post-infection rabbit corneas reported by Sery(6) may have coincided with similar spontaneous reactivation of infection—perhaps immediately prior to excision.

The repeated isolation of herpes simplex virus with and without attempts at induction and interspersed by long periods of absence of virus release is of considerable interest. Corneal herpes simplex in the rabbit thus closely parallels the syndrome of recurrent human cutaneous, ocular and mucous membrane herpes simplex infection and affords an accessible model for experimental study.

*Summary.* Herpes simplex virus was inoculated into corneas of 8 rabbits with the induction of herpetic keratitis which proceeded to healing. The animals were then sensitized to horse serum and an Arthus reaction was induced in the healed cornea. Herpes simplex virus was repeatedly recovered in rabbit kidney cell culture from swabbings taken from these corneas. This phenomenon was observed 7 times in 19 attempts at induction. While there were several instances of spontaneous virus release, none occurred during the 14 day post-Arthus period in the unchallenged eyes of animals in which bilateral infection had been induced previously.

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## Beta-2A (=Beta-3-II=Gamma-1A) Mouse Leukemia with "Flame Cells" in Leukemic Infiltrations and Degenerative Lesions in Muscles.* (26710)

RAGNA RASK-NIELSEN, J. F. HEREMANS, H. E. CHRISTENSEN AND R. DJURTOFT
(Introduced by N. A. Thorn)
*Inst. of Biochemistry, Inst. of Pathological Anatomy, Univ. of Copenhagen, Denmark, Clinique Universitaire St. Pierre, Univ. de Louvain, Belgium, and Research Laboratories, Carlsberg Breweries, Copenhagen, Denmark*

Gamma globulin-like paraproteins have been described in various mouse transplantation lines characterized by plasmacellular leukemic(1-6) or myelomatous (7-10) lesions. More recently a transplantation line associated with macroglobulinemia and with microscopic lesions comparable to those seen in Waldenström's macroglobulinemia in man was described(11). This report concerns the new observation that the paraprotein of a transplantation line of leukemia appeared immunoelectrophoretically to be of a type homologous to the recently described human beta-2A (=gamma-1A) myeloma proteins (12-13). In addition, some of the cells ("flame cells") present in the leukemic infiltrations resembled those very recently considered to be pathognomonic of human beta-2A myelomas(14). Degenerative lesions in cardiac and skeletal muscles similar to those described in thiamine-deficient mice(15) were also observed.

*Material and methods.* Methods of paper- and immunoelectrophoresis etc. have been described(5,16,17). For estimation of hexose content of paraproteins, total protein-bound hexose (anthrone method) was related to scannings of PAS-stained paper electrophoreses of whole sera(18). Ultracentrifugation was performed at 60,000 rpm in an oil-driven ultracentrifuge (Svedberg type) on serum diluted 4 times in phosphate buffer, pH 6.66, containing 0.2 M sodium chloride. The *transplantation line* originated in a 28-months old, untreated (CBA × DBA/2) $F_1$ mouse(19) with enlarged lymph nodes and

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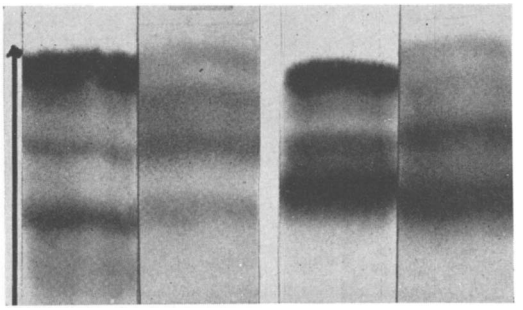

FIG. 1. Bromphenol blue-stained and PAS-stained electrophoreses of normal serum (left) and pathological serum (right); in the latter the heavily PAS-positive beta-fraction constituted 45% of total protein, in the former, 19%. Bromphenol blue-stained strips were run on 10 $\mu$l, PAS-stained on 20 $\mu$l of serum.

renal amyloidosis. Following subcutaneous inoculation of leukemic tissue, percentage of takes through 10 transfers was 80-100, survival time 9-10 months, gradually shortening to 2 to 3 months.

