserum (lower pattern, Text-fig. 6).

The Ouchterlony analyses illustrated in Text-fig. 7 were all made with Anti-CP (1) absorbed with normal serum. These analyses demonstrate reactions of identity among all CP samples with no detectable spurs to indicate structural differences. Also shown in the middle pattern in Text-fig. 7 are reactions of identity between two CP's and a component in normal tears, presumably lysozyme. The lower pattern in Text-fig. 7 demonstrates that the CP's in the serum, urine, and white blood cells (sonicated buffy coat preparation) of case 8 are antigenically identical. Although a visible precipitin arc did not develop

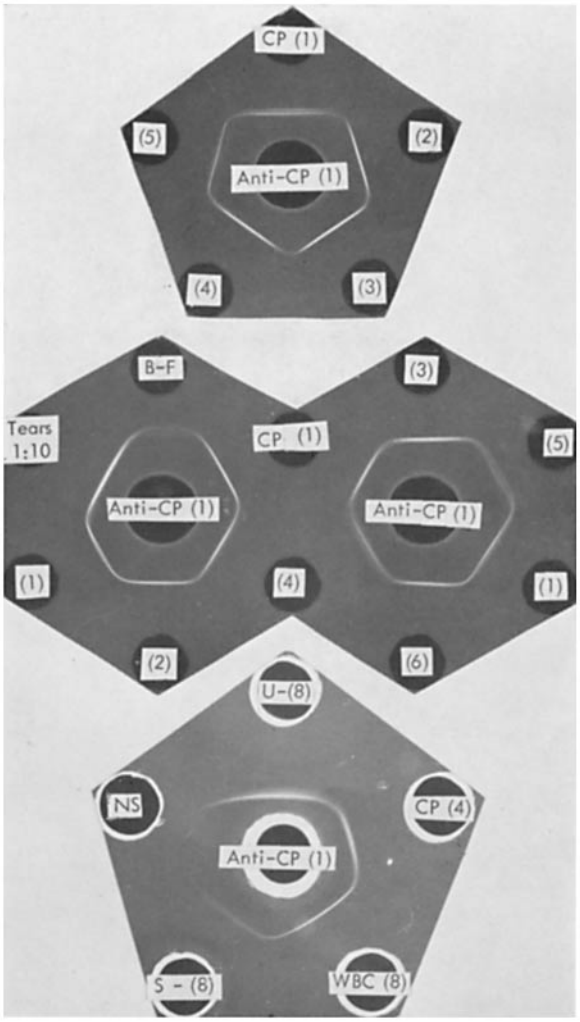


TEXT-FIG. 6. Immunoelectrophoretic analyses.

Top patterns, ionic strength, $\mu = 0.025$. Normal serum and whole urine of case 1 (concentrated 20 times), developed with horse antiserum to normal serum and rabbit antiserum to CP (1), prior to absorption. Unabsorbed Anti-CP (1) precipitates the gamma-G globulin of normal serum and case 1 urine. At this low ionic strength, CP precipitates as a chevron (*eu*-) on the cathodal side of antigen well.

Middle patterns, ionic strength, $\mu = 0.075$. Serum and urine of case 1 developed with Anti-normal serum and with Anti-CP (1) absorbed with normal serum (— NS). AntiCP (1) — NS demonstrates three components in the urine of case 1: a major constituent, *1*, identified as CP; a small component, *2*, in the same cathodal region; and a third arc, *3*, in the beta mobility range. Components *2* and *3* are unidentified.

Lower pattern, ionic strength, $\mu = 0.075$. DEAE-isolated CP (1), developed with Anti-CP (1), prior to and following absorption with normal serum.



TEXT-FIG. 7. Ouchterlony analyses, developed with rabbit antiserum to the DEAE-isolated CP of case 1 (Anti-CP (1)), absorbed with normal serum.

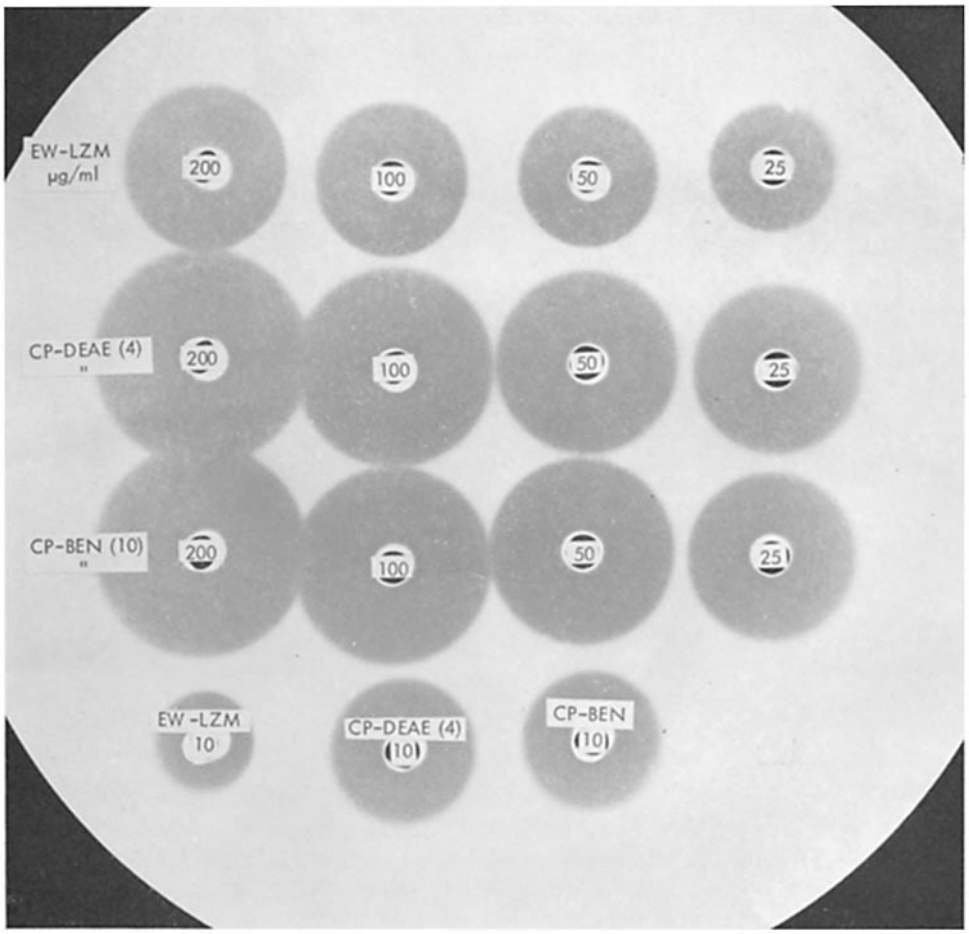
Top, reactions of identity among five of the DEAE-isolated CP's.

