B_6-group. The ratios of CSF levels to blood levels of these vitamins are shown in Table I.

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Macroglobulinemia in a Transplantable Mouse Leukemia.* (25676)

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(Introduced by G. Asboe-Hansen)

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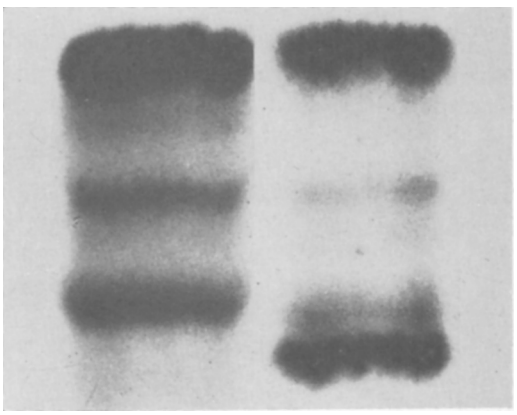
Gamma globulin-like paraproteins have been described in various mouse transplantation lines characterized by myelomatous(1,2) or plasmacellular leukemic(3-7) lesions. The subject of this report is the new observation that the paraprotein of a new transplantation line of leukemia appeared to be a macroglobulin and that the microscopic lesions seen in this line are comparable to those described in Waldenström's macroglobulinemia in man.

Material and methods. Methods of total protein estimation, paper- and immunoelectrophoresis, have been described(7). Ultracentrifugation was performed in a Spinco ultracentrifuge (Model E equipped with a rotor temperature indicator and control unit at 59,780 rpm at 20°C) on serum samples, diluted with 2 volumes of 0.9% NaCl. *The transplantation line,* originated in a 28-months old, untreated (CBA x DBA/2) F_1 mouse(8) with enlargement of lymph nodes and splenomegaly. Following subcutaneous inoculation of leukemic tissue, percentage of takes through 12 transfer generations was 100, survival time being 6 to 10 weeks, gradually shortening to 4 to 6 weeks.

Results. Total serum protein was found reduced to 3-4.5 g% (normal values 6.1-6.7 g %).

Serum protein pattern in (a) *paper electrophoresis* (Fig. 1) showed a pathologic globulin fraction of fast gamma mobility, in the first 5 passages constituting up to 40% of total protein; gradually fading, it disappeared in the tenth passage. b) *Immunoelectrophoresis* (Fig. 2) revealed an abnormal precipitation bow in the beta-1-beta-2- area located



6.1 g %	Total protein	5.1 g %
60%	Alb.	29%
5%	α_1	6%
9%	α_2	10%
15%	β	15%
	Path. fr.	35%
11%	γ	5%

FIG. 1. Paper electrophoresis of normal mouse serum (left), and of serum from a mouse No. 2420 of fourth transfer (right) characterized by large, abnormal gamma fraction.

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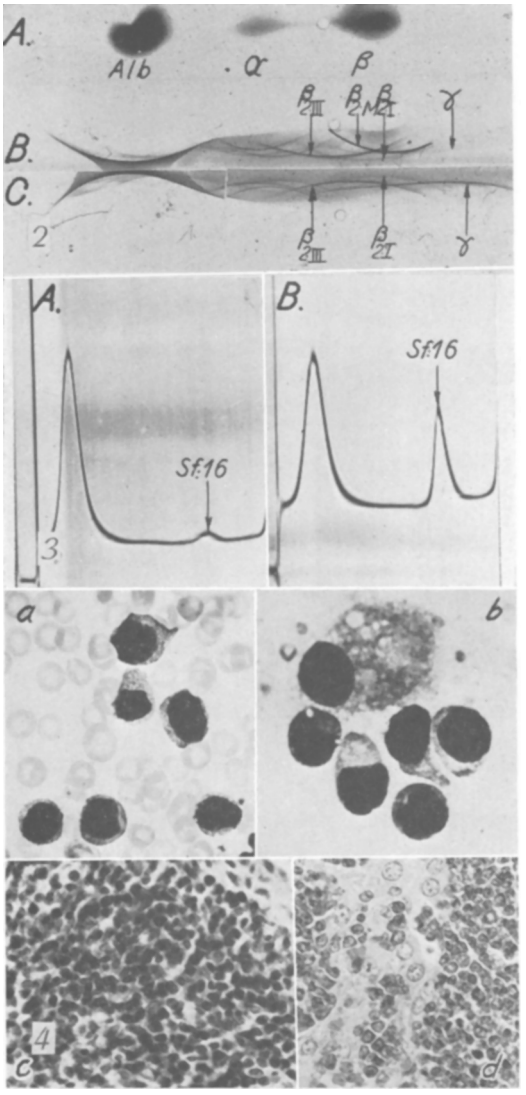

FIG. 2. *A.* Conventional agar electrophoresis of serum from a mouse of third transfer, showing electrically separated fractions. *B.* Immunoelectrophoresis of same serum; presence of a precipitation bow (beta-2-M) not usually seen in normal mouse serum, increased mobility and concentration of beta-2-III and highly reduced extent of gamma bow are evident from comparison with *C*. *C.* Immunoelectrophoresis of serum from normal mouse.

