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MACROGLOBULINÆMIA*

Effect of macroglobulins on prothrombin conversion accelerators

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MACROGLOBULINÆMIA was first reported by Waldenström in 1948.[1, 2] The main characteristic of this syndrome was the presence of serum globulins having an abnormally high molecular weight. Serum macroglobulins were easily detected in the presence of distilled water (euglobulins). The flocculation of the abnormal globulins readily disappears in a sodium chloride solution. The clinical features of "idiopathic macroglobulinæmia" have been well discussed by Waldenström,[2] Schaub,[3] Tischendorf and Hartmann,[4] Wuhrmann,[5] Wilde and Hitzelberger,[6] Kanzow,[7] and Layani and collaborators.[8] A progressive asthenia and an hæmorrhagic tendency are usually present. However, purpura is a very rare clinical manifestation and, according to Kanzow,[7] this permits differentiation of "idiopathic macroglobulinæmia" from "purpura hyperglobulinæmia", also described by Waldenström.[2] In addition, there are a moderate adenopathy, hepatosplenomegaly and pseudo-Raynaud syndrome manifestations.

The aminoacid composition of macroglobulins has been investigated by Pernis, Wuhrmann and Wunderly[9] and Mandema, van der Schaaf and Huisman.[10] Wilde and Hitzelberger[6] insist on the ultracentrifugation of the macroglobulins as the *sine qua non* for a diagnosis.

The interest of the present publication lies in the unusually good outcome in a case of macroglobulinæmia after splenectomy and in the definite effect of macroglobulins on blood coagulation. Some pathological and biochemical aspects of this syndrome are also presented.

CASE HISTORY

The patient was a 40-year-old white woman whose first complaints were those of an abdominal tumour, loss of weight, asthenia and dysuria. At the time of her admission to Hôtel-Dieu Hospital in Montreal in November 1953, she had noticed an increase in size of her abdomen for the past year. She never had any pain but complained of mechanical discomfort. The recurrence of herpes of the mouth was noted during ten years. Her past history was non-contributory except for an appendectomy and a subtotal hysterectomy for fibroids in June 1947. She had also had a tonsillectomy in 1938. Physical examination in 1953 revealed a generalized mild lymphadenopathy, a huge spleen and a slightly enlarged liver.

Laboratory data. — Peripheral blood: hæmoglobin value 7.4 g. %; red cell count 3,370,000; white cell count 4,850 with a differential count showing 54% neutrophils, 46% lymphocytes. Platelets numbered 91,200 (Rees and Ecker). Prothrombin concentration was 35% (bedside technique[11]). Thymol turbidity was 8.80 units; bromsulphalein 4%. Because of a positive Wassermann at 1 in 256, the patient was referred to the dermato-syphilologist for consultation. The only abnormal finding was that the reaction to light of the right pupil was greater than that of the left one. The fundi and C.S.F. were normal. She was temporarily discharged after a course of penicillin therapy (6,300,000 units) with a tentative diagnosis of infectious hepatosplenomegaly, probably luetic in origin.

She was again hospitalized in May 1954 for further investigation. Her complaints were identical with those on her first admission except for the loss of a few more pounds; she now weighed 95 lb. She was pale, asthenic and subfebrile. The liver was palpable 4 cm. below the costal margin, smooth and tender. The spleen was extremely enlarged; the inferior limit occupied the left pelvis and part of the right iliac cavity. Lymph nodes were palpable along the cervical chains and in the axillæ and groins. No purpura could be found and she never had any history of hæmorrhagic diathesis. Syphilis was ruled out by the Treponema immobilization test.[12]

Laboratory data.—Hæmoglobin value 6.6 g. %; red cell count 2,340,000; hæmatocrit 23%; white cell count 4,500 with a differential showing neutrophil promyelocytes 1%, neutrophils 44%, eosinophils 3%, lymphocytes 46%, monocytes 4%, plasma cells 2%. The erythrocyte sedimentation rate (Wintrobe, corrected) was 34 mm. in one hour and the prothrombin concentration fluctuated between 13 and 21%. Fibrinogen, 244 mg. %, total proteins 7.32 g. % ($CuSO_4$ technique); albumin 2.92, alpha globulins 1.28; β globulins 0.9 and γ globulins 2.18. Bromsulphalein 3.5% and thymol turbidity 12.80 units. The cadmium reaction[13] was negative, requiring eight drops before cloudiness appeared. Bence Jones proteins could never be demonstrated in the urine and the serum was free of cryoglobulins. Blood viscosity 1.65 (Ostwald viscositometer, 22-24° C., N-1.56). X-ray investigations of the chest, the gastrointestinal tract and kidneys were non-contributory. The peripheral blood smear showed a slight anisocytosis and marked rouleaux formation of erythrocytes. Platelets appeared to be slightly decreased and there was a relative lymphocytosis (2,070).

The myeloid-erythroid volume[14] of the bone marrow was 4%. The smears showed an invasion of the marrow by somewhat atypical lymphoid cells (50.4%). They were medium-size to large lymphocytes 9 to 13 micra in diameter. The nucleus, round or oval and sometimes eccentrically placed in the cell, occupied 2/3 to 4/5 of the cellular body. The chromatin stained violet and showed large irregular masses with occasional nucleolar vestiges. The parachromatin was distinct and pink in colour, but the cytoplasm was homogeneous without azure granules or vacuoles.

