Four cases of Waldenström's macroglobulinaemia

D'A. KOK, D. N. WHITMORE,¹ AND R. W. AINSWORTH

From the Departments of Medicine and of Pathology, University of Cambridge

SYNOPSIS The clinical course and pathological features of four cases of Waldenström's macroglobulinaemia are described. One was associated with a localized carcinoma of the bronchus and one with a chromophobe adenoma of the pituitary.

Trypsin digest preparations of the retina were examined in two cases, one with severe retinopathy and the other with no clinical evidence of ocular disease, and the findings are briefly described.

The aetiology of the anaemia is discussed. Haemolysis due to circulating autoantibodies was not demonstrated in any of the cases. Erythrophagocytosis was a prominent feature in case 4, of moderate degree in cases 1 and 2, and inconspicuous in case 3. It was not proportionate to the serum macroglobulin levels.

The cytological and histological features in all four cases are distinctive, and their value in differential diagnosis is stressed.

Waldenström's macroglobulinaemia, known also as primary or essential macroglobulinaemia, was first described in 1944. A great deal has since been written about the disorder, which has been reviewed recently by Kappeler, Krebs, and Riva (1958), by Zollinger (1958), by Mackay (1959), by Martin (1960), by Ritzmann, Thurm, Truax, and Levin (1960), and by Logothetis, Silverstein, and Coe (1960). Opinion is still divided as to whether the condition is a disease entity or a syndrome.

Of the 20 cases which have been reported in this country, and in which the diagnosis has been supported by ultracentrifugal studies, only four have been examined after death (McFarlane, Dovey, Slack, and Papastamatis, 1952; Martin, 1960). A detailed description of four cases which subsequently came to necropsy and have not previously been reported seems to be justified.

CASE 1

A 65-year-old man suffering from chronic bronchitis developed an endogenous depression in 1954. In May 1959 he developed symptoms of anaemia and was admitted to Addenbrooke's Hospital in July 1959 in congestive cardiac failure. A generalized superficial lymphadenopathy was found, the largest nodes measuring 4 × 3 cm. The liver was enlarged but the spleen was impalpable. Investigation revealed a normochronic

anaemia: haemoglobin 4.6 g%; reticulocytes 1.2%; Coombs test negative; serum bilirubin 0.6 mg.%; leucocytes 2,800 per c. mm. (42% lymphocytes); platelets 120,000 per c. mm.; E.S.R. 33 mm./hour (Wintrobe uncorrected); liver function tests normal; serum tests for syphilis negative. The urine contained no Bence-Jones protein. A chest radiograph showed hilar glandular enlargement.

Digitalis, mersalyl, and transfusion of 6 pints of blood relieved the cardiac failure within a week of admission.

Subsequently a chest radiograph showed a mass in the lower part of the right hilum consistent with a bronchial neoplasm. Radiographs of the skeleton showed osteoporosis only.

LYMPH NODE BIOPSIES Sections of lymph nodes obtained from the right groin and left axilla showed no evidence of carcinomatous involvement. The lymphoid follicles were obscured but reticulin preparations showed the architecture of the nodes to be preserved (Fig. 1). A cellular infiltrate was present, particularly in the pulp and in the pericapsular tissues (Fig. 2). It consisted of lymphocytes, plasma cells, cells intermediate in morphology between plasma cells and lymphocytes, reticulum cells, and occasional tissue mast cells, seen best in Romanowsky-stained imprint preparations. When stained by the Unna-Pappenheim method, the plasma cells were typically pyroninophilic, while the majority of the intermediate or 'lymphoid-plasma' cells were pyronin-negative. The sinuses of both nodes were haemorrhagic, and contained reticulum cells showing erythrophagocytosis. Haemosiderin granules were present in the inguinal node but not in the axillary node. The most striking feature of lymph node imprints subjected to the periodic acid-Schiff reaction was the intense background staining associated with a negative reaction in the majority of the lymphoid

¹Present address: Department of Clinical Pathology, Guy's Hospital, London, S.E.1.

FIG. 1. *Reticulin pattern of an inguinal lymph node. Silver impregnation (Gordon and Sweet) $\times 100$.*

FIG. 2. *Pulp and an adjoining sinus of an inguinal lymph node, showing diffuse pleomorphic infiltration. Haematoxylin and eosin $\times 100$.*

FIG. 3. Imprint preparation from an axillary lymph node showing the deeply stained background and only one positive lymphoid cell. Periodic acid-Schiff. $\times 1,000$.

FIG. 4. Marrow smear, showing the edge of a clump of pleomorphic cells. Scattered haemosiderin masses can be seen. M.G.G. $\times 400$.

FIG. 5. Marrow smear showing a plasma cell, a 'lymphoid-plasma' cell and five 'naked nuclei'. M.G.G. $\times 1,000$.

FIG. 6. Marrow smear showing a tissue mast cell and two lymphocytes. Leishman. $\times 1,000$.

cells. Less than 0.1% of the latter were positive, and these cells showed a rating of 1 only (Quaglino and Hayhoe, 1959, Fig. 3). The intranuclear P.A.S.-positive material described by Dutcher and Fahey (1959) was not seen.