Middle, further confirmation of reactions of identity among CP's and with a component, presumably lysozyme in normal tears. B-F is the concentrated (50×) urine of a case of Boeck's sarcoid with increased urinary lysozyme.

Lower, demonstration of CP in the serum (*S*), urine (*U*), and sonicated buffy coat (*WBC*) of case 8, and their identity with CP (4). No precipitin line is seen with normal serum (*NS*) but a bending of the end of the *U* (8) precipitin line adjacent to the *NS* well indicates the presence of a small amount of CP in normal serum.

with normal serum (NS), bending of the end of the U (8) precipitin line adjacent to the NS well indicates the presence of a small amount of CP in normal serum.

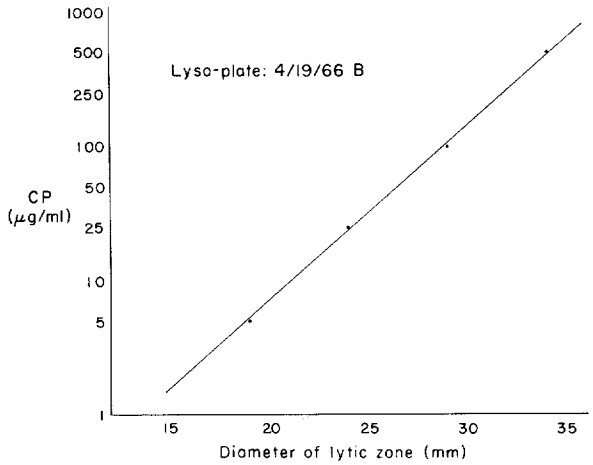
Enzyme Assays; Lysozyme Activity of CP.—Assays for enzymatic activities performed on the ammonium sulfate precipitates of the total urinary proteins of



TEXT-FIG. 8. Demonstration of lysozyme activities of DEAE-isolated CP (4) and bentonite-isolated CP (10), compared with equal concentrations (µg/ml) of twice-crystallized hens' egg white lysozyme (EW-LZM). Plate assay (lyso-plate) method, with heat-killed *M. lysodeikticus* incorporated in phosphate-buffered (pH 6.3) agar gel. Samples (25 µl) are placed in wells, 2 mm in diameter, and the plate photographed after 16 hr at 25°C. Dark zones around sample wells are zones of clearing due to bacterial lysis, and are proportional to the concentration of lysozyme proteins.

the monocytic leukemics showed extremely high lysozyme activity and very low levels of acid phosphatase, β-glucuronidase, cathepsin, and ribonuclease. Assays of the isolated CP's demonstrated only the lysozyme activity.

When the lysozyme assays were performed by standard procedures (6, 7), using suspensions of *M. lysodeikticus* organisms in phosphate buffer (pH 6.3) with 0.1% NaCl, the lytic activity of the isolated CP samples was 2 to 3 times greater per unit weight than the activity of twice-crystallized egg white lysozyme. With the lyso-plate assay method (Text-fig. 8), the lysozyme activity of the isolated CP's was 8 to 12 times greater than that of equivalent concentrations of egg white lysozyme.



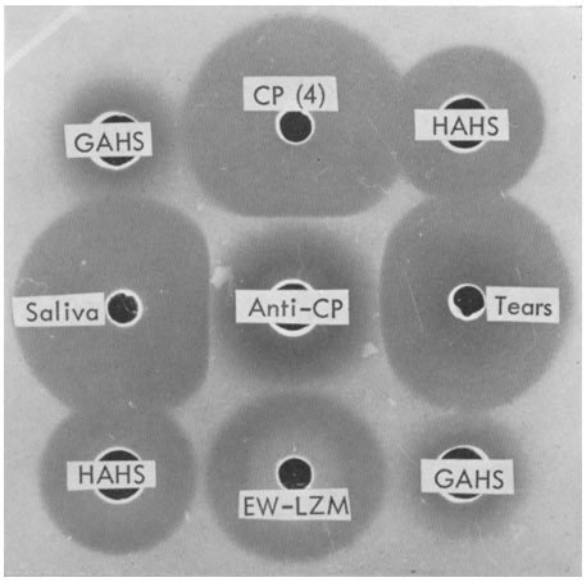
TEXT-FIG. 9. Lyso-plate analysis: semilogarithmic relationship between CP-DEAE (4) concentrations (5 to 500 μg/ml) and the diameters of the zones of clearing, measured from an enlarged photograph of the plate illustrated in Text-fig. 11.

Text-fig. 9 demonstrates the linear semilogarithmic relationship between the concentration of CP (ranging from 5 to 500 μg/ml) and the diameters of the lyso-plate lytic zones. The diameters plotted in Text-fig. 9 were measured from an enlarged photograph of the plate illustrated in Text-fig. 11. Equally satisfactory standard curves are obtained by direct measurements of lytic zone diameters using an enlarger-projector.

Inhibition of the Lysozyme Activity of CP by Anti-CP Antiserum.—The lyso-plate shown in Text-fig. 10 demonstrates that the rabbit antiserum to CP (1) inhibits the lysozyme activity of CP (4), human saliva, and tears as evidenced by flattening of their respective lytic zones on the side adjacent to the antiserum. In contrast, Anti-CP did not inhibit the activity of egg white lysozyme, indicating structural differences between the human and egg enzymes. No anti-lysozyme activity was found in a goat or horse antiserum to human serum, but

both of these sera as well as the rabbit serum (Anti-CP) showed intrinsic lysozyme activity.

Serum and Urinary Lysozyme in Monocytic Leukemia.—Text-fig. 11 illustrates lyso-plate analyses of normal serum and urine, and the serum and urine (unconcentrated) of three cases of monocytic leukemia. Normal serum shows approximately 7 μg/ml of CP-lysozyme activity, and the activity in normal urine is barely detectable. In the monocytic leukemics, the serum CP concentrations ranged from 40 to 150 μg/ml, and urine CP concentrations from 25 to 420 μg/ml.

