Agglutination of leukemic cells and daunomycin entrapped erythrocytes with lectin in vitro and in vivo

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Summary. Wheat germ agglutinin (WGA) agglutinated the L_{1210} leukemic cells and daunomycin entrapped erythrocytes in vitro. Comparing with control preparations, the greatest increase in survival was obtained in vivo when the daunomycin entrapped erythrocytes and WGA were given to BDF_1 mice bearing L_{1210} cells.

It has become apparent that erythrocytes could be useful as carriers of drugs and other biologically active agents[1]. Wheat germ agglutinin (WGA) was initially characterized by its activity to agglutinate erythrocytes. In 1963, Aub reported a crude wheat germ preparation which contained a component preferentially agglutinating tumor cells[2]. Since that time, WGA has been isolated and extensively studied.

Survival of BDF_1 mice bearing L_{1210} cells

Daily treatment (i.p.), days 1–5		Mean survival time (days), n = 10
Control		7.1
Daunomycin	2 mg/kg	10.0
WGA	0.1 mg/kg	7.3
Erythrocyte entrapped DM	2 mg/kg	12.6
Erythrocyte entrapped DM + 10 min after WGA	2 mg/kg 0.1 mg/kg	17.9
Daunomycin + 10 min after WGA	2 mg/kg 0.1 mg/kg	10.5
Resealed ghosts + 10 min after WGA	0.5 ml/kg 0.1 mg/kg	6.9
Resealed ghosts + Daunomycin + 10 min after WGA	0.5 ml/kg 2 mg/kg 0.1 mg/kg	9.8

We entrapped daunomycin in resealed erythrocyte ghosts and studied on the agglutination of L_{1210} leukemic cells and resealed erythrocytes with WGA in vivo and in vitro.

Materials and methods. Resealing of erythrocytes was performed by the method of Bodemann and Passow[3]. Heparin-treated erythrocytes of BDF_1 mice were washed 3 times. A medium composed of 150 mM NaCl, 4.8 mM Tris, pH 7 4, was used both for washing and erythrocyte suspension. 1 ml of erythrocytes (hematocrit 50%) was mixed rapidly with 10 ml of cold hemolyzing solution consisting of 4 mM $MgCl_2$. After 5 min, 50 mg of daunomycin was added to the solution and gently mixed. After a 1-min-equilibration in the cold, 1.2 ml of 1.66 M NaCl was added to restore isotonicity. The suspension was incubated at 37 °C for 60 min with gentle shaking The resealed erythrocytes were collected by centrifugation at 25,500 × g for 20 min and washed twice with isotonic NaCl-Tris and in vivo studies were performed within 30 min. To estimate entrapped daunomycin, after hemolyzing resealed ghosts, daunomycin and hemoglobin were separated by gel-filtration chromatography using Sephadex G-100 (1 × 15 cm). Elution was performed with 0.01 M Tris, pH 7.2. Concentration of daunomycin was assessed by OD at 495 nm, assuming an $E^{1\%}_{1cm}$ 196[4]. Hemoglobin concentration was measured by adding samples to ferricyanide-cyanide reagent to convert the hemoglobin to cyanmethemoglobin and then reading the OD at 540 nm[5] (figure 1).

To agglutinate L_{1210} leukemic cells and daunomycin entrapped erythrocytes in vitro, L_{1210} cells (1×10^3 cells/0.1 ml of isotonic NaCl-Tris) and daunomycin entrapped erythrocytes (1×10^4 cells/0.1 ml of isotonic NaCl-Tris)

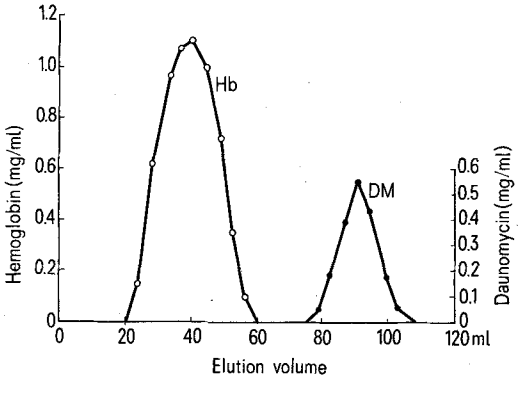

Fig. 1. Sephadex G-100 chromatography of hemolysate of daunomycin entrapped erythrocytes. Elution was performed with 0.01 M Tris-HCl, pH 7.2. DM, daunomycin (●—●); Hb; hemoglobin (○—○).