*Results.* A. *Serum proteins.* a) Total serum protein was found normal in earlier passages, then gradually decreased to about 4 g%. b) *Paper electrophoresis* (Fig. 1) revealed an increased beta globulin fraction, which in some mice, particularly of the earlier passages, appeared as 2 more or less merging bands. This fraction was highly PAS-positive (Fig. 1). c) *Hexose content* of the paraprotein from a mouse of the sixth passage was found to be 6.7%. Corresponding values for the gamma-type mouse paraproteins(4-6) ranged from 1.0 to 2.8%. d) *Immunoelectrophoretical analysis* of the pathological serum (Fig. 2A) showed the paraprotein as a heavy bow lying in the beta-2 area and apparently being (a) part of the gamma-globulin line. Evidence for some heterogeneity was visible from a deflection of the abnormally extended anodal end of the paraprotein bow. In addition a dense precipitate in the vicinity of the filling hole appeared to correspond to some amount of serum albumin migration with beta velocity. The anodal end of this precipitate could be followed into the cathodal end of the normal serum albumin bow. Both disappeared after absorption of the anti-mouse antiserum with purified mouse serum albumin, thus demonstrating their identity.

By using an antiserum produced against the microsome fraction of the leukemic tissue, a clearer and more intense picture of the serum paraprotein could be obtained (Fig. 2B). Confrontation of such a pattern with the one of normal white mouse serum, using the technic of interrupted slits(13,17), showed the paraprotein bow to fuse intimately with a normal bow previously described as beta-3-II(16). Recent studies on normal white mouse serum have indicated identity between this beta-3-II globulin and the protein of human serum known as beta-2A-globulin (=gamma–1A–globulin). The paraprotein also showed evidence for cross-reaction with the normal gamma-globulin (Fig. 2B). The same relationships between paraprotein on one hand and normal beta-2A and gamma-globulin on the other hand were also evident from an experiment (Fig. 2C) in which the antiserum against the microsome fraction was used for confrontation of a normal white mouse serum and a mixture of normal and pathological serum. The superimposed paraprotein bow here obviously cross-reacted both with normal beta-2A and normal gamma-globulin. The free cathodal tip of the paraprotein bow is to be interpreted as evidence for the existence of pathologic, antigenic determinants, whose corresponding antibodies were present in the homologous antiserum here employed. e) *Ultracentrifugation* of serum from one mouse (Fig. 3) revealed the following components: S 4.2 77%, S 6.2 10%, S 11.6 8%, S 16.8 5% (normal values: S 4.5 89%, S 7.8 2%, S 17.9 9%), thus demonstrating an excess of the S 6-8 component and 8% of a S 11.6 component. In serum from another mouse similar findings were observed.

B. *Macroscopic lesions* were marked enlargement of all lymph nodes, thymus, spleen and liver, but no growth at site of inoculation In some mice with severe leukemic infiltration, fine, sharp, white streaks were seen in the cardiac and lumbar musculature.

C. *Microscopic lesions* were infiltration of typical leukemic localization in the above mentioned organs, increasing in severity through the serial transfers. The cells of the infiltrations were of 2 types (Fig. 4A): a)

