PHOSPHOLIPASES A OF KREBS II ASCITES CELLS:
SPECIFICITY, RELEASE AND SUBCELLULAR LOCALIZATION

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The phenomenon of contact inhibition has proved to be closely related to growth control. It was demonstrated that insensitivity of cells in culture to contact inhibition is proportional to their malignancy in animals (6). In attempting to define the biochemical basis of contact inhibition, attention has naturally centered on the cell surface and its components, and particularly on phospholipids. It was stated that virus-transformed cells, thus having malignant potentialities renewed their phospholipids at a greater rate than normal controls. Among the enzymes of the plasma membrane, the phospholipases A have been mainly studied in normal hepatocyte (5) (11) and in cells infected with measles virus (8). We have a poor knowledge of them in the neoplastic cell and their role in the cell membrane is not clear. Therefore we investigated the specificity A₁ or A₂, the intracellular distribution and the release in the extracellular environment of phospholipases A in Krebs II ascites tumor cells, maintained in female Swiss strain mice (9-12 weeks aged) by weekly intraperitoneal transfer of ascitic fluid.

MATERIALS AND METHODS

Cells were separated from their exsudate by centrifugation at 700 xg (10 min) and washed with a phosphate buffer saline (PBS: 1.5 mM KH₂PO₄, 8 mM Na₂HPO₄, 137 mM NaCl, 1.8 mM KCl, pH 7.35). The cell pellet was suspended in nine volumes of homogenization medium for subcellular fractionation (Fig. 1) and then homogenized in a Dounce apparatus.

For optimum pH and release studies, cells were incubated in
WHOLE HOMOGENATE (H) in Tris 5 mM, MgCl₂ 0.2 mM, sucrose 0.25 M, pH 7.4

700 xg (10 min) → P₀.₇ (resuspended in the same medium)

700 xg (10 min)

NUCLEAR FRACTION (N)

40,000 xg (30 min)

MITOCHONDRIAL-
LYSOSOMAL
FRACTION (ML)
(resuspended in Tris 1 mM, MgCl₂ 1 mM, pH 8.6
1 mM, pH 8.6

98,000 xg (14 hrs) on 12 to 40 % w/v linear sucrose gradient in Tris

14 fractions

220,000 xg (60 min)

MICROSOMAL FRACTION (P)

SOLUBLE FRACTION (S)

Fig. 1: Subcellular fractionation of Krebs II ascites cells. All operations were carried out between 1-4°C. Acceleration forces are expressed as g max.

two volumes of PBS for 20 min at 37°C with shaking. Then cell suspension was centrifugated at 700 xg (10 min) and the pellet diluted with two volumes of new PBS before cells disruption. The incubation-PBS and the exsudate were centrifugated at 23,000 xg (10 min) to eliminate the remaining cells.

Acid and neutral phospholipases A₁ and A₂ activities (E.C.3.1.1.4) were determined with radioactive phosphatidylethanolamines or phosphatidylcholines, labelled on the fatty acid moieties with (1-¹⁴C) linoleic acid on the C₂ of the glycerol and (9,10-³H₂)-palmitic acid on the C₁ (4). In the subcellular localization of phospholipases A, we also considered the possibility of phospholipases C or D or A₁ + A₂ (on the same molecule) at pH 4.0 and pH 7.5. The substrate used in these cases (100 to 150 nMoles per assay) was a phosphatidylcholine labelled with (¹⁴C)-choline.