MARROW Sternal puncture produced a cellular marrow in which mitotic activity was not a prominent feature. Erythroid and myeloid elements were much reduced in number, but showed normal development. They were replaced by a pleomorphic cellular infiltrate (Figs. 4-6). The majority of the cells resembled mature lymphocytes, although some cells with pyknotic nuclei, almost devoid of cytoplasm, were also present. Moderate numbers of 'lymphoid-plasma' cells were also seen. They appeared to adhere to one another to a greater extent than did the other elements of the infiltrate. They were roughly twice the size of the mature lymphocytes; their nuclei were larger and their cytoplasm more abundant than those of typical plasma cells. They contained a slightly ovoid, eccentric nucleus, in which nucleoli were not visible; the chromatin was usually clumped. In Romanowsky preparations, the cytoplasm was stained a light blue, quite distinct from the deep blue of a typical plasma cell. The rest of the infiltrate consisted of small numbers of reticulum cells, typical plasma cells, and tissue mast cells. Many of the reticulum cells contained large amounts of haemosiderin. The P.A.S. reaction of the infiltrate was similar to that found in lymph node imprints.

SERUM PROTEINS Total serum proteins were 10.4 g.%. Paper electrophoresis (Flynn and de Mayo, 1951) showed a reduction in albumin and a marked increase of the γ globulin. The latter formed a dense, narrow band, which remained near the point of application of the serum. It stained intensely by the P.A.S. reaction (Björnesjö, 1955) and it failed to migrate after electrophoresis in a starch gel (Silberman, 1957). Cryoglobulins were not found in the serum. The Sia test was positive (Sia, 1924; Martin, 1960).

Ultracentrifugal analysis by Dr. P. Johnson² showed three main high molecular weight constituents, with sedimentation constants of 17.0 S, 19.2 S, and 28.9 S. in the proportions of 5:85:10% respectively. Together they accounted for 32% of the total serum proteins. Detailed physico-chemical and immunological studies are reported elsewhere (Albert and Johnson, 1961).

CLINICAL COURSE Blood transfusions had to be given at frequent intervals throughout the patient's illness. No evidence of blood loss was found and the Coombs test remained negative. Prednisone had no effect on the anaemia or macroglobulin level. He died in November 1959 of an acute chest infection.

NECROPSY

A generalized firm enlargement of the lymph nodes was present. The largest nodes were in the inguinal

and in the tracheo-bronchial groups ($4 \times 3 \times 2$ cm.). In the remaining sites the nodes were of moderate size. Their cut surfaces were pale brown and faintly striated, and some anthracotic mottling was present in the tracheo-bronchial nodes. The lymphadenoid tissue of the pyriform fossae and the posterior third of the tongue was increased, but the tonsils, Peyer's patches, and the solitary lymph follicles of the large intestine were normal.

Marrow, which was brownish and rather gelatinous, was present in the sternum, in a wedge of the vertebral column, and extended throughout the medulla of the right femur.

The spleen was enlarged and weighed 650 g. It was firm and the cut surface was dark red.

The liver weighed 2,250 g., and appeared normal.

The lungs were congested and oedematous. An abscess cavity, measuring $4 \times 3 \times 2$ cm., was present in the apex of the right lower lobe. All the basal segments were consolidated.

The heart weighed 395 g. The myocardium was normal apart from small patches of fibrosis. Gross atheroma, without occlusion, was present in both coronary vessels.

The prostate showed benign nodular hyperplasia.

The remaining organs, including the brain and kidneys, showed no macroscopic abnormality.

MICROSCOPIC FINDINGS

RETICULO-ENDOTHELIAL SYSTEM No evidence of secondary carcinomatous deposits could be found in any of the lymph nodes which were examined. The microscopic findings were characterized by the presence of a cellular infiltrate similar to that found in the lymph node biopsies and in the marrow aspirates which had been examined during life and which have already been described. The lymph nodes, the marrow (Fig. 7) and, to a lesser extent, the splenic pulp, were involved diffusely. Small focal collections of lymphocytes and plasma cells were present in the liver, the pericardium, the suprarenals, and the kidneys. Erythrophagocytosis was present in the lymph nodes and in the marrow, but was inconspicuous in the spleen. Reticulin preparations showed the architecture of the lymph nodes to be largely preserved. Reticulin formation in the marrow was normal. Sections stained by Perls's method showed large amounts of haemosiderin, chiefly within macrophages, in the marrow (Fig. 8), the spleen, and some of the lymph nodes. In the liver, finer granules were present within the majority of the hepatic cells but in very few of the Kupffer cells.

LUNGS All the sections showed severe congestion and oedema. The walls of the abscess cavity in the

²Serum diluted 1:10 in phosphate-NaCl buffer, pH 7.8, ionic strength 0.1, and centrifuged at 59,780 r.p.m. in a Spinco model E ultracentrifuge at 20°C.

FIG. 7

FIG. 7. Marrow section stained with methylene blue, toluidine blue, erythrosine, and orange G (Barrett, 1944). $\times 100$.

FIG. 8

FIG. 8. Marrow section. Perl's stain $\times 100$.

lung contained a poorly differentiated squamous carcinoma.

