Polymethacrylic Acid: Effects on Lymphocyte Output of the Thoracic Duct in Rats

Abstract. *The synthetic polyanion, polymethacrylic acid, was applied intravenously to thoracic duct cannulated rats. An increase of the lymphocyte count occurred in the lymph and in the peripheral blood. Polymethacrylic acid mobilized the lymphocytes from the reservoirs.*

The influence of polysaccharide sulfates and other compounds on the mobilization of lymphocytes has been receiving increased attention. Heparin induces lymphocytosis in animals (*1*). Lymphocytosis is produced by synthetic polysaccharide sulfates (heparinoids) in rats (*2*). The synthetic polyanion, polymethacrylic acid (PMAA), possesses antiviral activity and is less cytotoxic than the heparinoids (*3*); and it causes lymphocytosis in rodents (*4*).

The purpose of this study was to confirm the effect of PMAA on peripheral blood lymphocytes after thoracic duct cannulation and to determine whether this compound increased lymphocyte output under these conditions.

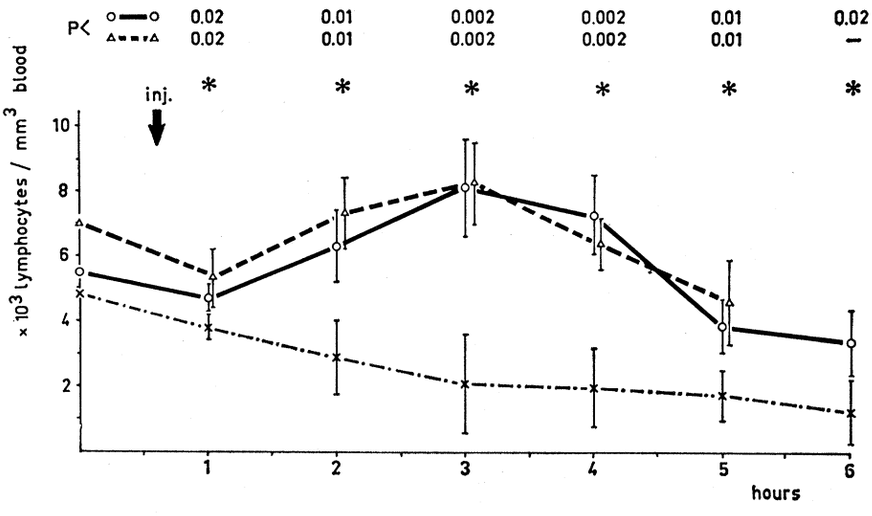
Male rats (WAG/Rij strain; 200 to 240 g) were used. The experiments were all done at the same time of day to avoid diurnal variation in leukocyte count. The animals were anesthetized with Nembutal (5 mg/100 g) intraperitoneally, and cannulas were inserted in the thoracic duct (*5*).

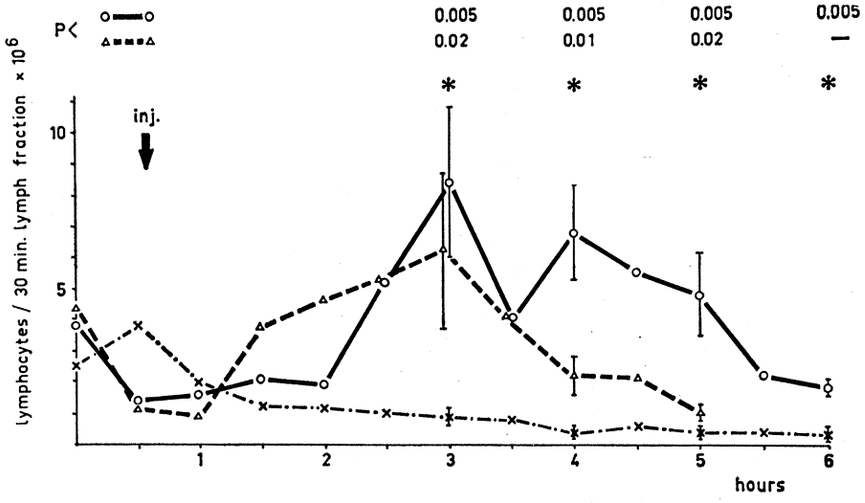
The lymph was collected at 30-minute intervals over a 6-hour period. Volume and cell count of every fraction were recorded to calculate lymphocyte output during each period. Blood samples were taken from the tail vein before the start of the lymph-flow and hourly thereafter. A differential cell count was made to estimate lymphocyte count.

