

VAT copy

MAR 1967

33

American Journal of Medicine

MACROGLOBULINEMIA WITH BONE DESTRUCTION.

Difficulty of Distinguishing between Macroglobulinemia and Myeloma.

John Welton, M.D., Samuel R. Walker, M.D., Gordon C. Sharp, M.D.,
Leonard A. Herzenberg, Ph.D., Richard Wistar, Jr. M.D.
William P. Creger, M.D.

John Welton, M.D., Fellow in Medicine (Hematology)
Samuel R. Walker, M.D., Fellow in Medicine (Infectious Disease)
Gordon C. Sharp, M.D., Assistant Professor of Medicine (Immunology)
Leonard A. Herzenberg, Ph.D., Associate Professor of Genetics
William P. Creger, M.D., Associate Professor of Medicine and Chief,
Division of Hematology,
Stanford University School of Medicine, Palo Alto, California.
Richard Wistar, Jr. M.D., Fellow in Immunology, Walter and Eliza
Hall Institute, Melbourne, Australia.

This work was supported in part by - Public Health Service Training
Grant, T1 AM 5239-07.

Requests for reprints should be addressed to Gordon C. Sharp, M.D.
Stanford University School of Medicine, 300 Pasteur Drive, Palo Alto,
California. 94304.

The purpose of this report is to suggest that current characterization of immunoglobulins, presence or absence of bone destruction, and cell morphology may not serve to separate macroglobulinemia and myeloma as sharply as has been thought.

The syndrome of Waldenström's macroglobulinemia (1-3) is characterized by hemorrhagic, visual, and neurologic symptoms. The ocular fundi are typical and lymphadenopathy and hepatosplenomegaly may be present. The erythrocyte sedimentation rate may be very high, and blood viscosity is usually elevated. Anemia is common and is frequently accompanied by large numbers of lymphocytoid cells in the marrow. Electrophoresis of the serum demonstrates a homogeneous band usually in the β - or γ -globulin region. Ultracentrifugation reveals a large amount of protein with a sedimentation constant of approximately 19S.

The rarity of destructive bone lesions in macroglobulinemia has been repeatedly emphasized (4-8); indeed, absence of such lesions has been used as an aid in distinguishing the condition from myeloma, in which they occur with great frequency (9,10). The few reports of bone destruction in macroglobulinemia are inconclusive, for they lack sufficient protein characterization to support the primary diagnosis.

We have recently studied a patient who initially had the features of macroglobulinemia and who later developed destructive bone lesions and, terminally, plasma cell leukemia. Autopsy and detailed protein studies were performed.

Case Report

A fifty-three year old accountant was admitted to the Stanford Medical Center in October, 1963 with a two-week history of weakness, dizziness, epistaxis, and dark stools. He had occasionally noted the sensation of objects floating across his field of vision, and symptoms suggesting Raynaud's phenomenon had been present for many years. His weight had been stable and his last blood count in 1961 had been normal. The past history was negative except for a small, intermittently draining pilonidal cyst, and "kidney stones removed from the bladder" 20 years ago without recurrence. He had not had ulcer symptoms or easy bruising, and there was no family history of hematologic disease.

Except for the presence of pallor, the patient was a healthy-appearing, middle-aged, white man. Fundoscopic examination revealed segmental venous engorgement and numerous retinal hemorrhages (Fig.1). The liver and spleen were not palpable and there was no adenopathy. Except for a draining pilonidal cyst, the remainder of the examination was normal.

Laboratory studies revealed a normochromic, normocytic anemia with hematocrit of 21%. Erythrocytes were morphologically normal. The stool guaiac reaction was 4+. Approximately 75% of bone marrow cells were plasma cells characterized by large, eccentric nuclei with perinuclear clear zone, nucleoli, and deeply basophilic cytoplasm (Fig.2A). The cells appeared consistent with those seen in myeloma. Erythroid, myeloid, and megakaryocytic elements were diminished but morphologically normal. The 24-hour urine protein excretion ranged from 500 to 1,000 mg., 75% being gamma globulin as shown by paper electrophoresis. The Bence Jones thermal reaction of a concentrated urine specimen was negative. Roentgenographic examinations of the skull, chest, stomach, small bowel, and colon were normal. The vertebrae showed only demineralization. The bleeding time was 19 minutes by the method of Borchgrevink (11); the Quick prothrombin time was 30%, and the prothrombin-proconvertin and thromboplastin generation tests were within normal limits. The total

protein was 11 gm.% with 8 gm.% globulin. The Sia test was strongly positive. Serum paper electrophoresis showed a narrow band in the gamma region. Ultracentrifugation demonstrated a large 19S component as well as smaller amounts of materials with higher sedimentation constants. White blood cell and differential, platelet and reticulocyte counts, fasting blood sugar, and serum urea nitrogen, creatinine, alkaline phosphatase, cholesterol, calcium, and uric acid were normal.

The hematocrit rose to 40% with transfusion and remained stable. Twenty units of plasma were removed over a period of two weeks, without change in serum protein concentration. The patient's condition improved without significant changes in the fundal veins or in the serum protein concentration, and he was discharged in November, 1963 on oral cyclophosphamide, 100 mg. daily.

Within two months the total serum protein had decreased from 11 to 7 gm.% with 2 gm.% globulin (Fig.3), the marrow plasma cells had fallen to 25% (Fig.2B), and the fundoscopic findings were normal (Fig.1, lower row). A fifteen-pound weight gain occurred during the following year. The remission was maintained with 50 mg. of cyclophosphamide daily (Fig.3).