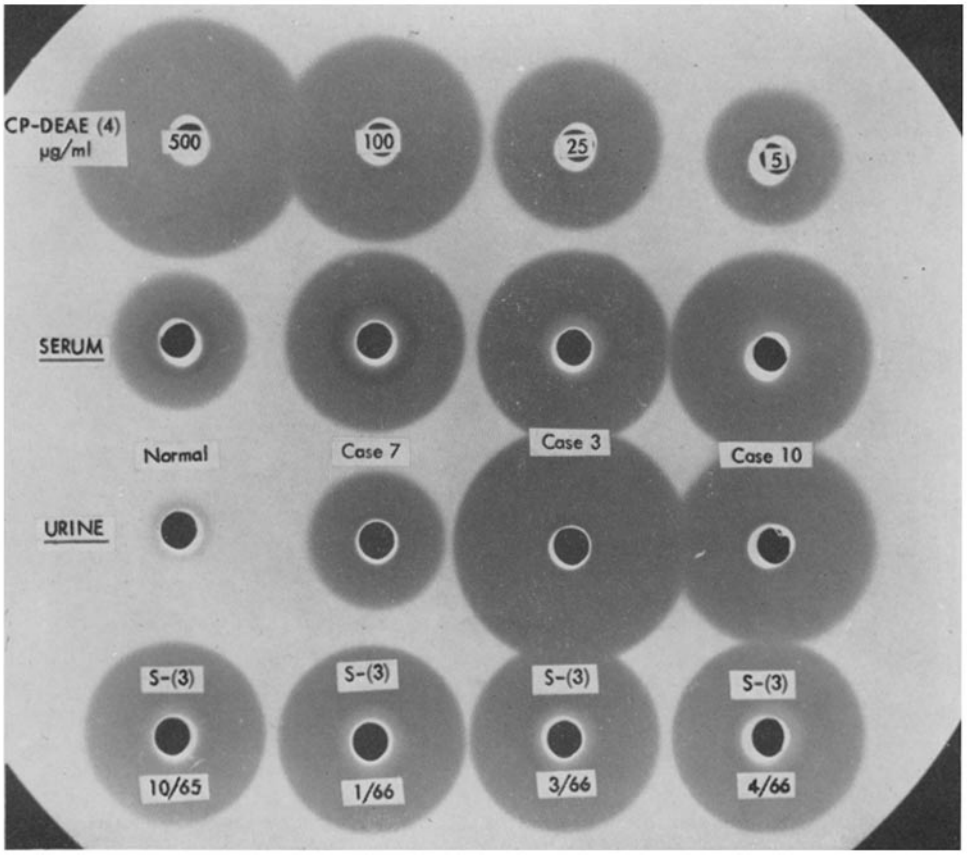


TEXT-FIG. 10. Lyso-plate demonstration of inhibition of the lysozyme activity of CP (4), human saliva, and tears by Anti-CP (5) antiserum, and the absence of detectable inhibition of egg white lysozyme activity by this antiserum. Goat (GAHS) and horse (HAHS) antisera to human serum show no antilysozyme activity but both of these sera as well as the rabbit serum (Anti-CP) show intrinsic lysozyme activity. Photographed after 30 hr development.

As shown in the bottom row in Text-fig. 11, serial serum samples from case 3 over a 6 month period demonstrated a small but significant increase in CP concentration from 48 to 59 μg/ml.

Serum and Urinary Lysozyme in Other Disease States.—With the lyso-plate technique, an increase in lysozyme activity was demonstrated in the urine of patients with chronic renal diseases, particularly the nephrotic syndrome and urinary tract infections, but the amount of lysozyme in these urines was considerably lower (3 to 5 μg/ml) than that of the monocytic leukemics. Moderately elevated (10 to 30 μg/ml) serum and urinary lysozyme levels were also

found in other chronic infections and sarcoidosis. In some of these cases, the serum lysozyme activity approached the lower range but not the median or higher values observed in the monocytic leukemics. Normal levels were found in myelocytic leukemics without associated monocytosis, and reduced levels in



TEXT-FIG. 11. Lyso-plate assays of normal serum and urine and the serum and urine of three cases of monocytic leukemia. The serum and urine samples are unconcentrated. Concentrations of CP-DEAE (4), ranging from 5 to 500 µg/ml, are in the top row for comparison. In the bottom row, serial serum samples from case 3, over a 6 month period show a small but significant increase in lysozyme activity.

cases of lymphatic leukemia. Lysozyme levels in infectious mononucleosis were within the normal range.

DISCUSSION

Chemistry and Distribution of Lysozymes.—In 1922, Fleming (10) reported to the Royal Society of London the discovery of a "remarkable bacteriolytic ele-

ment found in tissues and secretions" in a communication equally remarkable for its clarity and scope. The substance was designated "lysozyme" and the strain of gram-positive cocci found to be particularly susceptible to its lytic action as *Micrococcus lysodeikticus*. Among the observations reported in this first paper (10) were the widespread distribution of lysozyme and its particularly high concentration in hens' egg white, human tears, saliva, sputum, and nasal secretions. Significant activity was also found in blood serum and leukocytes. A particular association of lysozyme with leukocytes was suggested by its presence in "sero-pus from a cold abscess" (presumably tuberculous) and in abnormal urine "containing much albumin and pus," but not in normal urine. In a second paper in 1922, Fleming and Allison (11) reported the presence of lysozyme in empyema pus as further evidence of its relationship to leukocytes. In human tissues, the highest concentrations were found in cartilage and stomach, and lower concentrations in intestines, kidney, liver, tonsil, and brain. No activity could be demonstrated in normal cerebrospinal fluid. In tears, the lysozyme concentration was estimated as 100 to 150 times greater than that of serum.

The apparent similarity or identity of the lysozymes in different human tissues and body fluids and their similarity to the lysozyme of egg white was indicated by observations with lysozyme-resistant strains of *M. lysodeikticus*. Organisms grown in the presence of and resistant to egg white lysozyme were found to be comparably resistant to the lysozyme activity of human tissues and secretions. In subsequent studies (12), lysozyme-resistant strains of streptococci were found to be resistant to intracellular digestion by leukocytes, and it was postulated that lysozyme was the substance in leukocytes responsible for bacterial digestion.

Lysozyme was characterized by Fleming (10, 11) as nondialyzable, soluble in water and weak saline solutions, insoluble in alcohol and ether, resistant to heat and desiccation, stable on prolonged storage at room temperature, and readily absorbed by charcoal and fibrin. Most of the properties of the lysozyme in human tissues and secretions were apparently similar to those of egg white lysozyme.