FIG. 3. Ultracentrifugation diagram: *A.* of normal mouse serum, only a small S 16 peak is present. *B.* of serum from a mouse No. 2420 of fourth transfer, showing significant increase of S 16 component.

FIG. 4. *a.* Smear of blood from mouse of ninth transfer — Giemsa stain — × 550; leukemic cells showing large, somewhat eccentric nuclei and scarce, finely vacuolized cytoplasm may be classified as intermediate forms between mouse lymphocytes and plasmocytes. *b.* Imprint preparation of infiltrated lymph node from mouse of third passage, "naked nuclei" cells, plasmocytoid lymphocytes and a large reticulum cell with abundant, vacuolized cytoplasm are seen, the latter probably identical with PAS-positive reticulum cells seen in sections. *c.* Tumor at site of inoculation—fourth passage No. 2420—Hem.-Eos. stain—× 225; majority of cells appear as naked nuclei, but diffusely scattered larger reticulum cells with more abundant cytoplasm and paler nuclei are seen. *d.* Liver metastasis from the same mouse as in *c*—methyl-green-pyronine method—× 225 showing chromatin structure and pyroninophilic cytoplasm of the tumor cells.

most centrally in all bows, anodically limited by the central filling hole and cathodically superimposed on the beta-2-I (transferrin) bow. In the terminology for human serum this bow is named beta-2-M. In addition, a hypogammaglobulinemia decreasing through the passages to agammaglobulinemia, the latter present from the 5th transfer, and a constantly increased mobility and concentration of beta-2-III bow were observed; this last change, however, is unspecific in that it is seen with all types of malignant growth (6,7, 9,10). In the 7th transfer the pathologic bow was but faintly developed and was invisible from the tenth passage. c) *Ultracentrifugation* (Fig. 3) of serum from a mouse of the fourth transfer showed a large, abnormal globulin peak with sedimentation coefficient 16.2 S constituting 27.5% and located correspondingly to a minimal peak with sedimentation coefficient 16.9 S of normal serum. In conformity with electrophoretic findings, ultracentrifugation of serum from a mouse of the 9th transfer showed an almost normal picture.

Morphologic blood changes. Total number of white blood cells was increased (up to 50,000/cmm) as the result of granulocytosis and presence of numerous (up to 98%) rather small, abnormal cells (Fig. 4a), mostly resembling mature lymphocytoid cells with more or less marked tendency towards plasmacellular differentiation; moreover, a few large dark-staining "blast"-like cells were seen. From the tenth passage lymphocytoid cells appeared more immature, more "blast"-like with dark blue cytoplasm and more reticularly structured nuclei.

Macroscopic lesions in the grafted mice were enlargement of thymus, peripheral and mesenteric lymph nodes, severe hepato- and splenomegaly and perirenal infiltration.

Microscopic tissue lesions were infiltration

of typical leukemic localization widely spread, and most markedly developed in the above mentioned organs. Fig. 4 b-d shows morphology of the tumor cells. In imprint preparations of leukemic tissue (Fig. 4 b), the dominating cells were of the same appearance as those seen in the blood. In addition, a varying number of large reticulum-cell like cells were present. Stained with hematoxylin-eosin (Fig. 4 c), the tumor cells presented themselves as naked nuclei. With methyl-green-pyronine staining (Fig. 4 d), they were seen as closely packed, rather small cells with scarce, moderately to heavily pyroninophilic cytoplasm and with relatively large, round or oval nuclei with a distinct nuclear membrane and a fine chromatin network, often with one or 2 small nucleoli. Between the tumor cells large reticulum-cells with vacuolized and or PAS-positive cytoplasm were located. This picture persisted through 10 transfers. Thereafter the tumor growth showed dedifferentiation into a polymorphous reticulo-sarcomatosis killing the host at a time when only moderate infiltrations had developed; this applied particularly to the liver. In the majority of hosts of all transfers, the Kupffer cells of liver were found PAS-positive, swollen, containing granules, globules and vacuoles.

Discussion. These findings suggest the existence of a macroglobulinemic disease in mice. Localization in paper- and immunoelectrophoresis of the abnormal globulin fraction and its sedimentation coefficient in ultracentrifugation correspond to the findings in macroglobulinemia in man(11-15). The similarity also applies to reduction of the non-influenced immunoglobulins usually seen in paraproteinemic diseases in man(16), and to occurrence of characteristic, more or less plasmacellularly differentiated, lymphocytoid cells (11,17,18), appearing as naked nuclei in hematoxylin-eosin stained sections.

Summary. A transplantable mouse leukemia characterized by presence of a large, macromolecular serum globulin fraction and of more or less plasmacellularly differentiated, lymphocytoid cells is reported. The disease shows definite similarity to human macroglobulinemia.

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