Beginning in March, 1965 gradual exacerbation was noted, with decrease in the hematocrit to 35% and increase in the total serum protein and marrow plasma cells despite increase of cyclophosphamide to 150 mg. daily (Fig.2c). Transfusions were occasionally required.

Early in 1966 plasmapheresis was repeated, with removal of 10 units of plasma over a period of one month without significant change in the serum protein concentration. The patient then suffered severe low back pain, and over a two month period developed roentgenographic changes consistent with compression fractures of several vertebrae (Fig.4a).

Following a three-day period of poor fluid intake, the patient became oliguric and confused and was hospitalized. Anemia and thrombocytopenia were noted, and plasma cells were observed in the peripheral blood for the first time (Fig.2d). The serum creatinine was 7 mg.%, having been 1.5 mg.% five months earlier. Chest roentgenograms revealed bilateral pulmonary infiltrates, and sputum cultures grew Klebsiella-Aerobacter. The patient exhibited an acute brain syndrome without localizing signs. Spinal fluid and electroencephalogram were normal. His mental status fluctuated with some clearing, but never a return to normal. The urine output rose to normal following admission, but renal function gradually deteriorated, the serum creatinine rising to 12 mg.%. Serum calcium ranged between 8.8 mg.% and 12.0 mg.% with a normal serum albumin. Epistaxis began soon after admission. Rapidly changing pulmonary infiltrates were demonstrated roentgenographically and hemoptysis was noted. The bleeding time was greater than 45 minutes, with a prothrombin time of 46% and a prothrombin-proconvertin value of 43% (normal 60 to 140%). The partial thromboplastin time was normal and Factor VII assay was 30% of normal.* Bleeding decreased following two exchange transfusions. Antibiotic therapy initially consisted of methicillin, followed by chloramphenicol after determination of the sputum culture sensitivity. Subsequent sputum and blood cultures grew Candida albicans. Terminally, the white blood cell count rose to 66,000 with 50% plasma cells. In spite of additional therapy, including platelet concentrates, low molecular weight dextran, and vincalcikoblastine, the patient's condition worsened and he died one month after admission.

Autopsy examination revealed almost total replacement of the vertebral marrow with plasma cells, accompanied by marked bone destruction (Fig.4b). There was moderate hepatic periportal and marked splenic and lymph node infiltration by plasma cells (Cf.Fig.5a). The kidney cortices were infiltrated with plasma cells and the tubules contained large numbers of casts (Fig.5b). The lungs showed hemorrhage, edema, pneumonitis and plasma cell infiltration. Permission for brain examination was denied.

* Performed by Dr. Judith G. Pool.

Methods of Protein Analysis*

Serum protein electrophoresis was performed by paper electrophoresis in 1963 and by the cellulose acetate method in 1966.

Ultracentrifugal analysis of the patient's serum, diluted 1:10 in physiological saline, was carried out in a double-sectored cell at 56,000 rpm in a Spinco Model E ultracentrifuge at 21°C. The second cell contained normal human serum diluted 1:10 in saline. We are indebted to Dr. Jack Remington for the performance of these studies.

Immuno-electrophoretic analysis was performed in 0.05M barbital buffer containing 0.1M NaCl by the micro-method of Scheidegger (14).

Gel diffusion was performed on microscope slides coated with 1% "Tonagar" (Oxoid No.2) in 0.05M barbital buffer containing 0.1M NaCl.

Quantitative determination of the immunoglobulins was kindly performed by Dr. Mathea Allansmith, using the single radial immunodiffusion procedure outlined by Fahey and McKelvey (15).

Antisera against human γ A, γ G and γ M were shown to be specific by gel diffusion with appropriate purified antigens. Fab fragment, kappa (κ) and lambda (λ) chains and antisera to them and to normal human γ M were generously supplied by Dr. Hugh Fudenberg. The purified γ M fractions from this patient (J63 and J66) and from a patient without macroglobulinemia [γ M(Sc)] were taken from the first peak of a G-200 sephadex column. Antiserum against the patient's 1963 γ M (anti-J63) was produced by immunization of a rabbit with material from the G-200 sephadex γ M peak in

* The terms recommended by the World Health Organization (12) for the human immunoglobulins are used in this report: Gamma A globulin = γ A = IgA, γ 1A, B2A; Gamma G globulin = γ G = IgG, γ 2, γ ss, 7s; Gamma M globulin = γ M = IgM, B2M, γ 1M, 19S. Gamma D is not discussed because of inadequate clinical experience with γ D myeloma (13).

complete Freund's adjuvant. Purified heavy (H) and light (L) chains were prepared by reduction and alkylation followed by G-100 sephadex filtration in 1M propionic acid, essentially by the method of Fleischman et.al. (16).

Acrylamide gel electrophoresis was performed in 8M urea at pH9-10 using the buffers described by Reisfeld and Small (17). The samples were reduced in either .01M or 0.1M dithioerythritol.

Results

Analysis of the serum protein by electrophoresis, ultracentrifugation, and immunoelectrophoresis (Fig.6) showed a greatly elevated γM serum level in 1963, a marked diminution in 1964 (after cyclophosphamide therapy), and then a return to a very high level in 1966. Quantification was obtained by radial immunodiffusion (Table 1), showing a marked elevation of γM in 1963 and 1966 and a level somewhat above normal even in 1964. The other immunoglobulins remained below normal levels throughout the course, but there was a clear rise in γG level coincident with the γM decrease in 1964.