Lymph node biopsy of the left axilla revealed a non-specific lymphadenitis, and, when repeated in the right axilla, a lipomelanotic reticulosis. Because of the marked mechanical discomfort, splenectomy was advised and was carried out on July 5, 1954. Besides a marked hepatosplenomegaly, there was a generalized periaortic lymphadenopathy. Lymph nodes about 1 to 3 cm. in

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diameter were palpated in the hepatic and splenic hila. A spleen weighing 2,975 g. was removed, and abdominal lymph node and liver biopsies were performed at the time of operation. The histological data will be discussed below. The operation and the postoperative course were uneventful. The patient was seen at regular intervals, and ten months after splenectomy had gained 38 lb. She is now living a normal life and is back at work. The liver is no longer palpable and there is no adenopathy.

BLOOD COAGULATION STUDIES

A routine coagulogram was as follows: bleeding time (Duke) 1 min. 45 sec., coagulation time (Duke) 5 min. 15 sec., platelets 91,200, prothrombin time (Quick) 22".2/12".0. The protamine titration[15] did not reveal any abnormal heparin-like anticoagulant and gave a heparin concentration of 5 mg. for one hundred c.c. of blood.

Because of the prolonged Quick prothrombin time, it was decided to rule out any deficiency of prothrombin conversion accelerators by the techniques described by Owren[16] and Stefanini.[17] These tests were carried out on whole plasma and on plasma treated with distilled water to flocculate the macroglobulins. The supernatant fluid of the latter was used as macroglobulin-free plasma.

TABLE I.

STEFANINI'S AND OWREN'S TESTS ON PATIENT'S PLAIN PLASMA COMPARED WITH PATIENT'S PLASMA TREATED WITH D.D.H_2O. THE RESULTS IN SECONDS ARE COMPARED WITH NORMAL CONTROLS

Factor determined	*Patient's plain plasma diluted 1/5 with saline*	*Patient's plasma diluted 1/10 with D.D.H_2O.*
Quick P.T.	41.6/23.0	44.0/43.0
Prothrombin	30.4/19.2	24.2/23.6
Proconvertin	42.4/26.8	31.5/33.0
Proaccelerin	37.4/23.4	22.8/23.0

Table I indicates the abnormal coagulation times obtained when the plasma was diluted with saline. The prothrombin, proaccelerin and proconvertin concentrations appear to be decreased when compared with those of normal plasma. On the other hand, when the patient's plasma was diluted with distilled water (D.D.H_2O) to be free of macroglobulins, all the concentrations in prothrombin, proaccelerin and proconvertin were identical with concentrations of a normal plasma diluted with D.D.H_2O. The prolongation of coagulation times apparently was due to the presence of macroglobulins. To test this assumption, macroglobulins were added to a normal coagulation system, and the modified Quick prothrombin time was again determined. The data are summarized in Table II. Coagulation of normal plasma was delayed when the patient's plasma containing macroglobulins was added. Macroglobulin-free plasma did not prolong Quick prothrombin time of a normal plasma. The addition of 0.1 c.c. of thromboplastin in the Quick coagulation system does not affect the coagulation time of the patient's plasma, and this presumes an absence of antithromboplastin. The presence of an active antithromboplastin would also have prolonged the coagulation time.

TABLE II.

EFFECT OF MACROGLOBULINS ON QUICK P.T. OF A NORMAL PLASMA

Quick P.T. +	*Coagulation time in sec.*	
	Normal	*Patient*
Nothing added	12.0	22.2
0.1 c.c. of saline	13.4	23.2
0.1 c.c. of thromboplastin	13.8	21.8
0.1 c.c. of patient's plasma	41.8	
0.1 c.c. of macroglobulins dissolved in saline (1/5 initial vol.)	20.6	
0.1 c.c. of patient's plasma deprived of macroglobulins		13.8

EFFECT OF MACROGLOBULINS ON PROCONVERTIN AND PROACCELERIN TIMES

These tests were performed according to the following technique, and the results are summarized in Table III and graphed in Fig. 1. To demonstrate the anti-

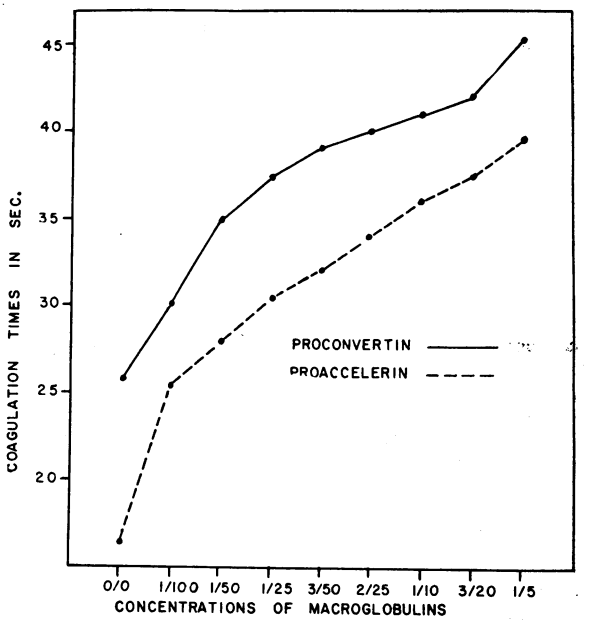

Fig. 1.—The effect of macroglobulins on proconvertin and proaccelerin.

proconvertin effect of patient's plasma precipitate (macroglobulins), different concentrations of this precipitate dissolved in saline were added to a coagulation system which consists of plasma from a patient with hypoproconvertinæmia,[18] stored ox serum, thromboplastin and $CaCl_2$. On the other hand, to demonstrate the antiproaccelerin effect of the patient's macroglobulins, different concentrations of macroglobulins were added to a coagulation system consisting of normal plasma incubated 20 minutes at 50° C., plus $BaSO_4$ treated ox plasma, thromboplastin and $CaCl_2$. The purpose of using such a system was to see whether the precipitate had any action on the correcting effect of proconvertin and proaccelerin on systems deprived of prothrombin-conversion accelerators. It can be seen that the proconvertin and proaccelerin times increase with an increase of the precipitate concentrations, as if macroglobulins had an antiproconvertin and antiproaccelerin action.