EYE Dr. A. M. Barrett reported that 'foci of lymphocytic infiltration were present in the choroid and to a lesser extent in the ciliary body. Focal haemorrhages were found in the choroid. Several colloid bodies, which were heavily stained by the P.A.S. method, were present on the membrane of Bruch. Many small retinal blood vessels contained plugs of a coagulum which was also strongly P.A.S. positive. Patches of a P.A.S.-positive coagulum, which contained pigment like that of the pigment layer of the retina, were situated between the latter and the rest of the retina. There was much vacuolation of the retina, especially of the ganglion cell layer. The innermost fibre layer of the retina and the internal limiting membrane stained more strongly than usual with eosin, and were moderately P.A.S. positive. The eosinophilic deposit noted in the outer plexiform layer by Cagiant and Theiler (1959) was not present.'

BRAIN Sections of the cortex showed multifocal perivascular accumulations of an amorphous P.A.S.-positive material, not associated with cellular infil-

tration. No other significant abnormalities were found.

REMAINING TISSUES (INCLUDING KIDNEYS) No significant abnormality was seen.

Death was due to bronchopneumonia, and associated conditions were lung abscess, carcinoma of the bronchus, and reticulosis with macroglobulinaemia.

CASE 2

A 73-year-old woman was seen in December 1960 complaining of recurrent nose bleeds for two and a half years, dimness of vision for six months, and undue fatigue for two and a half months. Physical examination revealed pallor, a few scattered petechiae, and bilateral retinopathy. Blood pressure was 130/80 mm. Hg. No enlarged lymph nodes were felt; the liver and spleen were just palpable. Investigations showed a hypochromic anaemia: haemoglobin 5.8 g.%; M.C.H.C. 26.3%; reticulocytes 1.9%; Coombs test negative; leucocytes 4,500 per c.mm. (25% lymphocytes). Marrow specimens obtained from the sternum and vertebral spine were of poor cellularity, consisting mainly of lymphocytes. The erythroid and myeloid cells which were present showed normal maturation. A number of reticulum and a few plasma cells were present. The urine contained no protein. Total serum

proteins were 10 g. %, and paper electrophoresis showed a dense narrow band in the γ globulin region and reduced albumin.

She continued to deteriorate despite iron by mouth and a blood transfusion and was admitted to Addenbrooke's Hospital on 16 May 1961 in mild congestive failure. The peripheral blood picture was relatively unchanged. The platelet count was 100,000 per c. mm. Clotting time was normal, but clot retraction was grossly abnormal. The E.S.R. was 16 mm./hr. (Wintrobe uncorrected) and 164 mm./hr. (Westergren). The blood urea was 50 mg. %. Radiographs of the skeleton showed slight osteoporosis only. The serum proteins had risen to 11.5 g.%; the electrophoretic pattern was unchanged. Cryoglobulins were absent, the Sia test positive. The viscosity of the serum relative to water at 25°C. was 9.0 (normal range 1.5 to 1.8). Ultracentrifugal examination of the serum (Dr. P. Johnson) showed three constituents with sedimentation constants of approximately 19S, 26S, and 33S comprising 51%, 6.5%, and 1% respectively of the total serum proteins.

CLINICAL COURSE The patient was digitalized and given a further blood transfusion. A course of D-pencillamine had to be abandoned after the development of a sensitivity reaction and was replaced by treatment with prednisone, tetracycline, and chlorambucil. Five days later she suddenly developed a high fever and became deeply comatose, with brisk jerks. This was rapidly followed by peripheral circulatory failure and death in a few hours.

NECROPSY

The para-aortic lymph nodes were slightly enlarged ($2 \times 1 \times 1$ cm.), and showed variegated dark red and grey cut surfaces. The tracheobronchial nodes were slightly enlarged and anthracotic. Nodes at other sites were not enlarged and their cut surfaces were pinkish-grey.

Red marrow was present in the sternum and in a wedge of the vertebral column, and extended throughout the right femur except for a narrow zone of fatty marrow at the lower end.

The spleen was enlarged and firm, and weighed 482 g. The cut surface was mottled by dark red and reddish-grey areas.

The liver weighed 1,565 g., and appeared normal.

The lungs were congested and oedematous. Patchy consolidation of the left lower lobe was present.

The heart weighed 392 g. The myocardium appeared to be normal and the coronary vessels showed very little atheroma.

The anterior lobe of the pituitary gland was compressed on its left antero-lateral aspect by a mass of friable grey tissue, measuring $0.8 \times 0.5 \times 0.3$ cm., and extending through the capsule of the gland.

The thyroid gland was enlarged and nodular and weighed 40 g.

The remaining organs, including the brain, showed no significant abnormality.

MICROSCOPIC FINDINGS

RETICULO-ENDOTHELIAL SYSTEM Imprints obtained from the marrow and lymph nodes showed the presence of a highly cellular pleomorphic infiltrate, similar to that found in case 1, except that fewer plasma cells were seen in the lymph nodes. Sections showed the marrow and lymph nodes from all the main groups to be involved diffusely. The infiltrate was present to a lesser extent in the spleen, which showed evidence of extramedullary haemopoiesis as well. Follicles were not seen in the lymph nodes, but reticulin preparations showed the architecture of the nodes to be largely preserved. Reticulin formation in the marrow was not excessive. The sinuses of many lymph nodes were haemorrhagic. Erythrophagocytosis was active in these nodes and in the marrow but was much less marked in the spleen. Considerable quantities of haemosiderin were present in the spleen marrow, liver, and lymph nodes.