Gel filtration of both the 1963 and 1966 sera with G-200 sephadex in each case resolved a large peak emerging with the void volume and a second peak corresponding to the usual albumin position. Because of the low γG level, a distinct 7S peak was not seen. Analysis of a pool of the first two-thirds of the peak by agar gel diffusion (Figs.7a, b, c) showed that it contained γM but no detectable γA or γG . The final part of this peak contained some γG , and albumin was found in the second peak. The G-200 fractions from 1963 and 1966, as well as the purified L chains, were shown to have kappa, but no lambda, specificity (Figs.7d, e). Utilizing both a commercial antiserum to γM and a rabbit antiserum to the patient's 1963 γM , there was a reaction of identity of the 1963 and 1966 γM proteins (J63 and J66 in Figs. 7c, d, g, h). The J63 and J66 γM proteins spurred over the γM from a normal pool and from another (non-macroglobulinemic)

patient $\gamma\text{M}(\text{Sc})$ when reacted against anti-J63; the latter finding is demonstrated in Fig.7f. This reaction of partial but not complete identity was shown more clearly by the technique of in-well absorption (Fig.7g, h) in which absorption of anti-J63 by $\gamma\text{M}(\text{Sc})$ or by normal γM failed to abolish the reaction with J63 and J66. As a further test of the identity of J63 and J66 γM proteins, the antiserum to the J63 protein was absorbed with the J66 protein, and there was no remaining reaction with either the J63 or J66 γM proteins on Ouchterlony analysis.

Since the electrophoretic banding pattern of H and L chains in alkaline urea gels is different for individual immunoglobulins (18,19), the finding of identical patterns with J63 and J66 on acrylamide gel (Fig.8) is strong evidence for the identity of these two proteins.

Thus the patient's abnormal serum protein was shown by immunochemical methods to be γM and not γG or γA . Further, the isolated L chains were of kappa and not lambda specificity. Ouchterlony analysis and acrylamide gel electrophoresis of the whole proteins, as well as the separated H and L chains, did not detect any difference between the 1963 and 1966 γM .

Discussion

In the past, ultracentrifugal analysis of the serum was regarded as sufficient for the identification of γM , and several reports of bone destruction in association with macroglobulinemia have relied solely on this technique (20-22). It is now known that some sera contain aggregated or polymerized γG or γA , and ultracentrifugation may show a pattern resembling that of true γM (23-25). Therefore, more sophisticated methods, such as immunoelectrophoresis and agar gel diffusion using monospecific antisera, are necessary to resolve possible confusion between such aggregates and true γM .

We are aware of only three cases of macroglobulinemia with bone destruction which have been studied with techniques beyond that of ultracentrifugation to prove the presence of an excess of true γM . Wanner and Siebenmann (26) and Adner (27) used a serologic precipitation reaction to

demonstrate a large amount of γ M. These studies did not describe purification and immunochemical analysis of the abnormal protein. The frequently cited patient of Waldenström (7,28) had breast carcinoma, and therefore the bone destruction could have been caused by metastases from that tumor. Since it has been shown that two or more "M-proteins" may coexist (13,29-31), it is pertinent that none of the above studies ruled out the possibility that aggregation of γ A or γ G might have accounted for part of that portion of the ultracentrifuge pattern which was attributed to γ M.

Immuno-electrophoresis and agar gel diffusion with monospecific antisera demonstrated that our patient's abnormal protein was indeed γ M and not an aggregate of γ A or γ G. These studies, plus L and H chain analysis, also revealed that his purified serum protein of 1963 (the time of initial clinical presentation of macroglobulinemia) was identical to that of 1966 (the period of myeloma-like bone destruction). The protein was of kappa specificity on both occasions. These observations suggest that no new immunoglobulin-producing cell line arose during the course of the patient's illness.

The "classical" picture of myeloma includes destructive bone lesions, anemia, and large numbers of plasma cells in the marrow, together with excessive amounts of γ A, γ G, γ D or micromolecular (Bence Jones) protein in the serum (10,13). Some patients, however, have only the protein disturbances for years (13,32-35). Similarly, patients with macroglobulinemia may have no symptoms, or they may exhibit all of the features of the syndrome originally described by Waldenström. Our patient's serum contained a large amount of γ M both at the time of his initial presentation as "typical" macroglobulinemia and at the time of the later myeloma-like bone destruction. It would therefore appear that although a number of patients with elevated serum γ M exhibit the usual clinical characteristics of macroglobulinemia, the same protein abnormality may be associated with a clinical picture which has features of myeloma.

In addition, it should be emphasized that at the beginning of his illness, when his signs and symptoms resembled Waldenström's original description of macroglobulinemia, the patient's marrow contained plasma cells rather than lymphocytoid cells. These cells did not change their morphologic characteristics as the disease progressed to bone destruction suggestive of myeloma (Figs.2c,4b).

In the experience of others as well, light and electron microscopic studies of cell morphology and in vitro culture efforts have thus far not related specific cell types to the class of immunoglobulin being produced (36-40). Further evidence is provided by the recent report of a γ M-producing mouse plasma cell tumor (41). This tumor is morphologically indistinguishable from other mouse plasma cell tumors producing γ A and γ G (42). At the present time, cell morphology does not appear to distinguish myeloma from macroglobulinemia.