The prothrombin time of the patient's plasma was studied by the two-stage technique[19] to appreciate the effect of macroglobulins on prothrombin. The normal prothrombin unit concentration per c.c. of plasma by the Ware and Seegers two-stage technique is approximately 250 units per c.c. of plasma in our laboratory. It was found that the two-stage prothrombin time of the pa-

TABLE III.

EFFECT OF MACROGLOBULINS ON PROCONVERTIN AND PROACCELERIN

		MACROGLOBULINS/cc	0 0	0 01	0 02	0 04	0 06	0 08	0.1	0.15	0.2
		SALINE/cc	0 2	0.19	0.18	0.16	0 14	0.12	0.1	0.05	0.0
THROMBOPLASTIN	0.1cc										
CACL2	0.1cc										
CONGENITAL HYPO-PROCONVERTINEMIA PLASMA	0.1cc	167 0" 13 4"	25.8"	30 0"	35.0"	37 4"	39 8"	40.0"	41.0"	42.0"	45.2"
STORED OX SERUM	0.1cc										
THROMBOPLASTIN	0.1cc										
CACL2	0.1cc										
PLASMA 50°/20'	0.1cc	60 0" 13.6"	16 4"	25.4"	28 0"	30 4"	34.0"	36.0"	37.4"	39.4"	41 4"
BaSO4 OX PLASMA	0.1cc										

tient's plasma gave a prothrombin concentration of 170 units per c.c. Fig. 2 indicates that diluting 1/10 of a volume of a normal plasma with D.D.H_2O does not influence the number of prothrombin units in a normal plasma if the same dilution is corrected in step two. The final yield is 267 units per c.c. compared to 250 units for an undiluted normal plasma. If, however, this test is repeated on the patient's plasma deprived of macroglobulins, the final yield is 142 units per c.c. of plasma compared to 170 units per c.c. if the test is performed on plain patient's plasma. It is believed that this difference may be covered by the indices of error inherent in the test itself and is not statistically significant. In step one of the Ware and Seegers technique, when a normal plasma is defibrinated in the presence of a solution of macroglobulins instead of saline, the final yield of prothrombin approaches 240 units of prothrombin per c.c. of plasma compared to 250 units for an undiluted normal plasma. The yield of a normal plasma was 257.5 units of prothrombin even when in step two 1 c.c. of a macroglobulin solution was added to 0.1 c.c. of defibrinated plasma plus 1.4 c.c. of normal saline. The finding that macroglobulins have no apparent action on the final prothrombin yield of a normal plasma is of interest. A slight delay in prothrombin-conversion action was noted when macroglobulins were added to the coagulation system. If there is a direct action by macroglobulins on prothrombin conversion—that is, an anti-proconvertin and antiproaccelerin action—this was not manifested by the two-stage technique, and the only explanation appears to be a question of dilution. No antiprothrombin effect stands out, according to the results found in the two-stage technique. There is, however, a definite prothrombin decrease in the patient's plasma according to the Ware and Seegers two-stage prothrombin time test which was not demonstrated by the one-stage method.

PAPER ELECTROPHORESIS*

Paper electrophoresis of the patient's serum was carried out at various intervals before and after the operation. The apparatus used was a modification of the horizontal method of Grassman, Hannig and Knedel.[20] Separations were obtained with 0.05M veronal buffer at pH 8.6 during 15-hour runs. The strips were stained for protein with amido-black,[20, 21] for carbohydrate with fuchsin,[22] and for lipids with sudan black NB. Optical densities of the stained strips were determined by means of the Eel densitometer.

Figs. 3 and 4 illustrate the marked changes in the electrophorograms of samples taken before and long after the operation, while the data for the complete series of samples are shown in Tables IV and V.

The preoperative serum is characterized by the presence of intensely staining fuchsin-reactive material in the gamma-globulin fraction. This material does not have the same mobility as normal gamma-globulin, since it remains exactly at the origin under the conditions used here. After operation, this material gradually disappears and gives way to a gamma-globulin fraction of more normal appearance. Some lipid also appears to be associated with the abnormal protein, but this could be an artefact. As mentioned above, dilution of the serum with distilled water causes precipitation of some of the protein. This material, recovered by centrifugation, dissolved in saline and placed in the electrophoresis apparatus, yielded only one band at the origin which stained intensely with fuchsin, but little or not at all with sudan black NB. Ultracentrifugation of this material showed the presence of a high molecular weight protein of about 19 Svedberg units.*

Minor changes were also observed in the other fractions. Beta-globulin increased, while alpha$_2$-globulin decreased. There was no significant change in the alpha$_1$ fraction. Albumin gradually increased to nearly normal values with a concomitant increase in the albumin-globulin ratio as determined either by electrophoresis or by the sodium sulphate fractionation method of Gornall.[22]

While there was no change in the total serum protein during the entire study, a gradual and very marked decrease in fuchsin-staining material occurred. Six months after operation only about 30% of the original carbohydrate remained.