Following these observations, no significant progress was made in the further characterization of lysozyme until Meyer and his coworkers (13) developed a procedure for purifying the enzyme from acetone-dried egg white by precipitation with flavianic acid. The enzyme thus obtained was identified as "a basic polypeptide, having a nitrogen content of 15.3 per cent and giving a number of protein reactions." Subsequently, Alderton, Ward, and Fevold (2) utilized the adsorbability property of lysozyme to effect its isolation from egg white, employing bentonite (finely divided morillonite clay) as the adsorbent, and eluting with 5% pyridine adjusted to pH 5.0 with sulfuric acid. By this procedure, lysozyme was isolated in high yield (85 to 90%), and characterized as a basic protein (isoelectric point between pH 10.5 and 11.0) of low molecular weight (about 17,000). It was further demonstrated that the enzyme could be crystallized at the isoelectric region (pH 10.8), at pH 7.0, and in acid solu-

tions (pH 3.5 to 5.0). The isolation procedure devised by Alderton et al. (2) has been extensively employed in subsequent investigations, including the present studies. Wetter and Deutsch (14), using this method, prepared 7 times–crystallized egg white lysozyme which was found to have an isoelectric point of 11.35 at 0.1 ionic strength, an electrophoretic mobility at pH 8.6 in diethyl-barbiturate buffer of $+2.90 \times 10^{-5}$ cm^2 v^{-1} sec^{-1}, and a sedimentation constant ($S_{20,w}$) of 2.11 at a protein concentration of 1.3 g per cent. From the sedimentation and diffusion constants and the apparent specific volume, a molecular weight of 17,200 was calculated. A rabbit antiserum to the purified lysozyme was found to inhibit its enzymatic activity.

More recently, detailed studies of the amino acid composition, tryptic and chymotryptic peptides and amino acid sequence of egg white lysozyme have been carried out by Jollès (8, 15–19), and Canfield (9, 20–22), and their associates. These studies have established that egg white lysozyme is a single polypeptide chain of 129 amino acid residues (Table I) with lysine in the N-terminal and leucine in the C-terminal position. The molecular weight of egg white lysozyme based on structural determinations is 14,307 (9). There are six half-cystines (three disulfide bridges) and six tryptophane residues. Bewley and Li (23) have demonstrated inactivation of the enzyme if the tryptophanes are hydroxymethylated and have suggested that tryptophane(s) may be present in the active site. A three-dimensional structure of hen egg white lysozyme has been derived by X-ray crystallographic analyses at 2 A resolution (24, 25), and preliminary evidence suggests that the tryptophane residues in positions 62, 63, and 108 may be involved in the substrate binding site.

Because of the availability of large quantities of hen egg white lysozyme, this protein has been the most extensively studied to date. Some studies, however, have been performed on lysozymes isolated from other sources, including plants, *Ficus* and *Papaya latex* (26, 27), rabbit, rat and dog spleen and kidney (28, 8, 17), human kidney (29), placenta (30), saliva (8, 31), and normal and leukemic leukocytes (8, 32) by Jollès and his associates. These studies, which have been reviewed by Jollès (33), have established certain chemical and structural similarities among different lysozymes, including acid and heat stability, basicity with isoelectric points in the range of pH 10 to 11, and comparable substrate specificities. The lysozymes of rabbit and dog spleen have been shown to have the same N-terminal lysine and C-terminal leucine as the hen egg white enzyme, but distinctly different tryptic peptide maps.

Amino acid analyses of lysozyme isolated from human placenta (30), saliva (31), and leukocytes (8, 32) have yielded comparable results corresponding closely with the analyses of CP-lysozyme in the present studies (Table I). These data, along with the additional immunochemical evidence of the identity of CP-lysozyme with the lysozyme of normal tears (Text-fig. 7), indicate the probable identity of the enzyme in all human tissues and secretions.

The dissimilarities between the human and egg white lysozymes are evident in their amino acid compositions (Table I) and were further substantiated by the failure of the antiserum to CP-lysozyme to inhibit the activity of the egg

enzyme (Text-fig. 10). In similar studies, Glynn and Parkman (34) have reported that a rabbit antiserum to rat lysozyme inhibited the homologous enzyme but failed to inhibit egg white or human lysozyme.

With respect to their relative enzymatic activities, Jollès and his associates (30, 31, 33), found that the human lysozymes had approximately 3 times the activity (units per μg) of hens' egg lysozyme when assayed with suspensions of *M. lysodeikticus* in phosphate buffer (pH 6.2). Comparable results were obtained in the present studies when similar assay methods were employed. However, by the lyso-plate method, CP-lysozyme was found to have 8 to 12 times the activity of hen's egg white lysozyme at equivalent concentrations. This difference may in part be due to differences in the diffusability of the two enzymes in the agar medium, possibly reflecting differences in molecular size, polymer formation, or interactions with acidic groups in the agar matrix. These parameters are presently being studied.

The Lysozyme Substrate.—The specific substrate of lysozyme was first isolated by Meyer et al. (35) and characterized by these workers (35, 36), and by Epstein and Chain (37) as a polymerized mucopolysaccharide which is depolymerized by the enzyme through the hydrolysis of glucosidic linkages with the release of a reducing sugar and acetylhexosamine. Subsequent studies of Salton and Ghuysen (38) demonstrated that the lysis of cell wall preparations of *M. lysodeikticus*, *B. megatherium*, and *Sarcina lutea* yielded dialysable and nondialysable products; the former consisted in part of a disaccharide of *N*-acetylglucosamine and *N*-acetylmuramic acid, and the latter of compounds with molecular weights of 10,000 and 20,000. Salton and Ghuysen (38) identified the disaccharide as *N*-acetylglucosaminyl-$\beta(1 \rightarrow 6)$-*N*-acetylmuramic acid. They (38) also detected a tetrasaccharide which is a dimer of the disaccharide joined by a $\beta(1 \rightarrow 4)$ linkage. At present, this tetrasaccharide is considered to be the specific substrate of lysozyme with the enzyme having $\beta(1 \rightarrow 4)$-*N*-acetylglucosaminidase and/or $\beta(1 \rightarrow 4)$-muramidase action. The bacterial cell wall is thought to have a basic skeleton of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine residues with alternating $\beta(1 \rightarrow 4)$ and $\beta(1 \rightarrow 6)$ linkages. Some of the muramic acid residues have substituent peptides accounting for the nondialyzable products yielded by lysozyme action on the cell wall substrate (38).