LUNGS Oedema and bronchopneumonic changes were present in both lower lobes.

BRAIN Small focal perivascular accumulations of a strongly eosinophilic, P.A.S.-positive coagulum similar to that within the blood vessels, were present in the cerebrum and to a lesser extent in the basal ganglia. Very few lesions were found in the cerebellum, pons, or spinal cord. Occasional perivascular foci of small round cell accumulations were seen. There was no free fat and no increase in glial cells or fibres.

ENDOCRINE ORGANS The nodule in the pituitary gland was a chromophobe adenoma.

The changes seen in a nodular colloid goitre were present in the thyroid gland.

EYE The globe was opened coronally to reveal greatly engorged veins and numerous haemorrhages in the peripheral retina with a few punctate haemorrhages at the macula. There was no evidence of an actual thrombosis within the vessels. The retina was removed from the eye and examined flat, where the haemorrhages were seen to be globular. A trypsin digest preparation of the retinal vessels showed on the peripheral capillaries innumerable microaneurysms, which gradually disappeared towards the disc so that only a few could be found at the posterior pole. Capillary nuclei were normal posteriorly, more

numerous equatorially and totally absent peripherally. Flat sections of the retina (stained with P.A.S. and haematoxylin) confirmed the above observations and clearly showed the microaneurysms as sacculations in the capillary basement membrane, which was sometimes greatly thinned and at other times greatly thickened. Periodic acid-Schiff-positive material could be seen in the lumens of many of the vessels due to the presence of the abnormal globulin, and numerous haemorrhages were present in the surrounding retina'. (Professor N. Ashton)³

REMAINING TISSUES (INCLUDING KIDNEYS) No significant abnormality was seen.

Death was due to bronchopneumonia, and associated conditions were reticulosis with macroglobulinaemia, chromophobe adenoma of the pituitary, and nodular colloid goitre.

CASE 3

A 71 year-old man was admitted to Lewisham Hospital in January 1959 in congestive cardiac failure. He had noticed swelling of the ankles for two years and dyspnoea for one year. His blood pressure was 140/90 mm. Hg. The liver and spleen were felt 8 and 4 cm. respectively below the costal margin. Small lymph nodes were present in the neck and large rubbery nodes in both axillae and groins.

He had a hypochromic anaemia; haemoglobin 5.6 g%; M.C.H.C. 26.8%; reticulocytes 3%; Coombs test was not done; the faeces contained no occult blood; leucocytes 4,100 per c.mm. (32% lymphocytes); platelets 196,000 per c. mm.; E.S.R. 138 mm./hour (Westergren). Sternal puncture produced a moderately cellular marrow specimen, showing normal erythroid, myeloid, and megakaryocytic development, excessive red cell aggregation and increased background staining. A pleomorphic infiltrate was present in clumps and as free cells, consisting of large numbers of lymphocytes and smaller numbers of lymphoid plasma cells, tissue mast cells, and non-phagocytic reticulum cells. The lymphocytes amounted to approximately 20% of the nucleated cells in the smear. Some of the lymphocytes were undergoing mitosis and occasional plasma cells were binucleated. Very few 'naked nuclei' were seen, although cytoplasmic fragments, resembling the cytoplasm of the lymphoid-plasma cells, were present. Large amounts of free iron were seen.

Biopsy of a lymph node showed an infiltrate to be present throughout the pulp, obliterating the lymphoid follicles. The infiltrate consisted predominantly of cells resembling medium-sized lymphocytes, together with small numbers of plasma cells, cells intermediate between lymphocytes and plasma cells, and reticulum cells. Erythrophagocytosis in the sinuses was not as prominent a feature as in the first case. A reticulin preparation showed the architecture of the node to be largely preserved.

³A detailed account of these pathological findings will be published in the *Journal of Pathology and Bacteriology* (in press).

Total serum proteins were 8.7 g.%. Paper electrophoresis showed a considerable reduction in albumin associated with a marked increase of γ globulin. A dense narrow band was present in the β - γ region. Cryoglobulins were not present. The Sia test was positive. Viscosity of the serum relative to water at 25°C. was 2.36 (normal range 1.5-1.8). Ultracentrifugal examination of the serum (Dr. P. Johnson) showed one constituent with a sedimentation constant of approximately 18S. It comprised 14% of the total serum proteins. The γ globulin level was increased and amounted to 37% of the total serum proteins. The urine was normal. The blood urea was 40 mg.%. A chest radiograph showed changes compatible with the presence of emphysema but was otherwise normal.

CLINICAL COURSE Blood transfusion, chlorambucil (12 mg./day), and prednisolone (30 mg./day) resulted in subjective improvement and a considerable reduction in the size of the lymph nodes. The haemoglobin level rose, but was associated with progressive leucopenia and thrombocytopenia.