Again, while the distribution of protein in all the fractions except the gamma globulin was not markedly altered, important changes in the relative distribution of carbohydrate in all the fractions were observed. The preoperative serum showed 45% of the total carbohydrate in the gamma globulin, 40% in the alpha$_1$ and alpha$_2$ globulins, 15% in the beta globulin, and none in the albumin. Immediately after operation, the gamma-

*This part of the work was supported by Grant No. 27 of "La Fondation Rhéaume," Université de Montréal, and was performed by one of us, M.F.

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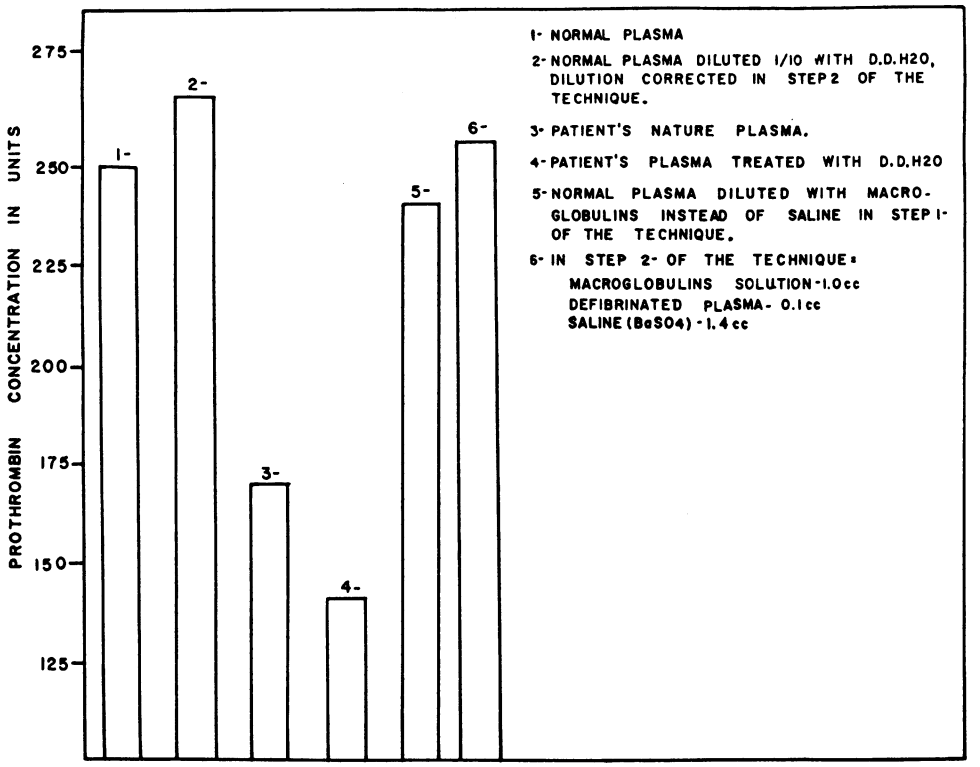

Fig. 2.—Effect of macroglobulins on prothrombin studied by the two-stage determination.

globulin level fell to 27% and the albumin rose to 13%, with no change in the other fractions. Gradually carbohydrate disappeared from the albumin, increased to 56% in the alpha$_1$ and alpha$_2$ fractions and to 20% in the beta globulin, and remained steady in the gamma globulin.

PATHOLOGICAL FINDINGS

Spleen.—Grossly the spleen was much enlarged, measuring 30 x 16 x 9 cm. and weighing 2,975 g. Its general shape was preserved, and deep notches were seen along its superior-external border. The capsule was tense, thin and transparent. On the cut surface the Malpighian bodies were conspicuous because of their number and large size. Their diameter varied between 1 and 2 mm. They were well demarcated and stood out on the background of the red pulp, which was normal in appearance.

Microscopical sections of the spleen (Figs. 5 and 6) showed an intact capsule and structurally preserved splenic tissue, consisting of a red and white pulp. However, there was an abnormal excess of white pulp, which to all appearances accounted for the splenomegaly. This excess of white pulp resulted predominantly from an

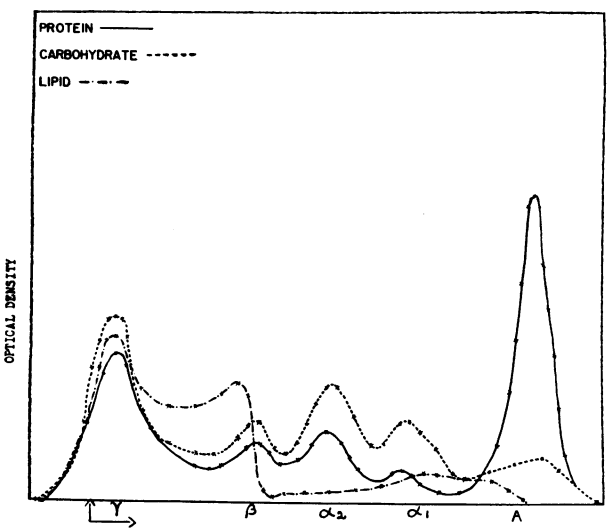

Fig. 3.—Electrophorogram of serum taken one month before operation: ———— protein (amido-Schwartz). - - - - - - - carbohydrate (fuchsin). --.--.--.--.--.--. lipids (sudan-black NB).