In *M. lysodeikticus*, *Sarcina lutea*, and other bacteria which are particularly susceptible to lysozyme action, the specific substrate is apparently located in a position directly accessible to the enzyme. In other organisms, the polysaccharide substrate may be present but inaccessible, because of surface lipids, proteins, and other polysaccharides. These organisms are resistant to lysozyme unless the substrate is rendered accessible by other enzymes (e.g. lipases, proteinases) or other agents (e.g. complement, antibody). Thus, in the host, lysozyme may act synergistically with other agents against a wide variety of bacteria including some which are apparently resistant when exposed to the enzyme in vitro (17, 33, 39, 40).

Leukocytes, Monocytes, Macrophages, and Lysozyme.—As previously noted, the original observations of Fleming (10, 11) established the relationship of

leukocytes to lysozyme. Subsequently, identification of the particular populations of leukocytes containing lysozyme has been the objective of several studies (34, 41–44) but the question of whether the enzyme is elaborated exclusively by a specific group of cells, possibly monocytic leukocytes, or whether both monocytes and polymorphonuclear leukocytes contain lysozyme, is still not clearly resolved. In great measure, the failure to clarify this issue is due to the inherent difficulties in obtaining "pure" cell populations for study, and this in turn is responsible for the fact that the precise interrelationship of monocytes and polymorphs is not clearly defined.

In 1940, Barnes (41) compared thoracic duct lymphocytes and peritoneal leukocytes of rabbits and cats with respect to their content of lysozyme, cathepsin, nuclease, and lipase and found significantly higher levels of all enzymes in the peritoneal leukocytes. Since these leukocytes were harvested from saline washings of the peritoneum, they clearly represented a mixed population of cells (polymorphs, monocytes, histiocytes, etc.), and the lysozyme-containing cells could not be specifically identified. The apparent absence of lysozyme in lymphocytes, however, was well documented and later confirmed by Flanagan and Lionetti (42) in studies of lysates of normal human blood. In these studies (42), lysozyme levels were found to correlate with the total leukocyte count, but not with any specific fraction in the differential count. Brumfitt and Glynn (43), in studies similar to those previously reported by Fleming and Allison (12), showed that human polymorphonuclear leukocytes and rat peritoneal macrophages had the capacity to phagocytize and destroy lysozyme-sensitive strains of *M. lysodeikticus*, whereas lysozyme-resistant strains were phagocytized but not destroyed. In subsequent studies, Glynn and Parkman (34) demonstrated binding of a rabbit anti-rat lysozyme antiserum to the cytoplasmic granules of rat peritoneal leukocytes and macrophages, using an indirect immunofluorescence method. These studies were interpreted as providing further evidence that both macrophages and polymorphs contain lysozyme. More recently, Speece (45) described an histochemical procedure for demonstrating lysozyme in tissues. Frozen sections of tissues are overlayered with a suspension of *M. lysodeikticus* in agar and stained with Alcian blue and basic fuchsin. Intact organisms were found to stain blue and protoplasts, red. Using a modification of this method, Briggs and associates (44) have studied peripheral blood, buffy coat, and bone marrow preparations and found lysozyme activity in monocytes, polymorphs, and polymorph precursors. No lysozyme was detected in lymphocytes, plasma cells, basophils, mast cells, or marrow reticuloendothelial cells. In these studies (44), monocytes appeared to have the highest content of lysozyme.

Considerable evidence has been assembled which apparently establishes that monocytes are the circulating precursors of tissue macrophages (46–52), alveolar macrophages (53–55), and the epithelioid and giant cells of tuberculous granulomas (49, 56, 57). Much of the earlier evidence for these cellular interrelationships was derived from morphologic observations of fixed tissues and cell cultures and from studies of the migration of phagocytic cells labeled with carbon particles, dyes, or other markers. More recent studies utilizing enzyme

markers, particularly lysozyme (43, 58–62) have provided further documentation of the transformation of monocytes into macrophages and certain of the cellular constituents of granulomas.

In 1961, Myrvik and coworkers (58) described a method for harvesting large numbers of alveolar macrophages from normal rabbit lungs and demonstrated (59) that the lysozyme content of these alveolar macrophages (2000 to 4000 μg/ml of packed cells) was many times greater than that of peritoneal macrophages. In subsequent studies (60), an even higher lysozyme content was found in alveolar macrophages of rabbits with granulomatous lung disease induced by vaccination with BCG strain tubercle bacilli. Cohn and Wiener (5) have also observed a 2- to 3-fold increase in lysozyme content of rabbit alveolar macrophages after BCG challenge and further demonstrated a comparable increase in several other enzymes including acid phosphatase, lipase, cathepsin, β-glucuronidase, ribonuclease, and esterase in these cell preparations. By differential centrifugation of cell lysates, 60 to 80% of these enzymes were localized in the postnuclear (lysosomal) fraction which sedimented at 15,000 *g*. In subsequent studies (63), release of enzymes including lysozyme into the soluble fraction was demonstrated following ingestion of heat-killed *E. coli* apparently as a consequence of granule (lysosome) rupture. More recently, Carson and Dannenberg (40) reported an increased lysozyme content in both alveolar and peritoneal macrophages of rabbits challenged intravenously with heat-killed *M. tuberculosis* but they could not demonstrate an increase in protease or lipase.

The multiplicity of enzymes found in leukocytes and macrophages and the variable changes in their concentrations in response to different stimuli raises the question of whether there are several genetically distinct cell populations with specific constituent enzymes, or whether a single cell line arising from a common precursor can respond to a particular challenge with a specific pattern of enzymes. At present, this question cannot be resolved with the evidence available.

Monocytes, Macrophages, Lysozyme, and Tuberculosis.—That monocytes, macrophages, and their contained lysozyme may play a significant role in host defenses against mycobacterial infections has been suggested by several observations in experimental animals (40, 57, 60, 64, 65).

In 1951, Myrvik and Weiser (64) demonstrated that the serum of rabbits immunized with heat-killed tubercle bacilli in Freund adjuvants was strongly tuberculostatic against the BCG strain of *M. tuberculosis* and that the active principle was similar to egg white lysozyme in its heat stability, adsorption behavior, and antibacterial spectrum. These observations were followed by the studies of the lysozyme content of alveolar macrophages of BCG-challenged rabbits, previously described (60–62). Myrvik's findings have been confirmed by Tsuji (65) who further demonstrated that the increase in lung (alveolar) macrophages in rabbits immunized with BCG and *Mycobacterium phlei* was associated with increased mycobacteriostatic activity of lung tissue. It is of further interest that de Wijs and Jollès (66) have identified the

polysaccharide lysozyme substrate in cell walls of three atypical mycobacterial strains, *M. phlei*, *M. fortuitum*, and *M. kansasi*, and have demonstrated the susceptibility of these strains to human milk lysozyme as well as hens' and duck egg white lysozyme. Similar studies on *M. tuberculosis* have been hindered by the difficulties in isolating cell walls of these organisms due to their high lipid content.