The patient stopped taking his drugs in September 1959 after developing a right-sided hemiplegia, and he was then treated with steroids only. The haemoglobin level remained between 9 and 10 g.% for the next 10 months, but the lymphadenopathy recurred and became generalized. The haemoglobin subsequently fell despite prednisolone therapy and repeated transfusions; he became leucopenic and thrombocytopenic once again, and developed purpura on the arms and thorax. In July 1961 the patient became unconscious after a transfusion, developed bronchopneumonia and died.

NECROPSY

The body was emaciated; petechiae and ecchymoses were present on the chest and arms.

A generalized firm enlargement of the lymph nodes was present. The para-aortic and left inguinal groups were the largest, and measured $5 \times 3 \times 3$ cm. Their cut surfaces were pinkish-grey.

Red marrow was present in the sternum and in a wedge of the vertebral column. The spleen was slightly enlarged, firm, and weighed 270 g. The liver weighed 1,900 g. and appeared normal.

The right pleural cavity contained 200 ml. of bloodstained fluid; the lungs were oedematous and the right lower lobe showed bronchopneumonic consolidation.

The brain weighed 1,537 g. Several areas of softening were present in the basal nuclei; the largest area affected the left thalamus and extended into the left internal capsule.

The remaining organs showed no significant abnormality.

MICROSCOPIC FINDINGS

RETICULO-ENDOTHELIAL SYSTEM Sections of the marrow, lymph nodes, and spleen showed the

presence of a pleomorphic cellular infiltrate similar to that seen in the lymph node biopsy. The marrow and the lymph nodes were involved diffusely, while small foci were present in the portal tracts of the liver, in the epicardial fat, and in the capsule of the pituitary. Lymphoid follicles were obscured by the infiltrate, but reticulin preparations showed the architecture of the lymph nodes to be well preserved. Reticulin formation in the marrow was normal. Small foci of extramedullary haemopoiesis were observed in the spleen. Large amounts of haemosiderin were present, particularly in the lymph nodes. Erythrophagocytosis was inconspicuous.

LUNGS Bronchopneumonic changes were present in the right lower lobe. Many of the remaining alveoli contained an eosinophilic coagulum.

BRAIN Sections taken from the left thalamic area showed the appearance of an old infarct. No significant changes were seen elsewhere.

KIDNEYS Small areas of ischaemic fibrosis were present, but no other significant abnormalities were seen.

Death was due to bronchopneumonia, and associated conditions were reticulosis with an excess of macroglobulin and of 7S globulin in the blood, and left cerebral infarction.

CASE 4

An 81-year-old woman, known to have an oesophageal hiatus hernia, suddenly developed complete loss of the sense of taste associated with incomplete loss of smell in 1958, and lost 28 lb. in weight during the next two years. Her haemoglobin level also started to fall progressively, despite intensive treatment with a variety of haematinics. She noticed paraesthesiae in both lower limbs early in 1960. During the winter of 1960-61 she developed congestive cardiac failure which responded poorly to treatment with digitalis.

She was admitted to Addenbrooke's Hospital in May 1961 with evidence of bilateral bronchopneumonia and congestive cardiac failure. The blood pressure was 145/60 mm. Hg. The fundi showed a mild degree of arteriosclerosis only. Tiny superficial lymph nodes were palpable. A blood count showed a hypochromic anaemia: haemoglobin 7.8 g.%; M.C.H.C. 26%; reticulocytes 1.4%; Coombs test negative; serum bilirubin 0.2 mg.%; occult blood present in the stools; leucocytes 7,600 per c. mm. (23% lymphocytes); platelets 370,000 per c. mm.; E.S.R. 60 mm./hour (Wintrobe). Sternal puncture produced a moderately cellular specimen with a M/E ratio of 8:1. Granulopoiesis and erythropoiesis were essentially normal and development of megakaryocytes was normal. Lymphocytes were present in fairly large numbers; smaller numbers of lymphoid-plasma cells, plasma cells, and

reticulum cells were present with occasional tissue mast cells.

Total serum proteins were 8.0 g.%; paper electrophoresis showed a reduction in albumin and a dense narrow band in the γ globulin region. Cryoglobulins were not present, and the Sia test was positive. Viscosity of the serum relative to water at 25°C was 3.4 (normal range 1.5-1.8). Ultracentrifugal examination of the serum (Dr. P. Johnson) showed two main high-molecular weight components with sedimentation constants of approximately 19S and 28S, comprising 25% and 2% respectively of the total serum proteins.

Skeletal radiographs showed generalized osteoporosis.

CLINICAL COURSE The patient was treated with tetracycline, digitalis, antidiuretics, blood transfusions, and chlorambucil. Her pneumonia resolved and the cardiac failure was relieved. The serum macroglobulin level fell progressively during the next five months while the haemoglobin rose to 10.8 g.% by December 1961. This was associated with considerable subjective improvement, including relief of the paraesthesiae. She continued to have bouts of oesophagitis which were usually followed by fluctuations in the haemoglobin level.

In April 1962 she contracted an intercurrent gastroenteritis which was followed by pneumonia and death within six days of the onset of the illness.

NECROPSY

Lymph nodes in the para-aortic and tracheo-bronchial regions were moderately enlarged, discrete, and firm. The former group measured up to $4 \times 1.5 \times 1$ cm.; the latter group were smaller. Nodes at other sites were only very slightly enlarged. The cut surfaces were mottled and greyish, except for those of the tracheobronchial group, which were congested and anthracotic.