The PMAA was prepared by polymerization of monomers, as described (*3*). A polydisperse preparation with a molecular weight ranging from 25,000 to 1,175,000 was administered into the femoral vein at the 60th minute following the start of lymph-flow, in 1 ml of phosphate-buffered saline (*p*H 7.2) in doses of 2 to 4 mg/100 g. The control rats received 1 ml of phosphate-buffered saline instead. At the end of the experiments the rats were killed, and the spleen and lymph nodes were removed for histological examination. The PMAA treatment produced an increase of the lymphocyte count (Fig. 1).

The lymphocyte output from the thoracic duct (Fig. 2) increased significantly after the treatment with PMAA compared to the controls (Student's test). The maximum coincided with the maximum in the blood (Fig. 1).

Histological examination showed a reduction of lymphocytes in the cortex of the lymph nodes and the white pulp





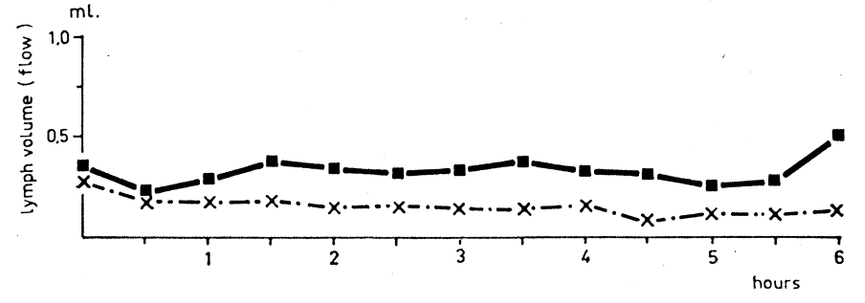


Fig. 1 (top right). Effect of polymethacrylic acid (PMAA) on the lymphocyte count in the blood of rats during thoracic duct drainage; n = five animals. Circles, 4 mg PMAA per 100 g of body weight; triangles, 2 mg PMAA per 100 mg of body weight; crosses, controls. Each point represents the mean, each * marked shows the mean ± S.D. Fig. 2 (bottom right). Effect of PMAA on the lymphocyte output (upper) and the lymph-flow (lower) of the thoracic duct of rats in 30-minute fractions; n = five animals. Circles, 4 mg PMAA per 100 g of body weight; triangles, 2 mg PMAA per 100 g of body weight; crosses, controls. Each point shows the mean, each * marked shows the mean ± S.D.

of the spleen in animals to which PMAA was administered. These findings resemble those recorded after treatment with dextran sulfate (*6*).

Like the polysaccharide polysulfates, PMAA mobilizes lymphocytes from the reservoirs into blood. The majority of the mobilized small lymphocytes reach the blood through the lymphatic vessels.

The finding of a simultaneous increase of the lymphocyte count in the peripheral blood of the rats with thoracic duct cannulas indicates another route from the lymphatic tissues to the blood, as described earlier (*7*).

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Secretory Activity and Oncogenicity of a Cell Line (MDCK) Derived from Canine Kidney

Abstract. *A cell line (MDCK) of dog kidney origin grows on a glass surface as a mosaic of epithelium with many multicellular hemispherical vesicles. The cells lining the blisters actively secrete into the cyst cavities. Suspensions of these cells injected intravenously in the chick embryo produce brain metastases resembling adenocarcinoma.*

The cell line MDCK (Madin-Darby canine kidney) was derived in 1958 from the kidney of a normal male cocker spaniel and has been used subsequently for virologic study (*1*). Confluent sheets of cells on glass developed multiple lesions described as "ulcers." We recognized that these focal lesions were not ulcers but hemispherical vesicles or blisters composed of numbers of cells. The blister-like character of the structures was appreciated best by examining the growth of living cultures on a microscope slide in a large flat tube (*2*; Fig. 1a).