In man, there is likewise considerable evidence of a relationship between monocytes, lysozyme, and mycobacterial infections.

After the observations of Cunningham et al. (57) of monocytosis in tuberculous rabbits, Medlar (67) and Flinn and Flinn (68) demonstrated a significant increase in peripheral blood monocytes in patients with active tuberculosis, and this has been confirmed in subsequent studies (69, 70). Gibson has reported (71) a remarkable case of disseminated tuberculosis associated with a monocytic leukemoid reaction and monocyte counts as high as 36,000 per mm^3. Post-mortem examination revealed extensive monocytic proliferation in the tuberculous granulomas.

With regard to lysozyme in human tuberculosis, Kerby and Chaudhuri (72) demonstrated increased lysozyme activity in the plasma and leukocytes of tuberculous subjects, comparable to that found in tuberculous rabbits by Myrvik and Weiser (64). Kerby and Chaudhuri's findings (72) have been confirmed by Finch, Lamphere, and Jablon (73) and by observations on a small number of tuberculous subjects in the present studies.

In Boeck's sarcoid, another granulomatous disease possibly related to tuberculosis, monocytosis has been reported in association with active progression (70, 74) and coincident with an unusual response to repeated challenge with Kveim antigen (75). In the present studies, significant increases in serum and urinary lysozyme were found in four of fifteen cases of Boeck's sarcoid (Text-fig. 7) but a correlation with peripheral monocytosis was not apparent.

Lysozyme in Other Chronic Infections and Renal Diseases.—With respect to the role of lysozyme in other disease states, Meyer and Prudden and their associates (76–78) demonstrated increased lysozyme activity in the gastric juice of patients with peptic ulcers and in the stools and colonic mucus of patients with ulcerative colitis and regional enteritis. On the basis of these observations, lysozyme was postulated to be an etiological factor in ulcerative intestinal diseases. However, subsequent studies by these workers (79) and by Hiatt et al. (80) established that the invading granulocytes were the principal source of lysozyme in these conditions rather than the gut wall, indicating that the increase in lysozyme probably represented a secondary rather than a primary factor.

An association between lysozyme and renal disease was initially suggested in Fleming's report (10) of increased lysozyme in abnormal urine "containing much albumin and pus." Subsequent studies (81–85) have further documented this association.

Wilson and Hadley (81) and Burghartz and Boosfeld (82) demonstrated increased lysozyme activity in the urine of some but not all nephrotic children. The degree of hyperlysozymuria could not be correlated with the extent of proteinuria or other parameters of renal functional impairment. These studies were extended by Prockop and Davidson (83) who reported increased urine and serum lysozyme levels in patients with a variety of renal diseases including pyelonephritis, glomerulonephritis, and nephrosis. Again, no correlation was evident between the amount of urinary lysozyme and the severity of proteinuria or pyuria. Hyperlysozymuria has also been reported in association with the nephropathy of cadmium poisoning in man (84) and rabbits (85), and in rats with mercuric chloride–induced renal tubular damage (83). A 70- to 100-fold increase in the lysozyme content of kidney tissue was found by Perri and associates (86, 87) in rats bearing the Jensen sarcoma. Initially, the increased lysozyme was considered to be specifically related to the Jensen tumor, but subsequent studies (88, 89) demonstrated comparable increases in kidney lysozyme with other transplanted neoplasms, and it was concluded that the lysozyme was related to the reticuloendothelial and macrophage response to the tumor, rather than to the tumor per se. In studies of lysozyme levels in post-mortem samples of kidneys of 29 human cancer subjects, Perri and Faulk (90) could find no consistent patterns.

In the present studies, we have confirmed an inconsistent increase in urinary lysozyme associated with renal disease, particularly pyelonephritis, of a much lower order of magnitude than that associated with monocytic leukemia.

Lysozyme and the Leukemias.—With respect to the hematologic dyscrasias, Finch (91) and Jollès (32, 92, 93) and their associates have previously reported increased serum lysozyme in cases of myelocytic and monocytic leukemia, and decreased serum lysozyme in lymphatic leukemia. Urinary lysozyme was not examined by these investigators. The present studies have confirmed the elevation in serum lysozyme in monocytic and monomyelocytic leukemia and have further documented the excretion of large amounts (0.6 to 2.6 g per day) of lysozyme in the urine of these patients.

In the present studies, a general correlation was evident between the amount of enzyme excreted and the absolute monocyte count in the peripheral blood. The degree of variability observed in this relationship probably reflects the multiplicity of factors involved in the production, cellular release, and ultimate renal excretion of the enzyme. Thus, although lysozyme was demonstrated immunologically in buffy coat preparations following the sonic disruption of cells, as shown in Text-fig. 7, it could not be demonstrated in saline washings of intact leukocytes. It is therefore likely that the lysozyme is primarily released when cells disintegrate. At present, it is not known if any enzyme escapes from intact cells and whether abnormal monocytes differ from normal cells in their quantity of lysozyme or in the manner in which it is released. Following release of lysozyme into the circulation, there unquestionably are also several additional factors (e.g. tissue binding, renal clearance) involved in its ultimate excretion. The availability of a more precise method for quantitating lysozyme

in small samples should enable elucidation of some of these factors, and these studies are in progress.

Another important question which remains to be clarified concerns the identification of the specific population of leukocytes which elaborate lysozyme. From the present studies, the cell type which appeared to be most consistently related to lysozyme elaboration had the cytologic and staining properties of monocytes. It must be recognized, however, that there is considerable variability in the morphologic features of individual cells of this series which at times makes it difficult to distinguish monocytic from immature granulocytic forms. Indeed, the exact nature of the relationship between these cell lines is still unclear. Unquestionably, much of the confusion in the classification of monocytic, monomyelocytic, and myelocytic leukemias relates to these factors and to the limitations of conventional morphologic criteria.

Of interest and possible significance is the fact that lysozyme was the only enzyme found in excess in the present cases, and that the other hydrolases (i.e. acid phosphatase, β-glucuronidase, cathepsin, and ribonuclease) which are demonstrable in mixed populations of leukocytes and macrophages were not found in abnormally high concentration in the whole urinary protein precipitates of these patients. By itself, this observation implies the proliferation of a population of cells which has, either constitutively or as a consequence of its abnormal proliferative (neoplastic) state, the capacity to elaborate lysozyme as its principal or sole product. Alternately, it is possible that other enzymes were produced in excessive amounts in these cases but not excreted in the urine because of different molecular parameters (i.e. greater size, charge differences, more rapid catabolism, etc.). These possibilities are presently under investigation.