Therefore it may be appropriate to recognize that we cannot always distinguish between macroglobulinemia and myeloma, and to realize that the disease course, including presence or absence of bone destruction, does not necessarily correlate with either the type of protein being produced or the presence of a particular cell line. A more fundamental distinction between myeloma and macroglobulinemia may ultimately result from examinations of other properties of the cells which produce the immunoglobulins.

Summary

A fifty-three year old man developed the signs and symptoms of macroglobulinemia, had a one-year remission with cyclophosphamide, and died two and one-half years after disease onset with destructive bone lesions, peripheral blood plasmacytosis, and organ infiltration. From the onset of illness until the time of death, the abnormal serum protein was γ M with L chains of kappa type, as shown by analysis of the chromatographically purified protein by agar gel diffusion and immunoelectrophoresis with monospecific antisera.

A number of previous reports have suggested, contrary to the generally accepted rule, that bone destruction may occur in macroglobulinemia. These reports, however, leave some doubt that the abnormal protein was γ M. With the improved immunochemical methods now available we have identified as true γ M the abnormal protein in a patient with bone destruction. If more reports like the present one appear, it could be concluded that the presence of a particular class of globulin is not necessarily correlated with a given set of clinical features.

Acknowledgments

The authors thank Drs. Morgan Berthrong and Stanton Eversole, of the Department of Pathology, for interpretation of the histologic sections of the vertebrae. We are also grateful to Mr. James Foerster for technical assistance, to Mrs. Joan Pollock for typing the manuscript, and to Dr. A.R. Bigler for referring the patient to us.

- Figure 1: The ocular fundi, right (a) and left (b). Upper row, pre-treatment; lower row, remission.
- Figure 2: a. Pre-treatment bone marrow (x 1200).
b. Remission, with normal marrow elements (x 1200).
c. Bone marrow, after exacerbation (x 1200).
d. Peripheral blood plasma cells, with adjacent normal neutrophil (x 1800).
- Figure 3: Clinical course. Shaded area in upper graph indicates normal range. The lower graph refers to the daily cyclophosphamide dosage.
- Figure 4: a. Arrow indicates vertebral fracture which was not obvious on the roentgenogram to the left, taken three months earlier.
b. Autopsy histologic section from site of fracture (x 175).
- Figure 5: a. Autopsy liver section with periportal plasma cell infiltration (x 190).
b. Kidney section with plasma cell infiltration and casts (x 200).
- Figure 6: Serum protein analysis comparing the patient's serum in 1963, 1964, and 1966. From left to right are cellulose acetate electrophoresis, ultracentrifugation carried out in a double-sectored cell with the patient's serum (1:10) on the bottom and normal human serum (1:10) on the top in each case, and immunoelectrophoresis reacting the patient's serum against antiserum to human γ M (in the bottom well is placed normal human serum). The decrease in γ M during remission in 1964 is demonstrated by the respective methods.

Figure 7: Demonstration of γ M specificity of J63 and J66.

J63 = patient's 1963 isolated γ M protein, 2 mg/ml:

J66 = patient's 1966 isolated γ M protein, 2 mg/ml.

(a) γ G = γ G, 1.7 mg/ml; anti- γ G = specific rabbit anti-human γ G.

(b) γ A = γ A, 0.5 mg/ml; anti- γ A = specific goat anti-human γ A.

(c) γ M(Sc) = isolated γ M of another (non-macroglobulinemia) patient, 2 mg/ml; anti- γ M = specific goat anti-human γ M.

Demonstration of specificity of J63, J66, and the light chains prepared from these proteins.

(d,e) λ = λ light chains, 2 mg/ml; κ = κ light chains, 2 mg/ml; Fab = Fab fragment of pooled normal γ G, 2 mg/ml; J(L) = light chains prepared from J63 and J66, 2 mg/ml.

Demonstration of antigenic identity of J63 and J66 (g and h utilize in-well absorption):

(f,g,h) γ M(Sc) = isolated γ M of another (non-macroglobulinemia) patient, 2 mg/ml; γ M = pooled normal human γ M, 2 mg/ml; anti-J63 = rabbit antiserum to the patient's 1963 isolated γ M protein.

Figure 8: Acrylamide gel electrophoresis in 8M urea at alkaline pH.

Samples were made up in 0.1M dithioerythitol and run at 0.5 milliamp. per tube for 12 hours.

TABLE 1

SERUM IMMUNOGLOBULIN CONCENTRATION OF PATIENT

Date	Immunoglobulins (mg./100 ml.)		
	γ G	γ A	γ M
1963	188	39	10,040
1964	480	46	800
1966	92	26	8,680
* Normal range (at <u>+2</u> S.D.)	711-1536	50-415	37-212

* Measured quantitatively by single radial immunodiffusion technique by Dr. Mathea Allansmith.