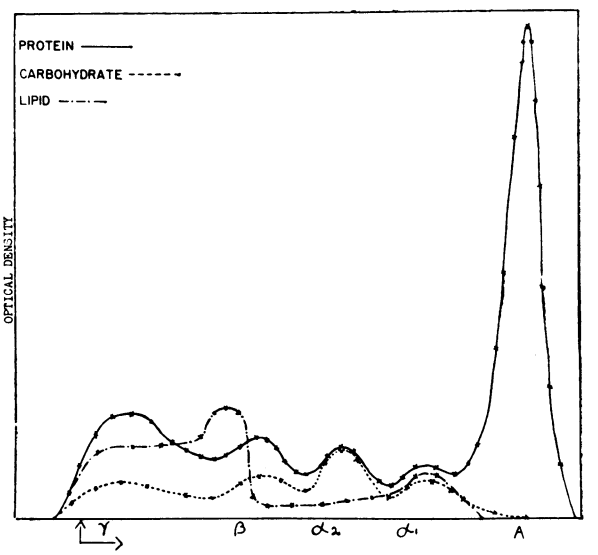

Fig. 4.—Electrophorogram of serum six months after operation, staining as above.

TABLE IV.

RESULTS OF ELECTROPHORESIS OF THE PATIENT'S SERUM AT VARIOUS PERIODS BEFORE AND AFTER OPERATION

Protein *Date*	*γ*	*β*	*α2*	*α1*	*Alb.*	*A/G*	*A/G biuret*	*Albumin*	*Total prot.*
6/12/54	33.8	9.6	12.2	4.3	39.0	0.64	0.52	122	365
8/ 4/54	26.0	10.6	13.5	5.1	44.4	0.81	0.81	161	360
9/20/54	22.0	14.6	12.0	3.4	48.0	0.91	0.93	189	390
11/29/54	24.9	12.6	12.9	3.1	46.4	0.87	——	——	——
1/17/55	23.0	14.0	9.5	4.1	49.0	0.97	1.04	190	378

TABLE V.

RESULTS OF ELECTROPHORESIS OF THE PATIENT'S SERUM AT VARIOUS PERIODS BEFORE AND AFTER OPERATION

Polysaccharide *Date*	*γ*	*β*	*α2*	*α1*	*Alb.*	*Total carbohydrate*
6/12/54	43.5	14.8	30.5	11.5	——	1200
8/ 4/54	26.7	17.0	28.2	14.5	13.4	1235
9/20/54	28.0	19.4	33.5	14.3	6.0	817
11/29/54	30.5	17.2	37.4	15.2	——	558
1/17/55	25.8	20.2	36.0	20.1	——	366

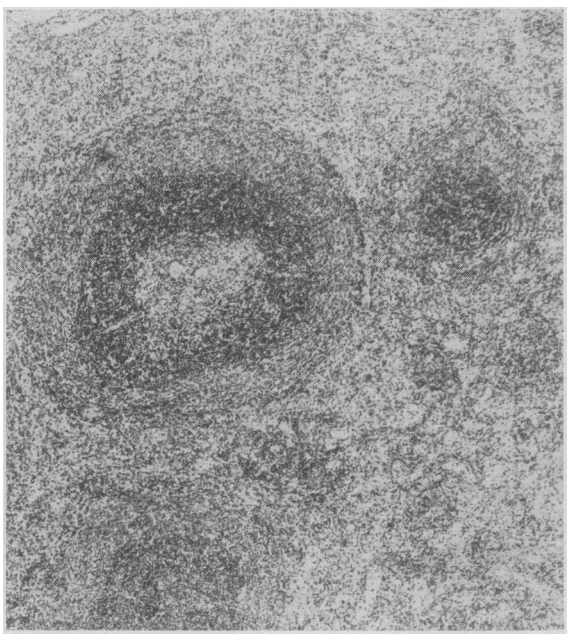

Fig. 5.—Spleen (×60). Enlargement of the lymphoid nodules of the spleen by proliferation of lymphoid tissue in the marginal zone of the nodule (halo). In the red pulp, presence of secondary nodules.

increase in volume and number of the lymphoid nodules of the spleen.

In favourable transverse sections, the enlarged Malpighian bodies had three easily distinguishable parts. There was a central, pale-staining portion consisting mainly of reticular cells, macrophages with phagocytosed blood pigment, and nuclear fragments, with a few plasmocytes. Irregular threads of hyalin were occasionally found in this central region, which was practically devoid of mitoses.

This clear centre was surrounded by a middle, dark-staining zone of densely crowded small lymphocytes where mitoses were infrequent.

All around this agglomeration of small lymphocytes, in the marginal zone of the nodes, there were a pale well-demarcated rim of lighter-staining round cells reminiscent of the halo formations described in so-called hypersplenism. In the peripheral rim, the predominant cells were medium-sized lymphocytes, admixed with lymphoblasts and reticular cells. Mitoses were numerous. The network of reticulin fibrils was denser than in the inner layers of the lymphoid nodules.

The red pulp was well preserved but studded with secondary lymphoid nodules, developed around the arterioles. The secondary nodules consisted of an inner layer of small lymphocytes and an outer rim of actively proliferating medium-sized lymphocytes, so that their architecture was similar to that of the Malpighian bodies.

Except for these secondary nodules, the framework of the red pulp was essentially normal, and there was no excess of free cells. However, iron staining revealed a notable amount of irregularly distributed hæmosiderin.

