Agglutination of cells by concanavalin A and pea phytohemagglutinin

Cells	0.1% trypsin treatment (min)	Concanavalin A concentration (μg/ml) 1500	1000	750	500	200	100	50	25	10	5	1	500 μg/ml in 30 m*M* sugar
LWF		−	−	−	−	−	−	−	−	−	−	−	
	6	+	+	+	+	−	−	−	−	−	−	−	−
HuEF		±	−	−	−	−	−	−	−	−	−	−	
	10	+	+	+	+	+	±	−	−	−	−	−	−
BLEF		+	−	−	−	−	−	−	−	−	−	−	
	10	±	−	−	−	−	−	−	−	−	−	−	−
LW13K2		+	+	+	+	+	±	−	−	−	−	−	−
[a])		+	++	++	+	+	±	−	−	−	−	−	−
LW13-RsK4		+	+	+	+	+	+	±	−	−	−	−	−
RsK4-A4		+	++	++	++	++	++	++	+	+	−	−	−
RsK4-A4Kl		+	+	+	+	+	+	+	+	−	−	−	−
CZW 1		+	+	+	+	+	+	−	−	−	−	−	−
		Pea phytohemagglutinin concentration (μg/ml)											
LWF		+	−	−	−	−	−	−	−	−	−	−	
	6	++	+	+	+	+	+	+	+	±	−	−	−
HuEF		+	+	±	−	−	−	−	−	−	−	−	
	10	++	+	+	+	+	±	−	−	−	−	−	−
BLEF		+	±	−	−	−	−	−	−	−	−	−	
	10	+	+	+	+	−	−	−	−	−	−	−	−
LW13K2		++	++	++	+	+	+	±	−	−	−	−	−
LW13-RsK4		++	++	++	+	+	+	+	±	−	−	−	−
RsK4-A4		+++	+++	+++	+++	++	++	++	+	+	−	−	−
RsK4-A4Kl		+++	+++	+++	++	++	++	++	+	+	+	−	−
CZW 1		++	++	++	++	+	+	+	±	−	−	−	−

The cell concentrations used in agglutination assay ranged from 0.8×10^6 to 2.5×10^6 cells/ml. The appropriate range of concentrations was determined by pretesting to meet rigid serological criteria for reading agglutination. One plus sign (+) designates only small macroscopically observable clumps resisting a gentle shaking with test tube; 2 plusses (++) = clumps at least twice big as one plus clumps; 3 plusses (+++) = very large clumps only. The plus-minus sign covers a range of microscopical clumps (3-20 cells) mixed with numerous free single cells. [a]) In this case con A prepared by the Agrawal and Goldstein procedure was tested for comparison with the all other results obtained with con A manufactured by Calbiochem.

'uncoupling' effect. The slightly higher and more pronounced agglutination activity of pea phytohemagglutinin is probably due to its higher molecular uniformity at physiological pH[1].

Zusammenfassung. Phytohaemagglutinin, aus der Erbse *Pisum sativum* L. isoliert, agglutiniert vornehmlich Tumorzellen, ähnlich wie Concanavalin A. Die durch RNS-onkogene Viren transformierten sowie spontan transformierten Zellen befolgen während der Agglutination das normale Muster der Tumorzellen-Interaktion mit Concanavalin A oder Weizenkeim-Agglutinin.

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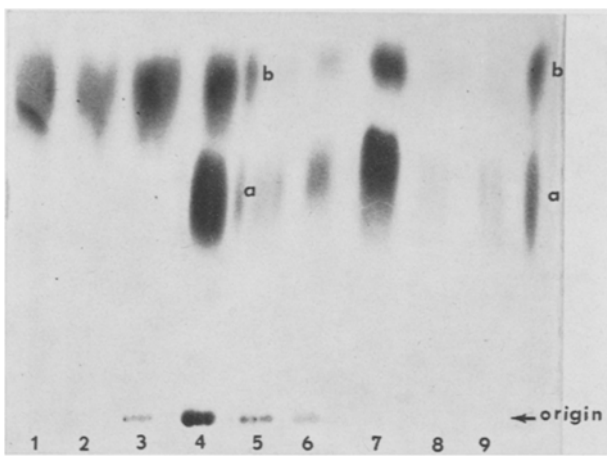
Phospholipid-Calcium Complexes in Experimental Tumors

Experimental tumors show a very high calcium uptake[1]. A large amount of the incorporated ^{45}Ca is localized in the microsomal fraction[2] which contains fragments of many cellular membranes[3]. It is possible that besides the contribution of the probable nucleic acid-calcium interaction[2] a very significant part of this incorporation is due to phospholipid-calcium complex formation. A recently reported increase in tumors of the membrane-bound calcium[4] appears to corroborate this assumption. Furthermore, it has been demonstrated that the binding of calcium by acidic phospholipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE), is enhanced in the presence of phosphate ion[5].