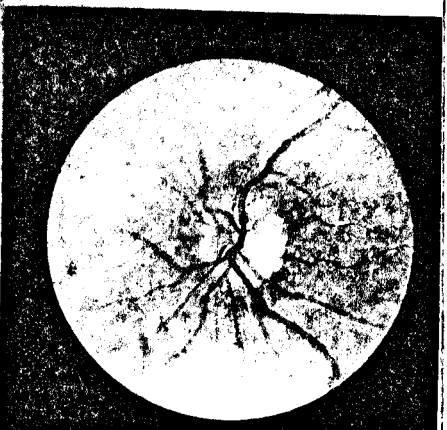
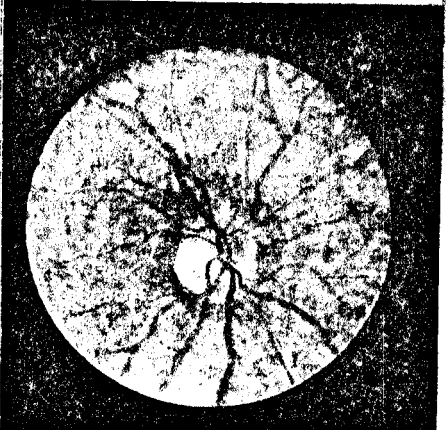
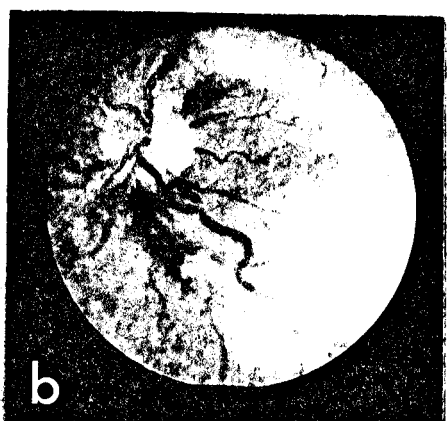
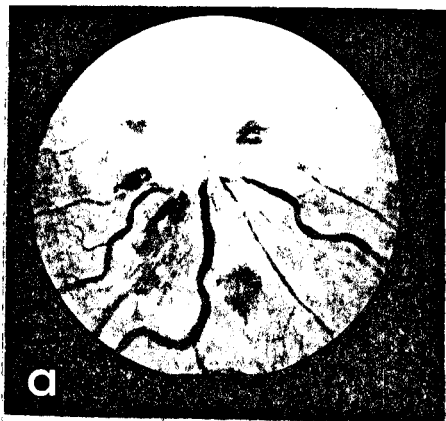
REFERENCES

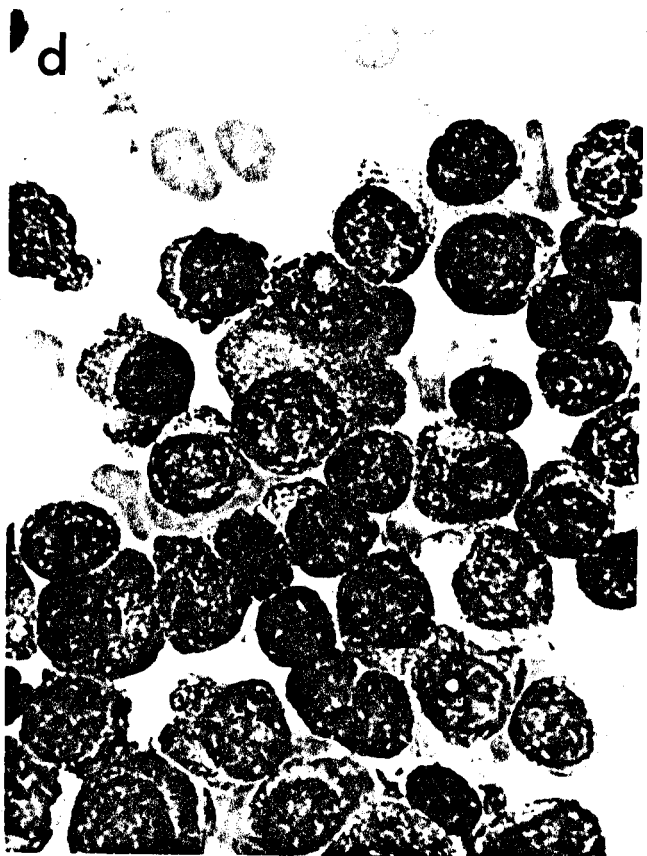
1. Waldenström, J. Incipient myelomatosis or "essential" hyperglobulinemia with fibrinogenopenia - a new syndrome? *Acta med. scand.*, 117:216, 1944.
2. Waldenström, J. Macroglobulinemia. In: *Immunological Diseases*, p. 364. Edited by Samter, M. Boston, 1965. Little, Brown and Co.
3. Rosen, F. S. The macroglobulins. *New Eng. J. Med.*, 267:491, 1962.
4. Jim, R. T. S. and Steinkamp, R. C. Macroglobulinemia and its relationship to other paraproteins. *J. Lab. Clin. Med.*, 47:540, 1956.
5. Imhof, J. W., Baars, H. and Verloop, M. C. Clinical and haematological aspects of macroglobulinemia Waldenström. *Acta med. scand.*, 163:349, 1959.
6. Kunkel, H. G. Macroglobulins and high molecular weight antibodies. In: *The Plasma Proteins*, vol. 1, p. 279. Edited by Putnam, F. W. New York, 1960. Academic Press.
7. Waldenström, J. Hypergammaglobulinemia as a clinical hematological problem: a study in the gammopathies. In: *Progress in Hematology*, vol. III, p. 266. Edited by Tocantins, L. M. New York, 1962. Grune & Stratton.
8. Waldenström, J. Macroglobulinemia. In: *Advances in metabolic disorders*, vol. 2, p. 115. Edited by Levine, R. and Luft, R. New York, 1965. Academic Press.
9. Snapper, I., Turner, L. B. and Moscovitz, H. L. Multiple myeloma. New York, 1953. Grune and Stratton.
10. Osserman, E. F. Multiple myeloma. In: *Immunological Diseases*, p. 353. Edited by Samter, M. Boston, 1965. Little, Brown & Co.
11. Borchgrevink, C. F. and Waaler, B. A. The secondary bleeding time. A new method for the differentiation of hemorrhagic diseases. *Acta med. scand.*, 162:361, 1958.
12. Ceppellini, R., Dray, S. and Edelman, G., WHO-meeting on nomenclature of human immunoglobulins: Nomenclature for human immunoglobulins. *Bull. World Health Organ.*, 30:447, 1964.
13. Bachmann, R. Studies on serum immunoglobulins. *Acta univ. lund.* II. No. 10, 1966.