Liver.—The liver biopsy specimen was a thin but relatively large tissue block of nearly rectangular shape, measuring 12 x 20 mm. This tissue block was fixed in

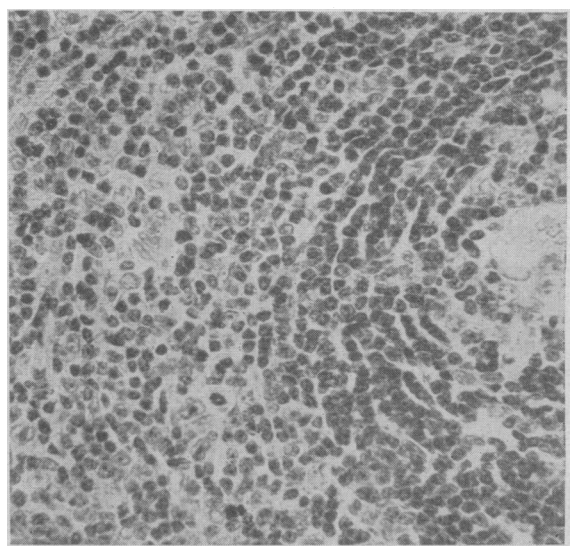

Fig. 6.—Spleen (×450). High magnification of a splenic nodule showing, from left to right, the pale centre of the nodule, the intermediate zone of closely packed small lymphocytes, and the outer or marginal zone composed of reticular cells, lymphoblasts and lymphocytes.

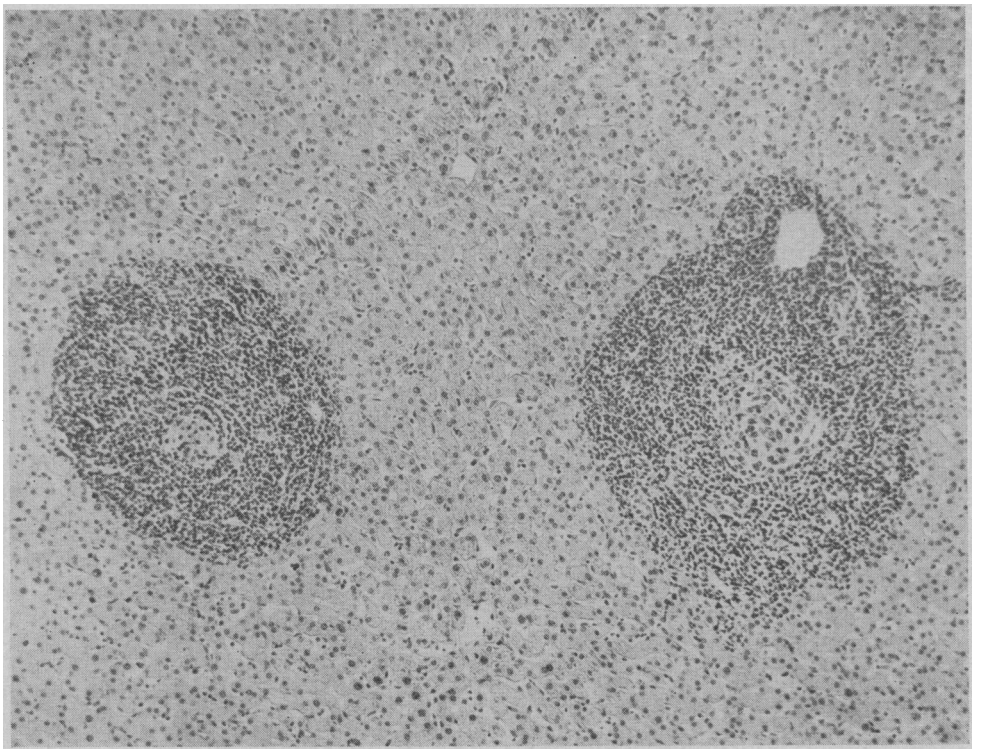

Fig. 7.—Liver (×120). Enlargement and diffuse lymphocytic infiltration of portal tracts. In the lymphoid masses one may see pale centres.

brazil and embedded in paraffin. At low magnification (Fig. 7), there was normally stained liver parenchyma of normal structure, while the ramifications of the portal tracts stood out because of their large size and dark staining. The alterations in the portal tracts were due to dense accumulations of round cells which obscured their normal architecture.

Smaller intralobular collections of round cells were occasionally seen, most of them close to the hepatic veins. At higher magnification, the round cells were seen to be mainly small and medium-sized lymphocytes, with an admixture of lymphoblasts and reticular cells. Here and there, clear centres, essentially made up of reticular cells, stood out in the nodular accumulations of lymphoid cells. In many places, numerous transitional forms were seen between lymphocytes and plasma cells. This tendency of lymphoid cells to change into plasmocytes was all the more striking in the liver because it was practically nonexistent in the spleen.

The intrahepatic biliary system was essentially normal. A scant amount of hæmosiderin was seen in the portal tracts and in the lobules.

Lymph nodes.—Two lymph nodes about 5 mm. long, with surrounding cellulo-adipose tissue, were excised for histological examination. At low magnification (Fig. 8), the normal markings of the medullary zone were obliterated by a mass of closely packed lymphocytes, while cortical sinuses were still apparent. The capsule and surrounding cellulo-adipose tissue were infiltrated by lymphocytes.

At higher magnification, numerous erythrocytes and macrophages laden with hæmosiderin were seen in the cortical sinuses. Reticulin stains showed that the medullary sinuses were still present, although obscured by the lymphoid infiltration. In summary, the anatomical changes were essentially characterized by an overproduction of lymphoid cells.

This overgrowth was most apparent in the spleen, where it resulted in an enormous splenomegaly. It was nodular and organoid, and resulted from an active proliferation of lymphoid tissue in the marginal zone of the nodules and lymphoid sheaths. The white pulp was thus seen surrounded by a clear halo. The microscopic picture was structurally the very reverse of follicular lympho-blastoma.