We anticipated that any polarization suggesting renal tubular organization of the cells making up the wall of the blister would find the surfaces of the cells bordering the vesicle contents covered with microvillus processes. Polarization was readily demonstrated in the cells of the cyst wall, as well as in cells adherent to glass, but the orientation was the opposite of our expectation. Numerous microvillus processes and tight junctions were consistently found on the convex surfaces of the blisters and on the surface of the epithelial sheets bathed by medium. No basement membrane was evident on either surface of the epithelial sheet (Fig. 1b).

To discover whether the vesicles once formed were relatively stable structures, we examined them for periods of 1 to 2 days with low magnification time-lapse microscopy. Innumerable vesicles were observed to form, expand, distend, and collapse. The picture resembled the bubbling surface of gently boiling oatmeal. The polarized functional cells seem to resemble renal tubular epithelium while the medium is anatomically and functionally analogous to a glomerular filtrate. The vesicles are interpreted as interstitial collections of "reabsorbed" components of the medium, "glomerular filtrate," which accumulate in a plane of cleavage between cells and glass until the distended secreting epithelial membrane covering the confined fluid develops a significant break. Then the vesicle collapses as fluid leaks into the medium. The cells return to the glass surface. The defect between cells is repaired, and the accumulation of fluid between the sheet of cells and the glass surface is resumed.

Oncogenicity of MDCK in vivo has been tested by injecting suspensions of single cells (removed from glass surfaces with EDTA-trypsin) intravenously in chick embryos. In one experiment, 3×10^6 cells were injected into each of a series of 12-day-old embryos. Histologic examination of the brain and liver of 12 animals was made 1 week later. Multiple foci of metastases were seen in 11 of 12 brains (Fig. 2); none was found in any of the livers. The lesions

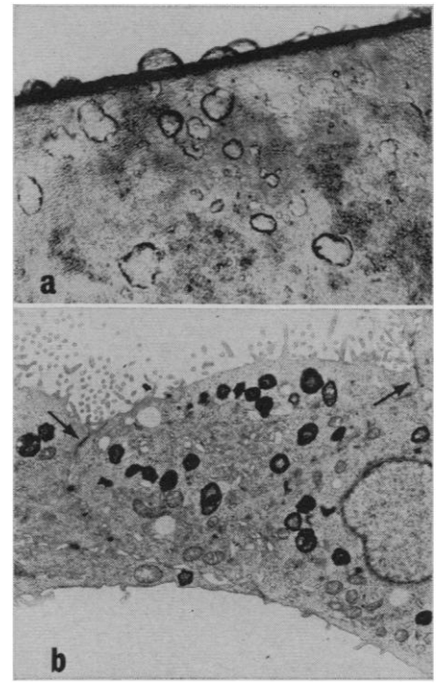

Fig. 1. (a) Living culture of MDCK growing on the surface of a microscope slide contained in a large flat tube photographed with an inverted microscope. In most of the field, the flat surface of the slide is seen completely covered with epithelium. The irregular, oval, scalloped structures (vesicles) seen on the flat surface appear as a series of hemispherical blisters on the edge of the slide (approximately × 25). (b) Vesicle wall grown on a glass slide. The convex surface on the top, the outer (medium-bathed) surface of the vesicle, is covered with numerous microvilli. Two tight junctions (arrows) join cell borders near the surfaces with many microvilli. The pattern of many microvilli and adjacent tight junctions indicate that the tissue culture medium is anatomically analogous to a glomerular filtrate. At the bottom of the figure, occasional microvilli are seen in the concave surface lining the fluid cavity of the blister. The fluid-filled cavity is perhaps analogous to an interstitial cyst. The dark structures in the cytoplasm are cytosomes (lysosomes) (× 3900).

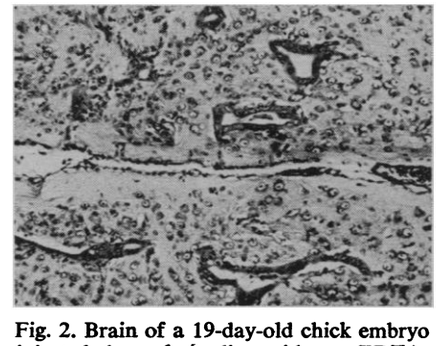

Fig. 2. Brain of a 19-day-old chick embryo injected 1 week earlier with an EDTA-trypsin produced, single-cell suspension of MDCK. Epithelial glandular structures are consistent with a histopathologic diagnosis of metastatic adenocarcinoma (hematoxylin and eosin; × 85).