Relationship between the Monocytic and Plasmacytic Dyscrasias.—In certain respects, the elaboration of large quantities of lysozyme by proliferating monocytes in the present cases appears similar to the elaboration of gamma globulins and gamma globulin subunits in cases of multiple myeloma and other plasma cell dyscrasias. A significant difference, however, is the apparent identity of the lysozyme in all the present cases and its identity with the normal enzyme, as compared with the structural specificities of the proteins produced in individual cases of plasma cell dyscrasia (94). This difference unquestionably relates to the heterogeneity of the normal immunoglobulin products of plasma cells as opposed to the apparent homogeneity of the enzymes elaborated by specific populations of leukocytes.

Additional similarities between the present group of cases and some of the plasma cell dyscrasias relate to certain clinical features and possible pathogenic mechanisms.

As previously noted, all the present cases had significant nonspecific proteinuria in addition to hyperlysozymuria, suggesting renal functional impairment, and five of

the cases exhibited hypokalemia and hyperkaluria, indicating renal tubular dysfunction possibly related to the excretion of the cationic lysozyme. Detailed renal and electrolyte studies are in progress.

Electrophoretic and immunoelectrophoretic analyses of the serum proteins in seven of the present cases demonstrated an increase in gamma globulins, particularly gamma-A globulin, and in two cases a small, M-type gamma-G peak was present in the serum. As further evidence of a possible association with the plasma cell dyscrasias, the first of the present cases has well documented plasma cell myeloma with amyloidosis and Bence Jones proteinuria of at least 4 yr duration prior to developing monomyelocytic leukemia as an unexpected terminal event.[3]

The patient's urinary proteins were serially studied to evaluate chemotherapy and for a period of 18 months prior to the terminal event, there was a significant decrease in Bence Jones proteinuria associated with *l*-phenylalanine mustard (melphalan, 'Alkeran') therapy. Coincident with the terminal development of monomyelocytic leukemia, the cationic protein, ultimately identified as lysozyme, appeared in the urine.

As previously reported (95), the plasma cell dyscrasia developed in this case against a background of long-standing urinary tract infection, and the possibility that the chronic infection might have induced the plasma cell dyscrasia was suggested (95). The sequence of events in this case, and the fact that significant chronic infections were documented as antecedent illnesses in five other cases in the present series has suggested the possibility of the operation of pathogenic mechanisms similar to those postulated for certain of the plasma cell dyscrasias (96). Further detailed clinical and pathologic studies are in progress in an effort to elucidate these mechanisms.

SUMMARY

Markedly increased quantities of lysozyme have been found in the serum and urine (ranging to 2.6 g per day) of ten consecutive cases of monocytic and monomyelocytic leukemia. The enzyme has been isolated from the urine of several cases and physicochemically and immunochemically characterized. It is apparently identical to the lysozyme of normal tears, saliva, leukocytes, and serum, but structurally different from the lysozyme of hen's egg white. The activity of the human enzyme assayed with *M. lysodeikticus* organisms is 3 to 12 times greater than egg white lysozyme at equivalent concentrations.

An agar plate method has been developed for quantitating lysozyme activity in small samples (approximately 25 μl) of serum, urine, or other biological

[3] A detailed description of this case prior to the terminal event was reported as case 4 (B. Eps.) in a study of amyloidosis and plasma cell dyscrasia (95).

fluids. The range and reproducibility of this method were found to be superior to previously available lysozyme assay procedures.

Present evidence indicates that lysozyme is the principal, if not the sole, product of the proliferating monocytes in monocytic and monomyelocytic leukemia, and quantitation of serum and urine lysozyme should be a useful diagnostic procedure for these leukemias.

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EXPLANATION OF PLATES

PLATE 91

FIG. 1. (A to D). Representative peripheral blood and bone marrow smears of monocytic (case 10) and monomyelocytic (case 6) leukemics. Wright Giemsa stain. × 300.

A. Peripheral blood (case 10). Total white blood cell count, 117,000, with 82% monocytic forms.

B. Bone marrow (case 10). Large monocytic forms with cytoplasmic granules and granule-free cytoplasmic buds.

C. Bone marrow (case 6). Monocytic and myelocytic forms. Total white blood cell count, 68,500, with 40% monocytes, and 42% polymorphonuclear leukocytes.

D. Bone marrow (case 6). Large monocytic cells with prominent nucleoli, nuclei with vacuoles, and azure-stained cytoplasmic granules; polymorphonuclear leukocytes and myelocytes.