In the liver, the lymphoid infiltration was mostly confined to the portal tracts and the histological picture was reminiscent of chronic lymphatic leukæmia. However, the tendency to formation of plasma cells was a feature not usually seen in leukæmia.

In the lymph nodes, diffuse lymphoid infiltration with invasion of the capsule could not be distinguished from that usually seen in lymphoma.

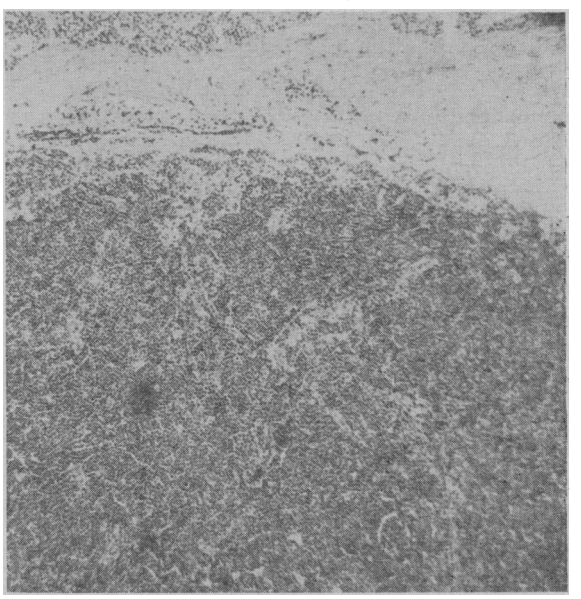

Fig. 8.—Lymph node (×60). Diffuse lymphocytic infiltration with obliteration of medullary sinuses. Preservation of cortical sinuses. Lymphocytic infiltration of the capsule and loose adipose tissue.

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The pathological diagnosis was malignant lymphoma primary in the spleen and spreading to the lymph nodes and liver.

COMMENTS

This new case of Waldenström's syndrome shows similarity to those previously reported, although the clinical features differ in several respects. External generalized mild lymphadenopathy of unspecified nature with a concomitant loss of weight, asthenia and hepatosplenomegaly is part of the picture of this syndrome, though not with a spleen as large as in the present case. The majority of investigators consider the hæmorrhagic tendency as an integral part of this syndrome, although Schaub and Wuhrmann's patients showed no hæmorrhages. No previously reported cases mention hypoprothrombinæmia except for one instance encountered by Frick.[24] Little is known about the exact mechanism of the blood coagulation defect in macroglobulinæmia. Our patient never showed a bleeding tendency even with a low prothrombin concentration. Because of an increase in tissue mast cells, Tischendorf and Hartmann were of the opinion that heparin or heparin-like substances accounted for the bleeding tendency of their patients. Stefanini[25] stipulates that the antithrombin mechanism resides in the pathological protein with subsequent deficiency in fibrin formation, and moreover that this foreign protein may also inhibit the formation of thromboplastin. Subsequently, macroglobulins would have two distinct effects, antithromboplastic and antithrombic. The presence of antithromboplastin was ruled out and no abnormal heparin concentration could be detected in the plasma of our patient.

It appeared, by routine prothrombin determination, that the blood coagulation defect concerned the yield of prothrombin, and it was demonstrated that macroglobulins have an action similar to that of an antiproconvertin and an antiproaccelerin. However, prothrombin determination by the two-stage technique showed a slight decrease which was not manifested by Owren's and Stefanini's techniques, perhaps because the prothrombin deficiency was not sufficient to be detected by the latter techniques. On the other hand, it was demonstrated by the one-stage method that macroglobulins had a direct effect on prothrombin conversion factors, and that this effect was not evident in the two-stage prothrombin determination. It is felt that this discrepancy is due to the great plasma dilution which decreases the action of the foreign protein, or that the time allotted to the prothrombin conversion by its factors is not sufficient for a complete conversion. In fact, in the different experiments, the yield of prothrombin is the same but the incubation time in experiment No. 6 (Fig. 2) is 5½ minutes compared to 2½ minutes for normal plasma.

The coagulation studies seven months after splenectomy did not reveal any abnormality, and the Quick prothrombin time was 14".2/13".4. The plasma showed no cloudiness in the presence of distilled water. At that time, paper electrophoresis showed a disappearance of the abnormal protein that gave way to a gamma globulin fraction of normal appearance. Follow-up studies 11 months after splenectomy revealed the following coagulogram: very slight plasma cloudiness in the presence of distilled water, Quick prothrombin time 17.9/13.8, prothrombin 14/12.1, proconvertin 19.8/13.6 and proaccelerin 19.7/11.9, thus indicating a return of the macroglobulins.

From the pathological viewpoint, the present observation falls in line with the large proportion of previously reported cases in which an abnormal proliferation of lymphoid-like cells was described. This proliferation was here interpreted as a variant of malignant lymphoma, apparently primary to the spleen and spreading to the lymph nodes and liver. Microscopically, the pattern was more reminiscent of chronic lymphatic leukæmia than of lymphosarcoma. No increase in mast cells was noted. Transition forms of lymphoid-type cells to plasma cells, a feature of sternal puncture smears, could be detected only in the liver, and not in the spleen where cellular multiplication appeared much more active. All in all, the process was essentially a lymphoreticular proliferation, with a slight tendency to formation of plasmoid cells.

SUMMARY

A case of macroglobulinæmia is described. Identification of macroglobulins was established by ultracentrifugation. Splenectomy in this case produced a temporary remission.