Brownish-red marrow was present in the sternum, in a wedge of the vertebral column, and throughout the right femur.

The spleen was firm and weighed 105 g. Its capsule showed patchy fibrous thickenings, and its cut surface was uniformly red.

The liver weighed 1,480 g., and appeared normal.

An oesophageal hiatus hernia was present, but there was no evidence of recent oesophagitis.

The lungs showed evidence of bronchopneumonia, which was confluent in the right lower lobe.

The heart weighed 292 g. The myocardium appeared normal. The coronary arteries showed only a slight degree of atheroma.

The remaining organs, including the brain and kidneys, showed no significant abnormality.

MICROSCOPIC FINDINGS

RETICULO-ENDOTHELIAL SYSTEM Imprints obtained from the bone marrow, and sections of the bone

marrow and lymph nodes all showed the presence of a highly cellular pleomorphic infiltrate, similar to that described in the preceding cases, except that plasma cells were less numerous and tissue mast cells were more plentiful. The marrow and the lymph nodes were involved diffusely. Infiltration of the spleen was relatively slight. Focal collections of lymphocytes were seen in the portal tracts of the liver. Erythrophagocytosis was conspicuous in the spleen, but less marked in the lymph node sinuses and in the marrow. Considerable haemosiderin deposits were present in the spleen, marrow, lymph nodes, and in the liver. The abdominal lymph nodes were fibrotic. Follicles were obscured in nodes at other sites but reticulin preparations showed that the architecture of these nodes was preserved. Reticulin formation in the marrow was not increased.

LUNGS Sections confirmed the presence of severe bronchopneumonia, which was confluent in the right lower lobe.

KIDNEYS The cortex contained a number of ischaemic glomeruli, with areas of tubular atrophy and fibrosis. Extensive focal lymphocytic infiltration was present in these fibrotic areas and beside the arcuate vessels. Evidence of recent tubular degeneration, with casts in both convoluted tubules and collecting ducts, was also present.

EYE Tryptic digestion of a flat preparation of the retina from one eye showed the peripheral vessels to be devoid of both pericyte and endothelial cell nuclei. These were present elsewhere, the endothelial cell nuclei frequently forming aggregations. Only two microaneurysms were found throughout the specimen (Professor N. Ashton).

REMAINING TISSUES (INCLUDING BRAIN) No significant abnormality was seen.

Death was due to bronchopneumonia and associated conditions were acute necrosis of the renal tubules, reticulosis with macroglobulinaemia, and an oesophageal hiatus hernia.

DISCUSSION

Macroglobulins have been shown to be glycolipoproteins, with a molecular weight of about 10^6 (Schrade, Böhle, Biegler, and Bruch, 1958). A small quantity, with a sedimentation constant of approximately 19S, is normally present in serum, and comprises about 2% of the total protein content. It consists of α_2 and γ globulin fractions, and includes the anti-A and anti-B iso-agglutinins as well as the naturally occurring agglutinating antibodies such as

anti-Rh, anti-M, and anti-P (Kunkel, 1960; Kekwick and Mollison, 1961). The α_2 fraction is elevated in nephrosis, while the γ fraction may be increased in haemolytic anaemias associated with cold antibodies, in rheumatoid arthritis, syphilis, infectious mononucleosis, cirrhosis of the liver, occasional cases of chronic lymphatic leukaemia or of lymphosarcoma, and in Waldenström's macroglobulinaemia. In the majority of these conditions the macroglobulin level rarely exceeds 10% of the protein content of the serum. The level is frequently found to exceed 15% in cases of Waldenström's disease (Ritzmann *et al.*, 1960), and Martin (1960) has suggested that the diagnosis of Waldenström's macroglobulinaemia should only be considered if 10% or more of the serum proteins can be shown to have a sedimentation constant greater than 16S.

Macroglobulin levels of 32%, 58.7%, 14%, and 27% respectively were present in the cases presented here. With the exception of case 3 the macroglobulins were heterogeneous on ultracentrifugal examination; the major peaks in all the instances had a sedimentation constant of about 19S. They failed to migrate after electrophoresis in a starch gel. These findings contrast with those obtained in myelomatosis, in which, in the majority of cases, the abnormal proteins have a sedimentation constant of 7S, and do migrate after starch gel electrophoresis (Silberman, 1957; Butler, Flynn, Harris, and Robson, 1961); occasional instances with sedimentation values of 10S and 12S may, however, be found (Kunkel, 1960).

Immunoelectrophoretic and gel diffusion studies, using antigens which have been obtained by the application of modern chromatographic techniques, suggest that pathological macroglobulins possess antigenic determinants common to normal macroglobulins but not present in 7S immune globulins; they also contain determinants lacking in normal macroglobulin and specific for each patient (Roulet, Spengler, Guyler, Bütler, Ricci, Riva, and Hässig, 1961); they differ from normal macroglobulins in being deficient in antigenic determinants against 7S immune globulins (Korngold, 1961). The cross-reactions between pathological macroglobulins and 7S immune globulins which have previously been reported (Kunkel, 1960) may have been due to the use of antigens containing appreciable amounts of 7S globulins as impurities (Albert and Johnson, 1961). Recent fluorescent antibody studies suggest that the macroglobulin is synthesized by the lymphoid-plasma cells of the infiltrate and not by the plasma cells (Zucker-Franklin, Franklin, and Cooper 1962).