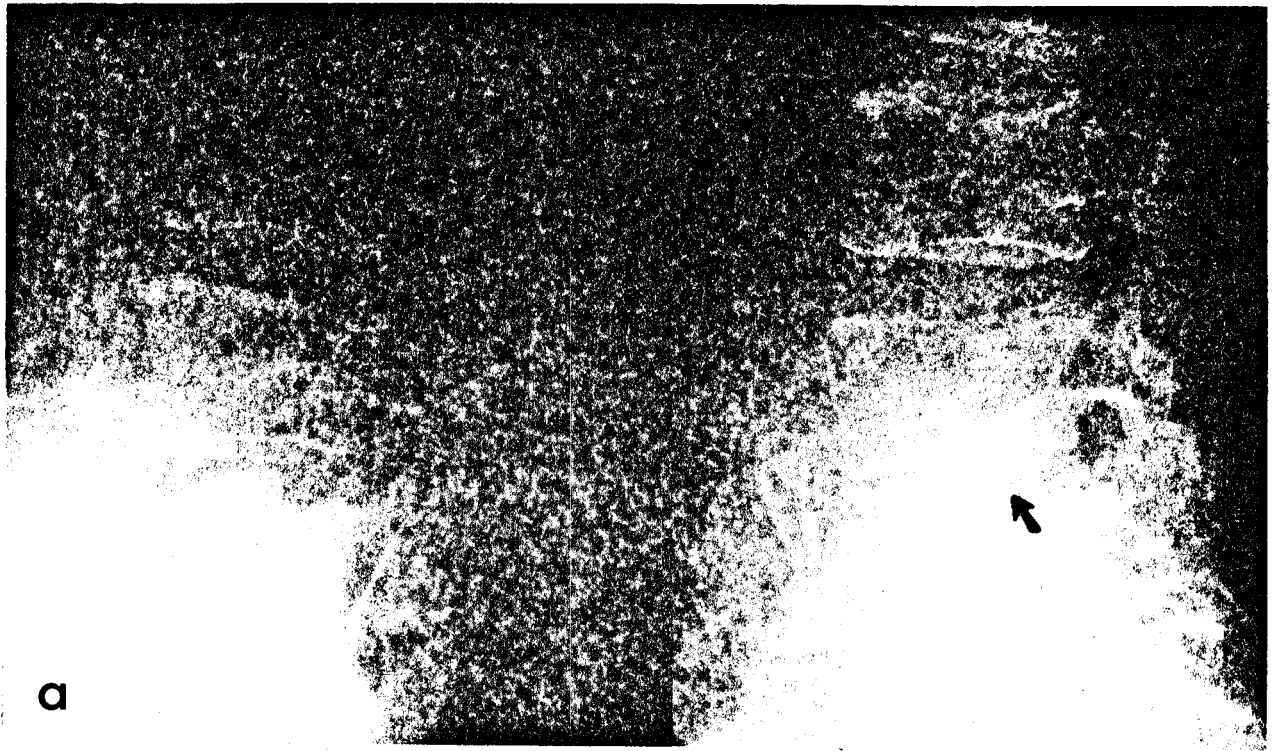
14. Scheidegger, J. J. Une micro-méthode de l'immuno-électrophorèse. *Int. Arch. Allergy*, 7:103, 1955.
15. Fahey, J. L. and McKelvey, E. M. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.*, 94:84, 1965.
16. Fleischman, J. B., Pain, R. H. and Porter, R. R. Reduction of γ -globulins. *Arch. Biochem. Biophys. Suppl. No. 1*, 174, 1962.
17. Reisfeld, R. A. and Small, P. A., Jr. Electrophoretic heterogeneity of polypeptide chains of specific antibodies. *Science*, 152:1253, 1966.
18. Cohen, S. and Porter, R. R. Heterogeneity of the peptide chains of γ -globulin. *Biochem. J.*, 90:278, 1964.
19. Terry, W. D., Small, P. A., Jr. and Reisfeld, R. A. Electrophoretic heterogeneity of the polypeptide chains of human G-myeloma proteins. *Science*, 152:1628, 1966.
20. Wunderly, C. and Piller, S. Die Färbung der im Blutserum enthaltenen Proteine, Lipide und Kohlenhydrate nach Papier-elektrophorese. *Klin. Wschr.* 32:425, 1954.
21. Lennert, K. Die pathologische Anatomie der Makroglubulinämie Waldenström. *Frankfurt. Z. Path.*, 66:201, 1955.
22. Droste, R. Histiomonozytäre Retikulose mit Makroglubulinämie und Knochendestruktion. *Medizinische*, 792, 1958.
23. Levin, W. C., Ritzmann, S. E., Seeuwen, J. P. and Nanninga, L. Some properties of B_{2A}-myeloma proteins. *Clin. Chim. Acta*, 10:12, 1964.
24. Vaerman, J. P., Johnson, L. B., Mandy, W. and Fudenberg, H. H. Multiple myeloma with two paraprotein peaks: an instructive case. *J. Lab. Clin. Med.*, 65:18, 1965.
25. Smith, E., Kochwa, S. and Wasserman, L. Aggregation of IgG in vivo. I. The hyperviscosity syndrome in multiple myeloma. *Amer. J. Med.*, 39:35, 1965.
26. Wanner, J. and Siebenmann, R. Über eine subakut verlaufende osteolytische Form der Makroglubulinämie Waldenström mit Plasmazellenleukämie *Schweiz. med. Wschr.*, 87:1243, 1957.
27. Adner, P. L., Wallenius, G. and Werner, I. Macroglobulinemia and myelomatosis. *Acta med. scand.*, 168:431, 1960.
28. Waldenström, J. Clinical diagnosis and biochemical findings in material of 296 sera with M-type, narrow, γ globulins. *Acta med. scand. Suppl.* 367, 110, 1961.