It is postulated that these macroglobulins act as an antiproconvertin and an antiproaccelerin. The reticulo-endothelial system and its lympho-

plasmocytic derivatives appear to be the main source of this foreign protein.

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REFERENCES

1. WALDENSTRÖM, J.: *Acta med. scandinav.*, **117**: 216, 1944.
2. *Idem*: *Schweiz. med. Wchnschr.*, **78**: 927, 1948.
3. SCHAUB, F.: *Schweiz. med. Wchnschr.*, **82**: 890, 1952.
4. TISCHENDORF, W. AND HARTMANN, F.: *Acta hæmat.*, **4**: 374, 1950.
5. WUHRMANN, F.: *Schweiz. med. Wchnschr.*, **82**: 937, 1952.
6. WILDE, H. AND HITZELBERGER, A. L.: *Blood*, **9**: 875, 1954.
7. KANZOW, U.: *Klin. Wchnschr.*, **32**: 154, 1954.
8. LAYANI, F., ASCHKENASY, A. AND BENGUI, A.: *Presse méd.*, **63**: 44, 1955.
9. PERNIS, B., WUHRMANN, F. AND WUNDERLY, C.: *Acta hæmat.*, **11**: 309, 1954.
10. MANDEMA, E., VAN DER SCHAAF, P. C. AND HUISMAN, T. H.: *J. Lab. & Clin. Med.*, **45**: 261, 1955.
11. SCHWAGER, P. G. AND JACQUES, L. B.: *Canad. M. A. J.*, **60**: 258, 1949.
12. NELSON, R. A., JR. AND MAYER, M. M.: *J. Exper. Med.*, **89**: 369, 1949.
13. WUNDERLY, C. AND WUHRMANN, F.: *Schweiz. med. Wchnschr.*, **75**: 1128, 1945.
14. SUNDBERG, R. D.: *Bull. Univ. Minnesota Hosp.*, **21**: 471, 1950.
15. ALLEN, J. G. *et al.*: *J. Lab. & Clin. Med.*, **34**: 473, 1949.
16. OWREN, P. A.: *Am. J. Med.*, **14**: 201, 1953.
17. STEFANINI, M.: *Am. J. Med.*, **14**: 64, 1953.
18. LONG, L. A., LETENDRE, P. AND COLPRON, G.: *Acta hæmat.*, **13**: 242, 1955.
19. WARE, A. G. AND SEEGERS, W. H.: *Am. J. Clin. Path.*, **19**: 471, 1949.
20. GRASSMAN, W., HANNIG, K. AND KNEDEL, M.: *Deutsche med. Wchnschr.*, **76**: 333, 1951.
21. PEZOLD, F. A. AND KOFES, A.: *Klin. Wchnschr.*, **32**: 504, 1954.
22. KÖIW, E. AND GRÖNWALL, A.: *Scandinav. J. Clin. & Lab. Invest.*, **4**: 244, 1952.
23. GORNALL, A. G., BARDAWILL, C. J. AND DAVID, M. M.: *J. Biol. Chem.*, **177**: 751, 1949.
24. FRICK, P.: Personal communication.
25. STEFANINI, M.: *Bull. New York Acad. Med.*, **30**: 239, 1954.

Case Reports

CALCIFIC ARTERIOSCLEROSIS OF INFANCY*

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THE RARE OCCURRENCE of vascular disease in infants of sufficient degree to cause death is of considerable interest to both clinician and pathologist. Some 65 cases of calcific arteriosclerosis in infancy have been reported in the literature since 1891, when Bryant and White[1] first drew attention to the disease.

Study and discussion of the subject is principally to be found in the sporadic reports in the literature. The majority of standard textbooks of pædiatrics and pathology merely mention its existence. Most of the cases so far reported, including the one presented here, appear to belong to a group whose pathological features are more or less distinctive. These consist of the deposition of amorphous calcium adjacent to the internal elastic lamina of medium-sized and small arteries accompanied by hyperplasia of the intimal fibroblastic tissue and consequent narrowing or complete occlusion of the lumen. The coronary, splenic and renal arteries are most often involved, although lesions have been described at many other sites. Involvement of the coronary arteries has commonly led to infarction of the myocardium, which is not infrequently quoted as the immediate cause of death.

Clinically, the age range is between birth and two years. The symptoms are often of rather sudden onset and are those of acute congestive heart failure in a previously well infant. A few cases reported appear to have run a more protracted course, lasting a few weeks or months. The diagnosis is rarely, if ever, made ante mortem.

The infant, aged nine months, was well until 36 hours before death. At this time, he developed mild fever and anorexia. He was not considered seriously ill and received an intramuscular injection of penicillin. During the following hours, he did not improve and was finally admitted to hospital.

On admission, the child's temperature was 102.6° F., pulse 154, respirations 70. Respiratory distress was marked and he appeared to be gasping for air by a series of grunting respirations in which all the muscles of respiration were brought into play. He was immediately placed under continuous oxygen therapy and given further intramuscular injections of penicillin, but died two hours after admission.

Enquiry into his past history revealed nothing except a mild attack of diarrhœa at the age of six months, lasting only a few days. He was a normal, full-term baby with an unremarkable ante-natal and post-natal course and had progressed well until the onset of his terminal illness. No history of excessive vitamin D or calcium intake was elicited.

AUTOPSY FINDINGS

The infant was a well-developed, well-nourished, nine-months' old male child. Approximately 15 c.c. of straw-coloured fluid was present in the pericardial sac. The visceral and parietal pericardia were smooth and glistening. The heart was of normal weight but the chambers,

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