The polymerism of the macroglobulin molecule is responsible for its high intrinsic viscosity (Jahnke,

Scholtan, and Heinzler, 1958). The presence of macroglobulin contributes to the viscosity of plasma and tissue fluids, which is considerably elevated in Waldenström's macroglobulinaemia (Waldenström, 1944). The increased cellularity of the marrow in this disease, combined with the increased viscosity of the tissue fluid in which the cells are imbedded, sometimes cause marrow aspiration to be unsuccessful and to result in a 'dry tap' (Kappeler *et al.*, 1958). This is well illustrated in the second case, in which a trephine biopsy of the marrow would have yielded an adequate specimen during life.

Fluorescent antibody studies in macroglobulinaemia have shown that both the red cells and the platelets are coated with the macroglobulin (Curtain, 1959; Pachter, Johnson, Neblett, and Truant, 1959). The resulting alteration in the biochemical and the biophysical state of the red cell surfaces presumably accounts for the increased erythrophagocytosis which is a prominent feature of the disease in severe cases (Zollinger, 1958; Windrum and Freeman, 1960). This presumably gives rise to the excessive haemosiderin depositis seen in the marrow and in the sinuses of the spleen and lymph nodes. Another factor in the development of anaemia is the reduction in the number of erythropoietic cells present in the marrow. These are replaced, possibly as a result of substrate competition, by the infiltrate, so that the normal erythropoietic response to excessive red cell destruction is prevented. Auto-immune haemolysis played no part in the genesis of the anaemia in any of the cases reported here, and reasonably aggressive courses of treatment with prednisone did not prolong the survival of the circulating red cells in case 1 or in case 3.

An increased tendency to red cell aggregation is a further consequence of the change in the state of the erythrocyte surfaces mentioned above. This is manifested by rouleaux formation, which is especially marked in the cold. The tendency to red cell aggregation combined with the viscosity effects of the plasma give rise to 'blood sludging', particularly if the velocity of the blood flow is reduced. These effects may result in complete stasis of the blood flow, followed by anoxia and endothelial damage (Donders, 1958). They will be accentuated by a fall in temperature in those cases where the macroglobulin behaves as a cryoglobulin also.

Coating of the platelets with macroglobulin is thought to interfere with their disintegration, and so with liberation of the platelet clotting factor (factor III, Pachter *et al.*, 1959). This is considered to be the cause of the impaired clot retraction which is frequently found in macroglobulinaemia. Waldenström observed a low fibrinogen level in the plasma in his first two cases, and a great variety of clotting

abnormalities have since been reported (Kappeler *et al.*, 1958; Henstell and Kligerman, 1958; Firkin, 1958).

The combined effects of anoxia, clotting abnormalities, and thrombocytopenia, if present, presumably account for the bleeding diathesis, which may be a prominent feature of the disease. This would result in the development of a hypochromic anaemia, and is well exemplified by the repeated epistaxes in case 2 and by the terminal haemorrhagic stage of case 3.

Logothetis *et al.* (1960) found that the nervous system was involved in 43 of 182 cases of macroglobulinaemia reported in the literature. Approximately 40% of these patients suffered from an encephalopathy due to diffuse multifocal lesions, which included the perivascular cell accumulations and plasma exudations found in the cases presented here. The altered oligodendrocytes and the bizarre enlargement of astrocytes seen in cases of progressive multifocal leucoencephalopathy (Richardson, 1961) have not been encountered in macroglobulinaemia so far. Although cases of macroglobulinaemia with neurological involvement may present with a psychosis, it is impossible to be certain of the relationship between the onset of the psychosis and that of the macroglobulinaemia in case 1, since plasma protein studies were not undertaken before the investigation of his anaemia. No changes were found in the brain and olfactory tracts to account for the loss of taste and smell in case 4.

The serum macroglobulin level is rarely found to be significantly elevated in patients suffering from a carcinoma, although a carcinoma has been found to be present in about 12% of the published cases of macroglobulinaemia (Kappeler *et al.*, 1958). This percentage may in fact be greater, since many of these cases have been incompletely investigated, and only a proportion of the total number have come to necropsy. The carcinoma may develop years after the onset of the macroglobulinaemia, which may, as a result of chemotherapy, be in remission at the time (Kok, personal observation). The carcinoma found in case 1 was limited to a segment of the right lung, and no secondary deposits were encountered anywhere in the body. A chromophobe adenoma of the pituitary was present in case 2. No evidence of an accompanying carcinoma was found in case 3 or in case 4. The significance of the association of carcinoma with Waldenström's macroglobulinaemia cannot be assessed at present, and it would seem reasonable to regard the tumours present in two of the four cases reported here as coincidental with a reticulosis.