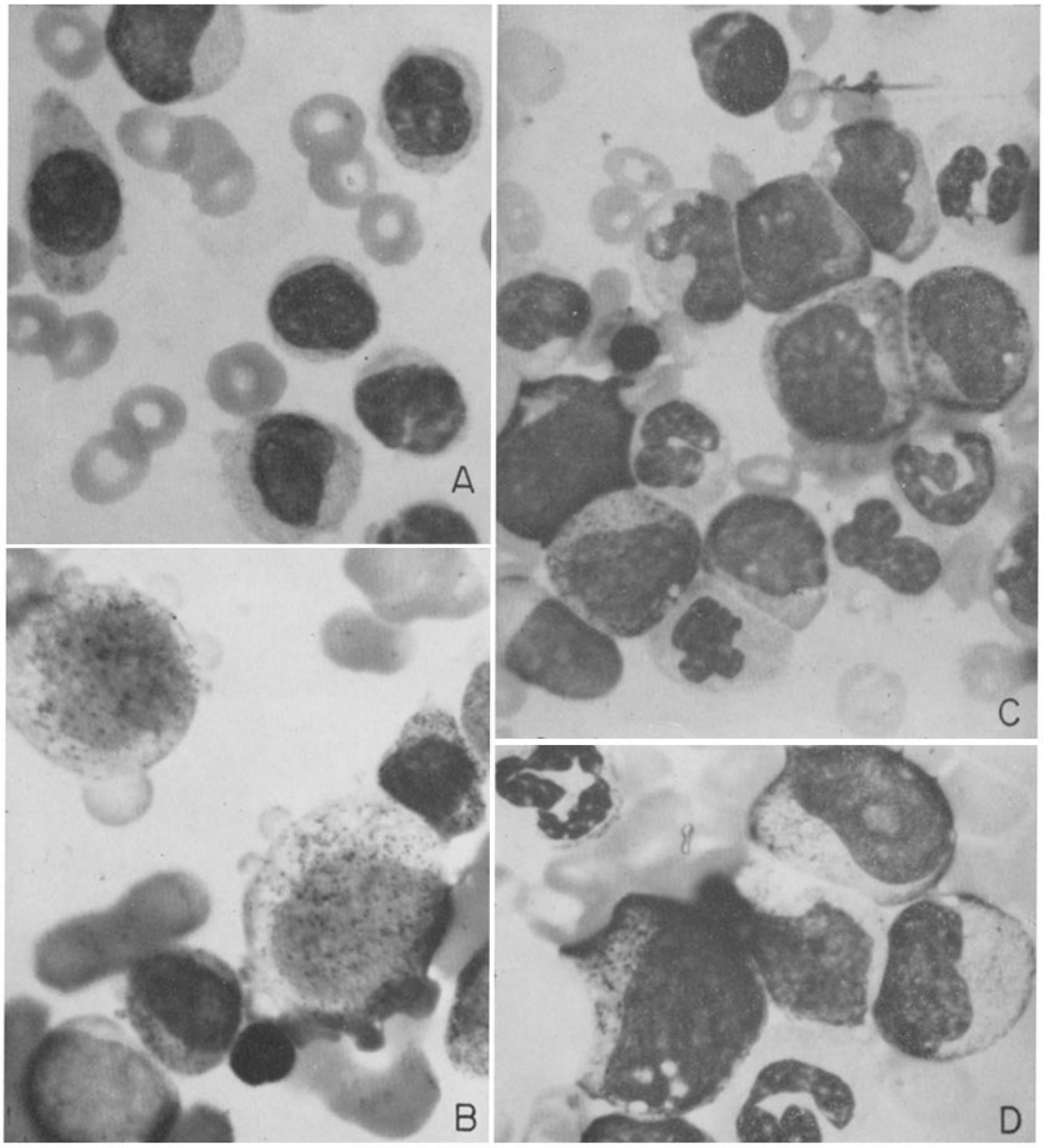

FIG. 1

(Osserman and Lawlor: Serum and urinary lysozyme in leukemia)

PLATE 92

FIG. 2. Electron photomicrograph of the predominant cell form in the bone marrow of case 3. Enlarged area (*B*) shows a prominent Golgi zone (*Go*), scattered endoplasmic reticulum (*ER*), mitochondria (*M*), and numerous lysosomal granules (*LyG*). × 1500.

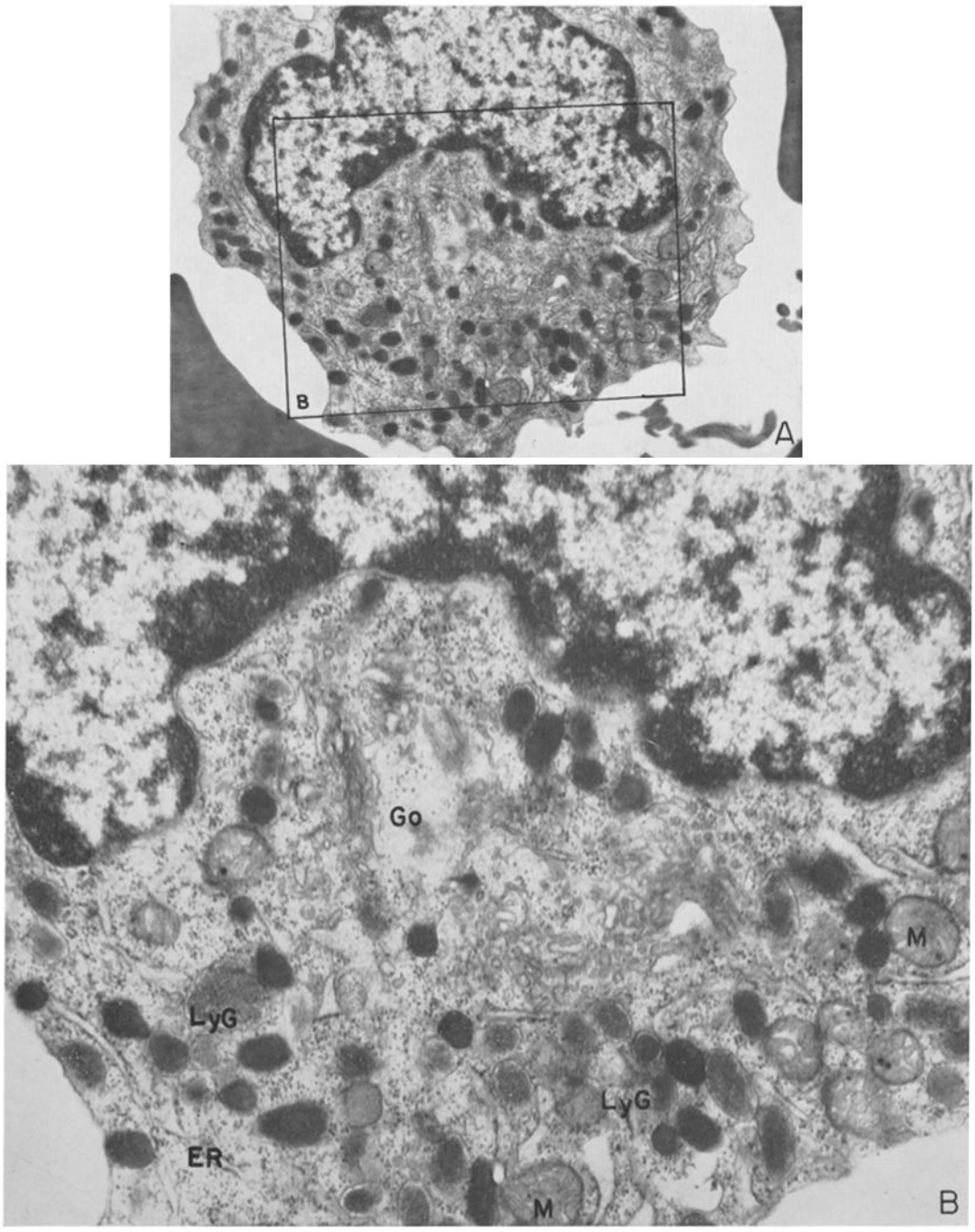

FIG. 2

(Osserman and Lawlor: Serum and urinary lysozyme in leukemia)