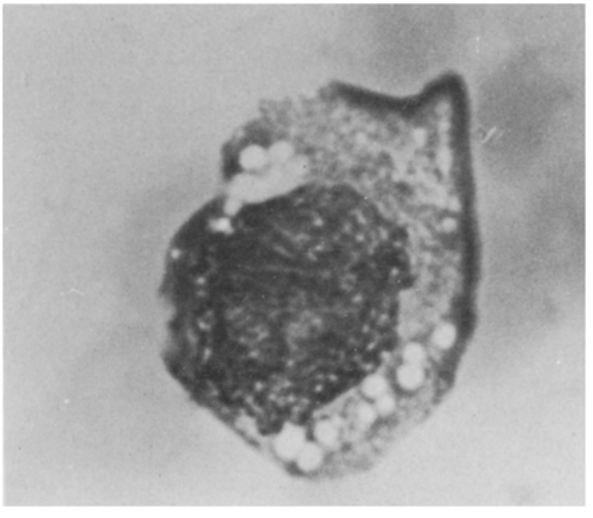

Fig. 2. Agglutination of L_{1210} cell and daunomycin entrapped erythrocytes. Giemsa staining.

were preincubated at 37 °C for 1 min. WGA (0.01 mg/0.1 ml of isotonic NaCl-Tris) was added to agglutinate the cells and agglutination was observed within 5 min. To study the effectiveness of agglutination in vivo, L_{1210} mouse leukemic cells (1×10^5 cells/mouse) were inoculated i.p. into BDF_1 mice. After 24 h the mice received the 1st of 5 daily injections of the daunomycin entrapped erythrocytes and WGA at intervals of 10 min.

Results and discussion. 1 ml of resealed erythrocytes entrapped about 4 mg of daunomycin. Resealed erythrocytes (0.5 ml) were suspended in 5 ml of isotonic NaCl-Tris at 37 °C and efflux of daunomycin was estimated at 1, 3, 6, 12 and 24 h and 23, 42, 78 and 84% of entrapped daunomycin was leaked out respectively.

WGA agglutinated the L_{1210} leukemic cells and resealed erythrocytes in vitro (figure 2). The greatest increase in survival time was obtained in vivo when the daunomycin entrapped erythrocytes and WGA were given (table).

WGA mediates attachment of resealed erythrocytes to tumor cells. The effectiveness of daunomycin entrapped erythrocytes against leukemic cells especially demands targeting of the erythrocytes to leukemic cells, and it may be possible through the use of the lectins which do not have agglutinability of normal erythrocytes and mitogenic activity to lymphoyctes but have agglutinability of leukemic cells and surface modified erythrocytes.

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Evidence for the presence of viable endothelial cells in cultures derived from dissociated rat brain[1]

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Summary. The morphology and histochemistry of dissociated newborn rat brain was studied in tissue culture. Direct microscopy of developing cells, electron microscopy and the alkaline phosphatase activity were used to identify the capillary endothelial cells.

Tissue cultures obtained from mechanically dissociated brain tissue have been used for many years[3]. As a consequence there is a cumulative information on the distinctive properties of neuronal and glial cells, and on the extent and nature of glial-neuronal interactions. However, none of the reports published so far have dealt seriously with the possibility that in dissociated cultures, the cells deriving from the brain capillaries can also grow under the same conditions.

In the present paper, we show how the capillary fragments obtained by mechanical disruption of brain tissue as inevitable contamination can undergo morphological changes in vitro, and result in growing cells of capillary origin. Furthermore, these cells show some histochemical characteristics typical for the endothelial cells of brain capillaries.

Cerebral hemispheres from 3-day-old rats were dissociated in sterile conditions by pushing the minced brain tissue through nylon sieves of 250 and 125 mesh pore sizes. Fragments being attached to the sieve were suspended in tissue culture medium and plated out immediately on the cover-slip, according to the culture method described in detail previously[4]. The homogenate obtained was centrifuged and processed further as described by Joó and Karnushina[5] for the isolation of capillaries from brains of adult animals. After differential and density gradient centrifugations, the pellet was resuspended and in part seeded. The ultrastructure of the pellet was checked in the electron microscope (figure 1). From the fragments of dissociated brain tissue, as expected, several different types of cells started to grow. Among the neuronal and glial cells, large (about 25 µm in diameter) occasionally elongated, but as a rule round and flat cells were growing (figure 2). The same type of cell of unknown nature was also present in those cultures, which were derived from the pellet of centrifugations. It was clearly seen that when the short segments or longer networks of capillaries settled in vitro, the large and flat cells originated from the smaller, elongated cells of the capillary tubes themselves (figure 3). The cells of capillary origin, possibly due to their stronger viability, have grown faster than other cells and within some days (varying from 2 to 5 days) formed a continuous monolayer (figure 2). Several neuronal and glial cells were observed to grow later on the surface of the monolayer, establishing contacts either with each other or with the capillary cells. To characterize the enzyme pattern in the cells of capillary origin, histochemical reactions were performed. Alkaline phosphatase[6] and dopa-decarboxylase activities confined to the capillary wall have been regarded as characteristic marker enzymes for the endothelial cells of brain capillaries. Many large and flat cells exhibited strong alkaline phosphatase activity (figure 4), which could easily be observed among the nonreactive neuronal and glial cells. There were, however, cells which did not show alkaline phosphatase activity, although they had the characteristics of capillary origin. L-dopa was taken up to varying extent by almost every cell regardless to its nature and origin (figure 5). Dopa-decarboxylase, though believed to indicate an

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