The cytology of the infiltrate in the marrow and the lymph nodes is remarkably similar in the cases reported here, and conforms to the descriptions given

by Kappeler *et al.* (1958), by Zollinger (1958), and by Rohr (1960), who regard Waldenström's macroglobulinaemia as a primary reticulosis, characterized by a pleomorphic infiltrate. This consists predominantly of cells resembling lymphocytes, together with varying numbers of lymphoid-plasma cells, plasma cells, naked nuclei, reticulum cells, and tissue mast cells. Some degree of erythrophagocytosis and of haemosiderosis is normally present, and may be prominent. This picture is too pleomorphic to suggest a diagnosis of chronic lymphatic leukaemia or of lymphosarcoma, while plasma cells are present in insufficient numbers to support a diagnosis of myelomatosis.

Histological examination reveals the bone marrow and the lymph nodes to be infiltrated diffusely in all four cases. Although lymph follicles are obscured by the infiltrate, reticulin preparations show that the architecture of the nodes is largely preserved and that the reticulin pattern of the bone marrow is normal. These findings contrast with the appearances in reticulosarcoma, lymphoid medullary reticulosis (Hodgkin's paraganuloma), chronic lymphatic leukaemia, and lymphosarcoma. The lymph node appearances are likely to be confused with those of non-specific chronic inflammation, to which they bear a superficial resemblance.

The demonstration in the marrow and in a lymph node, if available, of the cytological and histological features illustrated by the cases reported here would permit a diagnosis of Waldenström's macroglobulinaemia to be made, and this would be supported by the finding of an excess of a 19S γ macroglobulin in the blood.

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REFERENCES

- Albert, A., and Johnson, P. (1961). *Biochem. J.*, **81**, 658, 669.
 Barrett, A. M. (1944). *J. Path. Bact.*, **56**, 133.
 Björnesjö, K. B. (1955). *Scand. J. clin. Lab. Invest.*, **7**, 153.
 Butler, E. A., Flynn, F. V., Harris, H., and Robson, E. B. (1961). *Lancet*, **2**, 289.
 Cagianut, B., and Theiler, K. (1959). *Albrecht v Graefes. Arch. Ophthalm.*, **160**, 628.
 Curtain, C. C. (1959). *Aust. Ann. Med.*, **8**, 143.
 Donders, P. C. (1958). *Ophthalmologica (Basel)*, **135**, 324.
 Dutcher, T. F., and Fahey, J. L. (1959). *J. nat. Cancer Inst.*, **22**, 887.
 Firkin, B. G. (1958). *Amer. J. Med.*, **24**, 974.
 Flynn, F. V., and De Mayo, P. (1951). *Lancet*, **2**, 235.
 Henstell, H. H., and Kligerman, M. (1958). *Ann. intern. Med.*, **49**, 371.
 Jahnke, K., Scholtan, W., and Heinzler, F. (1958). *Helv. med. Acta*, **25**, 2.
 Kappeler, R., Krebs, A., and Riva, G. (1958). *Ibid.*, **25**, 54.
 Kekwick, R. A., and Mollison, P. L. (1961). *Vox Sang. (Basel)*, **6**, (n.s.), 398.
 Korngold, L. (1961). *Ann. N.Y. Acad. Sci.*, **94**, 110.
 Kunkel, H. G. (1960). *Macroglobulins and High Molecular Weight Antibodies in the Plasma Proteins*, edited by F. W. Putnam, vol. 1 p. 279. Academic Press, New York.
 Logothetis, J., Silverstein, P., and Coe, J. (1960). *Arch. Neurol. (Chic.)*, **3**, 564.
 Mackay, I. R. (1959). *Aust. Ann. Med.*, **8**, 158.
 Martin, N. H. (1960). *Quart. J. Med.*, **29**, 179.
 McFarlane, A. S., Dovey, A., Slack, H. G. B., and Papastamatis, S. C. (1952). *J. Path. Bact.*, **64**, 335.
 Pachter, M. R., Johnson, S. A., Neblett, T. R., and Truant, J. P. (1959). *Amer. J. clin. Path.*, **31**, 467.
 Quaglino, D., and Hayhoe, F. G. J. (1959). *J. Path. Bact.*, **78**, 521.
 Richardson, E. P. (1961). *New Engl. J. Med.*, **265**, 815.
 Ritzmann, S. E., Thurm, R. H., Truax, W. E., and Levin, W. C. (1960). *A.M.A. Arch. intern. Med.*, **105**, 939.
 Rohr, K. (1960). *Das Menschliche Knochenmark. Dritte Auflage*, p. 467. Thieme, Stuttgart.
 Roulet, D. L. A., Spengler, G. A., Guyler, E., Büttler, R., Ricci, C., Riva, G., and Hässig, A. (1961). *Helv. med. Acta*, **28**, 127.
 Schrader, W., Böhle, E., Biegler, R., and Bruch, H. (1958). *Folia haemat. (Frankfurt)*, **2**, 337.
 Sia, R. H. P. (1924). *Chin. med. J.*, **38**, 35.
 Silberman, H. J. (1957). *Lancet*, **2**, 26.
 Waldenström, J. (1944). *Acta med. scand.*, **117**, 216.
 Windrum, G. M., and Freeman, Z. (1960). *Med. J. Aust.*, **1**, 655.
 Zollinger, H. U. (1958). *Helv. med. Acta*, **25**, 153.
 Zucker-Franklin, D., Franklin, E. C., and Cooper, N. S. (1962). *Blood*, **20**, 56.