29. Harboe, M., Osterland, C. K., Mannik, M. and Kunkel, H. G. Genetic characters of human γ -globulins in myeloma proteins. *J. Exp. Med.*, 116:719, 1962.
30. Kjeldsen, K. and Asfeldt, V. H. Macroglobulinemia or multiple myeloma. *Acta med. scand.*, 174:407, 1963.
31. Bachmann, R. Simultaneous occurrence of two immunologically different M-components in serum. *Acta med. scand.*, 177:593, 1965.
32. Hällén, J. Frequency of "abnormal" serum globulins (M-components) in the aged. *Acta med. scand.*, 173:737, 1963.
33. Osserman, E. F. and Takatsuki, K. Plasma cell myeloma: gamma globulin synthesis and structure. A review of biochemical and clinical data, with the description of a newly recognized and related syndrome, "H γ -2-chain (Franklin's) disease". *Medicine*, 42:357, 1963.
34. Waldenström, J. The occurrence of benign, essential monoclonal (M type), non-macromolecular hyperglobulinemia and its differential diagnosis. IV. studies in the gammopathies. *Acta med. scand.*, 176:345, 1964.
35. Stevens, A. R. Evolution of multiple myeloma. *Arch. Int. Med.*, 115: 90, 1965.
36. Zucker-Franklin, D., Franklin, E. C. and Cooper, N. S. Production of macroglobulins in vitro and a study of their cellular origin. *Blood*, 20:56, 1962.
37. Solomon, A., Fahey, J. L. and Malmgren, R. A. Immunohistologic localization of gamma-1-macroglobulins, beta-2A-myeloma proteins, 6.6S gamma-myeloma proteins and Bence Jones proteins. *Blood*, 21: 403, 1963.
38. Brecher, G., Tanaka, Y., Malmgren, R. A. and Fahey, J. L. Morphology and protein synthesis in multiple myeloma and macroglobulinemia. *Ann. N.Y. Acad. Sci.*, 113:642, 1964.
39. Maldonado, J. E., Kyle, R. E., Brown, A. L., Jr. and Bayrd, E. D. "Intermediate" cell types and mixed cell proliferation in multiple myeloma: electron microscopic observations. *Blood*, 27:212, 1966.
40. van Furth, R., Schuit, H. R. E. and Hijmans, W. The formation in vitro of paraproteins in multiple myeloma and Waldenström's macroglobulinemia. *Brit. J. Haemat.*, 12:202, 1966.

41. McIntire, K. R., Asofsky, R. M., Potter, M. and Kuff, E. L.
Macroglobulin-producing plasma-cell tumor in mice: identification of
a new light chain. *Science*, 150:361, 1965.
42. Warner, N. L., Riblet, R. and Herzenberg, L. A. (unpublished).



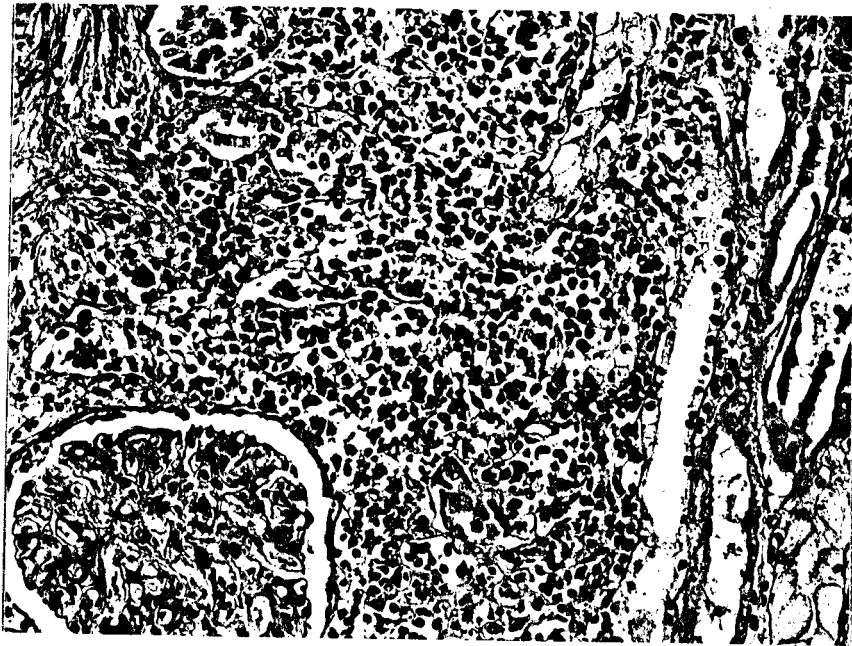
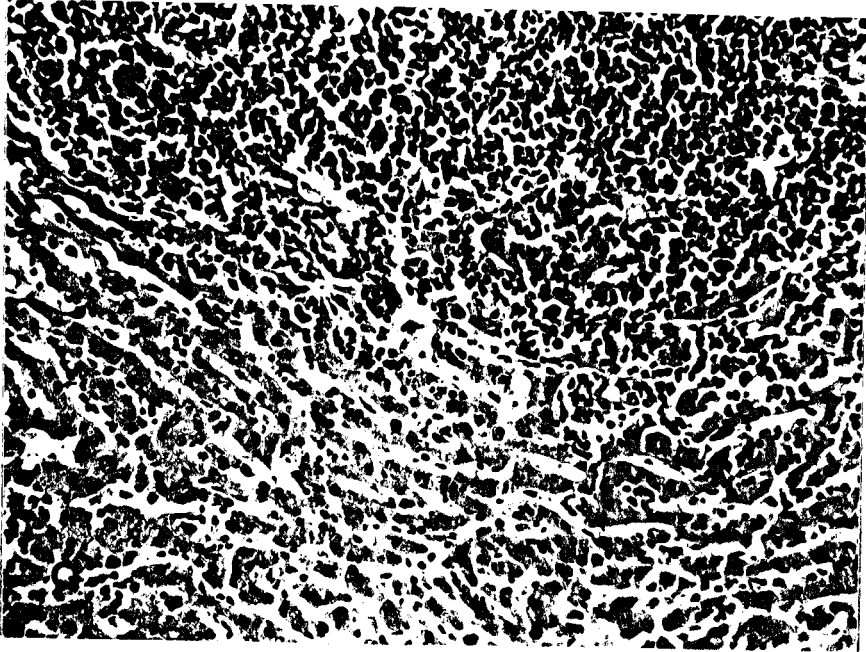