In the present experimental works the phospholipids from Ehrlich ascites, lymphatic leukemia BW5147 and lymphosarcoma 6C3HED were extracted using the organic phase of a mixture of chloroform-methanol-water (2:1:2 by volume). The extract was washed 3 times with

the aqueous phase, evaporated under vacuum and the excess lipid (non complexed) was separated washing 3 times with ethanol-diethyl ether (3:1 by volume). The insoluble complex was dissolved in concentrated formic acid and partitioned between the organic and aqueous layers of chloroform-methanol 1 *N* HCl (200:100:75 by volume) as described elsewhere[5]. Samples of the non complexed lipid, complex dissolved in chloroform and partition lipids remaining in the organic layer of the partitioned complex were analyzed by thin-layer chromatography on basic plates of silica gel without calcium sulfate binder, using chloroform-methanol-glacial acetic acid-water (50:25:7:3 by volume) as solvent[6]. Pure standards of PS and PE were chromatographed on the same plate. The spots were located, using ninhydrin reagent (Figure). The analysis indicates that practically the total PS and part of the PE are present in the tumor as phospholipid-calcium phosphate complex (Figure, 4, 5 6). These complexes show a non-migrating moiety which disappears by partition (Figure, 7, 8 and 9). Phosphorus was determined in the partition aqueous layer (inorganic phosphate) as well as in the organic partition layer and non complexed extract after hydrolysis with $HCLO_4$ by the method of MARTIN and DOTY[7]. Calcium was determined in the aqueous partition layer according to SOBEL and HANOK[8]. For Ehrlich ascites the molar ratio found was 64:8:15:15 (*P* as non complexed phospholipid: *P* as complexed phospholipid: *P* as inorganic phosphate: Ca).

Since it has been postulated that phospholipids may be involved in calcification at sites of primary mineralization[5], the importance of these experimental results in the interpretation of the tumor calcification process and of the carcinogenesis problem itself is clear when approached from the membrane phenomena point of view[9].



Thin-layer chromatography and ninhydrin detection of: uncomplexed phospholipids (1,2,3); complexed phospholipids (4,5,6) and partitioned complex (7,8,9); Ehrlich ascites: 1, 4 and 7; lymphatic leukemia BW5147: 2, 5 and 8; lymphosarcoma 6C3HED: 3, 6 and 9. a) Standard of phosphatidyl serine. b) Standard of phosphatidyl ethanolamine.

Zusammenfassung. Die chromatographische Trennung und chemische Analyse der Phospholipide von Versuchstumoren zeigte, dass die grösste Menge von Phosphatidylserin und ein Teil des Phosphatidylaethanolamin mit Kalzium und Phosphat-Ion komplexiert sind. Dieses Ergebnis beweist die mögliche Bedeutung dieser Komplexe in der Kalzifikation des Tumors und in den Eigenschaften der Zellenmembrane.

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Distribution of Particulate Elements at the Axolemma of Preterminal and Terminal Vegetative Nerve Fibers as Revealed by Freeze-Etching

Freeze-etching has proved to be a useful tool for studying the surfaces of biological membranes. Most of the membranes investigated by this method showed surfaces carrying particles of different means and amounts. It has been suggested that these particles may represent globular proteins located in the internum of the biomembranes[1-3]. The application of freeze-etching to nervous tissues has proved to be successful when studies of the synaptic region of the CNS were carried out[4-6]. No data are available about membrane faces of the peripheral autonomic tissue, especially of the terminal 'efficient part'[10]. Previous investigations of the myelin of peripheral nerves demonstrated a rather fine granulation of the single lamella at its exterior surface, which is explained by the metabolic inactivity of the myelin[7,8]. The present study was intended to clarify whether there is a significant change of the axolemma of autonomic nerves in their course from preterminal to terminal regions.

Ductus deferens obtained from adult white rats were dissected immediately after preparation and treated with phosphate buffer at pH 7.4, containing 25% glycerol. After having been frozen in liquid nitrogen, the specimens were freeze-etched and replicated in a Balzers high vacuum unit (BA 360 M). Measurements of the replicas were made by a quarz crystal thin film oscillator. The thickness of the replicas varied between 20–30 Å for Pt/C and 200–300 Å for carbon alone. All the specimens studied were taken from unfixed tissue. The fracture exposes the intracellular faces of the axolemma of preterminal autonomic nerves and of the cytolemm of the Schwann cell. The axolemma of the preterminal autonomic nerves does not differ very much from the neurilemm of myelinated nerves. Particles