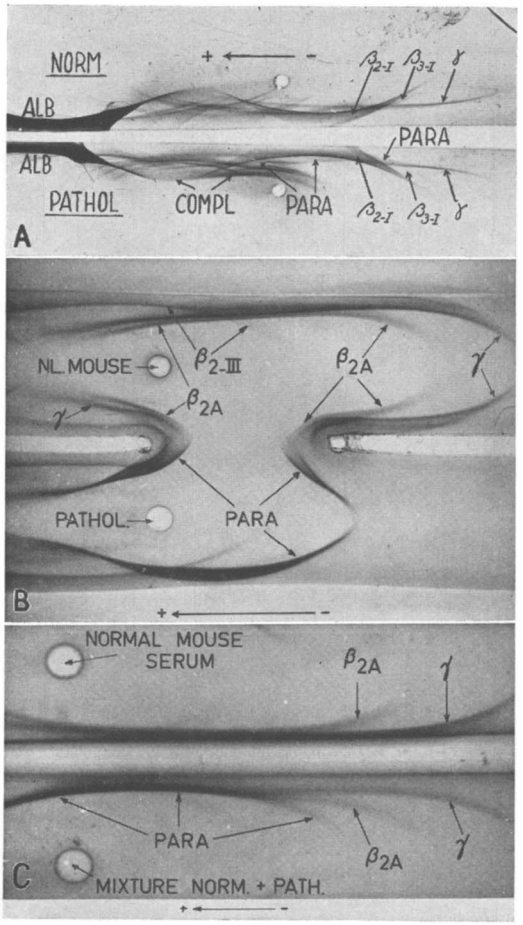


FIG. 2. Immunoelectrophoreses. A. Normal and pathological serum developed with anti-normal mouse serum antiserum. The lower pattern shows the heavy paraprotein line cathodally ending in the remnant of the normal gamma bow and anodally extending into the slow alpha-2 area, where the albumin-paraprotein complex is also visible. B. Interrupted slit technic using antiserum against homologous tumor microsome fraction and confronting the patterns of pathological (lower half) and normal white mouse serum (upper half). Fusion of paraprotein bow with both normal beta-2A-globulin and normal gamma-globulin is evident. C. Confrontation of normal white mouse serum alone (upper part) and a mixture of the latter with pathological serum (lower part), using anti-microsome fraction antiserum. Cross-reaction is obtained between paraprotein and both normal beta-2A- and normal gamma-globulin. The free cathodal tip of paraprotein corresponds to pathological antigenic determinants reacting with their homologous antibodies.

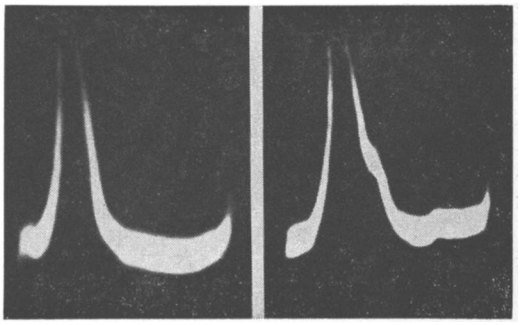

FIG. 3. Ultracentrifugation diagrams; exposure after 50 min. A. Normal mouse serum. B. Pathologic serum showing S 6 and S 11 components.

rather small, closely packed cells with scanty, slightly basophilic cytoplasm and relatively large, round, ova or angular nuclei with a strong nuclear membrane and with a low chromatin content, arranged in small clumps connected by fine threads, and b) much larger cells with sharply demarcated cell borders and with very abundant eosinophilic or neutrophilic cytoplasm, moderately positive to the PAS-reaction, occasionally of a glassy appearance and sometimes showing minute vacuoles; the nuclei were small, round, rather dark and definitely eccentrically located. In Giemsa-stained imprints (Fig. 4B) the first-mentioned cell type appeared as cells with large, round, intensely staining nuclei with delicate chromatin pattern and scarce, moderately basophilic cytoplasm. The other cell type was seen as light cells of varying size characterized by very small, dense, eccentrically placed nuclei, and abundant, inhomogeneous basophilic cytoplasm; in a varying number of these cells, however, the cytoplasm, except for a juxtanuclear space, was filled with minute eosinophilic spots, which in the peripheral part of the cytoplasm coalesced to smaller or larger eosinophilic areas (so-called "flame cells"). Numerous naked nuclei were always seen in the imprints.

In the latest passages the large, light cells were seen diffusely spread as single cells among the cells of the small type; they were most numerous in the lymph nodes, where they occurred in large numbers in the severest cases accumulating to whole coherent areas; they were less numerous in the spleen and more rarely seen in the perivascular liver infiltrations. After administration of cortisone to one mouse the number of these cells was found reduced. In the 5th to 7th transfers the large, light cells

were located as small clusters or streaks between the small cells, preferably near the nodal sinuses or where "free space" probably had been available; they were not present in all lymph nodes of each mouse. In the very earliest passages and in the spontaneous case only a few of the light cells were present in the leukemic infiltrations.