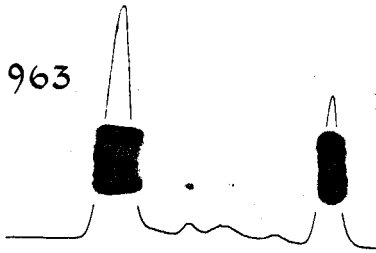
a



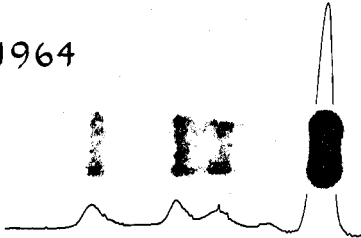
b



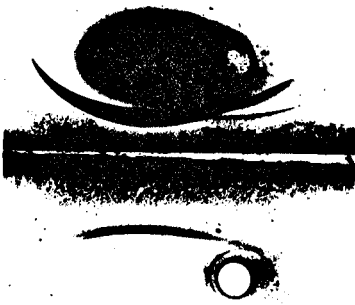
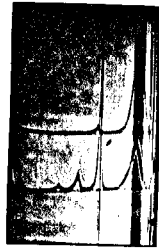
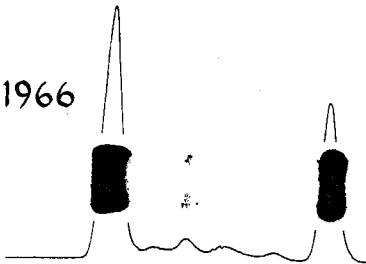
1963

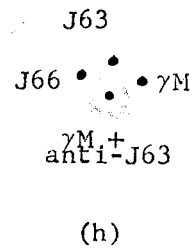
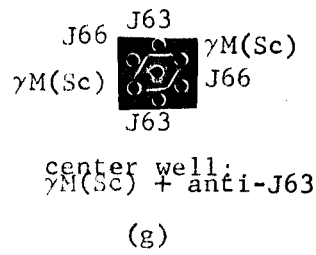
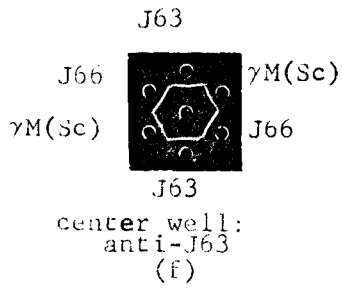
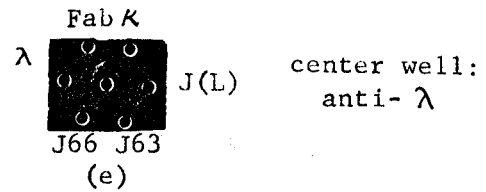
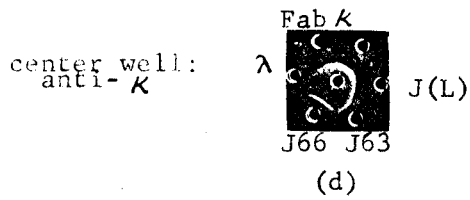
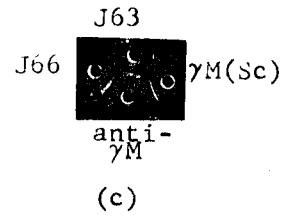
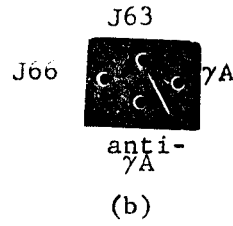
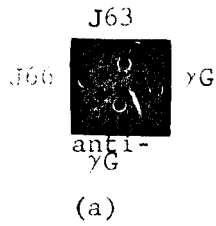


1964



1966





10
1963

1966