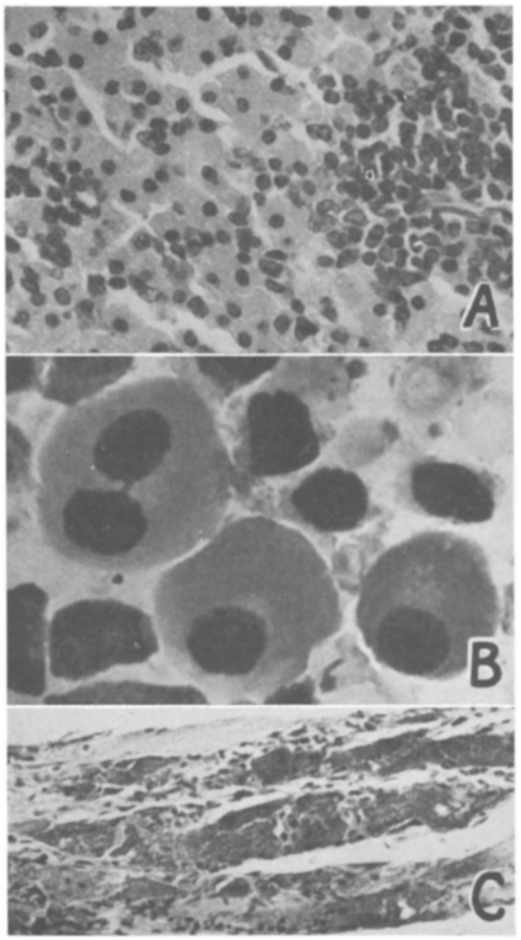


FIG. 4. A. Section of tumor infiltrated lymph node. Areas of large, polygoneal cells with abundant slightly eosinophilic or neutrophilic cytoplasm and with small dark nuclei intermingled with masses of small dark closely packed tumor cells with a rather large, round nucleus and with scarce, slightly basophilic cytoplasm with hardly visible cell borders. Hem.-Eos. × 210. B. Imprint preparation of tumor infiltrated lymph node. Three cells corresponding to the larger polygoneal cells in A; one of them binucleate. These cells are large with dense, ovoid, eccentrically located nuclei and abundant inhomogeneous cytoplasm with a clear perinuclear zone; eosinophilic areas of varying extent occupy part of the otherwise basophilic cytoplasm. The small cells with large dark nuclei and with scarce or damaged cytoplasm correspond to the small dark cells of A. Giemsa stain × 1400. C. Amorphous masses in striated muscle apparently replacing muscular fibers. The granular and hyaline appearance is visualized. Hem.-Eos. × 350.

In mice of the second transfer severe splenic amyloidosis of secondary type and in 2 mice heavy intestinal amyloidosis was observed.

The white streaks in the cardiac and lumbar muscles seen in mice with marked infiltrations appeared (Fig. 4C) as amorphous, dark staining masses often of hyaline, sometimes of granular appearance. They stained heavily with the PAS-reaction, not at all with methyl violet, only slightly with alcian blue and in the Hale reaction, and not with chromotrope 2R. Bacteria or fungi could not be detected. Moderate to severe calcification appeared in about half the cases. With few exceptions no tumor cells and no signs of inflammation were found in relation to the deposits. The same changes, although not macroscopically visible, were seen in most mice of the later passages, located as mentioned above and particularly in muscles around the larger joints.

*Discussion.* The following factors point to the mouse leukemia here reported as homologous to the beta-2A-type of human myeloma. 1) The immunological correspondence of the paraprotein with beta-2A-globulin and its cross-reaction with normal gamma-globulin, which are characteristic of human beta-2A-type paraproteins, were also found here. 2) As in many human beta-2A-myeloma cases, a slow moving albumin component probably corresponding to complex formation with the paraprotein(13) was also evident in this leukemic mouse serum. 3) The hexose content of the mouse paraprotein here described was considerably higher than in other mouse leukemias of the gamma-type, a situation corresponding to the findings in the homologous 2 human myeloma types(13). 4) The electrophoretic mobility of the mouse paraprotein here reported was of the order of magnitude of beta- and to some extent even of slow alpha-2-globulins. 5) The paraprotein appeared to be inhomogeneous in the ultracentrifuge and to contain components sedimenting with coefficients larger than 7S,

as also observed in human beta-2A-type myelomas(20). 6) The peculiar type of large light cells was consistently found to be associated with the leukemia described here, in contrast with the cytological findings in ordinary gamma-type mouse paraproteinemias. Similar cells have been found to be pathognomonic of human beta-2A-myelomas (14). The degenerative lesions in cardiac and skeletal muscles, so far of unknown etiology, have not been observed in other types of mouse leukemia.

*Summary.* A transplantable mouse leukemia with the following characteristics is described: Production of a paraprotein of beta-2A-type. Presence of peculiar large light cells ("flame cells") in the leukemic infiltrations and degenerative lesions in cardiac and skeletal muscles.

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## Heterologous Transplantation of Two Tissue Culture Lines of Glioblastoma Multiforme (TC 178 and TC 224).* (26711)

ELIAS E. MANUELIDIS (Introduced by H. S. N. Greene)
*Department of Pathology, Yale University School of Medicine, New Haven, Conn.*

It is extremely difficult to distinguish between "normal" and neoplastic cells growing in monolayer tissue cultures on a morphological basis(2,5). Furthermore, the histological features of neoplastic tissue culture cells do not give accurate information about the tumor of origin. It was thought that heterologous transplantation of tissue culture cells might solve these problems. The present communication deals with the successful heterologous transplantation of 2 permanent lines of human glioblastoma multiforme cultured in this laboratory. Glioma TC 178 has been growing since Sept. 4, 1957, and has been reported in detail in this journal(3). The line TC 224 has been in continuous cultivation since Feb. 28, 1959. A

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