CONTACT INHIBITION - PLASMA MEMBRANES ENZYMATIC ACTIVITIES IN CULTURED CELL LINES

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Two phenotypically stabilized lines of plasmocytoma MOPC 173 - one of which (ME_2) is contact inhibited; the other (MF_2) is not - have different patterns of plasma membrane enzymatic activities. Mg^{++} K^{+} Na^{+} dependant ATPase and 5' nucleotidase activities of ME_2 decrease sharply when cells come into contact.

The term contact inhibition was coined by Abercrombie and Heaysman (1, 2) for a particular form of locomotory behaviour shown by fibroblast-like cells in culture on a plane surface. It implies the arrest of the continuous movement of cells at time of contact in such a manner as to avoid cell overlap. The term contact inhibition was also used as inhibition of mitosis leading to growth control when cells come into contact. (Stoker and Rubin .3).
In a cell population, tendency to stop growth was studied using 3T3 line (4,5), mouse embryo cells (6), mouse fibroblast line chemically transformed (7), chinese hamster ovary clone (8) and human cells strains (9, 10).
We have succeeded in obtaining murine myeloma cells in continuous lines and in inducing in vitro differentiation leading to two phenotypically differentiated cells :
one, MF_2, is fibroblast-like, with multiple cell layers,able to induce tumors in mice ; the other one, ME_2, stops growing when cells come into contact and cannot be transplanted back to mice (11). This communication deals with the study of enzymatic activities linked to the plasma cell membrane.

MATERIALS and METHODS

Cell lines and tissue culture.

Two continuous cell lines originated from MOPC 173

were used : ME_2 and MF_2 cells growing on plastic or glass. Cells are cultivated in a basic medium (Earle's saline Yeast Extract, Lactalbumine, vitamins, Glutamine .) adjusted to pH 7.4 with HCl-Tris. Calf-serum is added : 2% in the case of ME_2 and 10% for MF_2 . Cells are washed three times in saline and then harvested by scraping the surface of the flask with a piece of rubber.

Isolation of membranes.

Cells are incubated at 4° C in a medium containing 1 mM $NaHCO_3$ and 2 mM $CaCl_2$ to obtain a complete lysis without damaging the nuclei. The pellet obtained after 15,000 g centrifugation was layered on a discontinuous sucrose gradient of density 1.12 to 1.22. Plasma membrane material present at the various densities was repurified on an identical sucrose gradient . The yield of membrane proteins was between 2 to 4% of the total proteins whatever the cell lines were (12) . Densities of plasma membranes (PM) and ergastoplasmic reticulum (ER) were found to be 1.12 - 1.14 and 1.16 - 1.18 respectively (for more details see ref. 12).

Enzymes activities.

Na^+ K^+ Mg^{++} Adenosine triphosphatase and 5'Nucleotidase could be considered as specific PM functions (13). Mg^{++} dependent ATPase, activated by Na^+ and K^+ was assumed to be the difference between the phosphorus released in the presence of Na^+, K^+ and Mg^{++} and the phosphorus liberated in the presence of Mg^{++} alone ; both measurements being made at pH 7.4.

The reaction medium was made of 100 mM Tris buffer pH 7.4 ; 2 mM Tris-ATP ; 4 mM $MgCl_2$; 5 mM KCl ; 60 mM NaCl and approximately 150 µg of membrane proteins in a final volume of 2 ml for the first measurement ; the same medium devoid of KCl and NaCl was employed for the second measurement.

5'Nucleotidase activity was determined by incubating 200 µg of membrane proteins in 2 ml of a medium containing 5 mM of AMP in 0.05 M Tris-HCl buffer pH 7.4 with 5 mM $MgCl_2$. Phosphorus present in the reaction medium was determined by a modification (12) of the Fiske and Subbarow's method (14).

Alkaline Phosphatase is considered to be attached to the ER (15) and its activity was measured using p.nitrophenyl phosphate as substrate at a concentration of $2\ 10^{-3}$ M in a

50 mM Tris buffer pH 8.9. Paranitrophenol (PNP) was estimated by absorbancy at 410 mμ. Protein concentrations were determined by the method of Lowry et al. (16).
The enzyme activity is expressed in μM of product (Pi or PNP) liberated per hour per mg of protein.

RESULTS.

Growth curves.

We have compared the growth curves in 2% and 10% serum in both cell lines MF_2 and ME_2 on plastic surface (Fig 1, Fig. 2). It can be seen that MF_2 cells presented a clear exponential growth phase with a doubling time of 12 hours. The slope of the growth curve decreased when only 2% of serum was added. In contrast, ME_2 cells did not show evidence of a clearcut exponential phase, and the slope of the growth curve is unaffected whether the medium contained 2 or 10% of calf serum.

Fig. 1 :

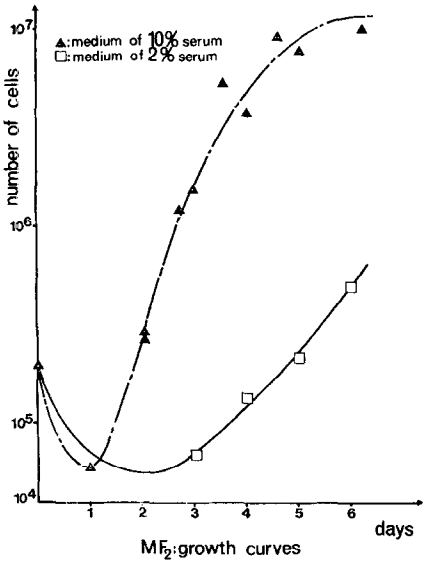
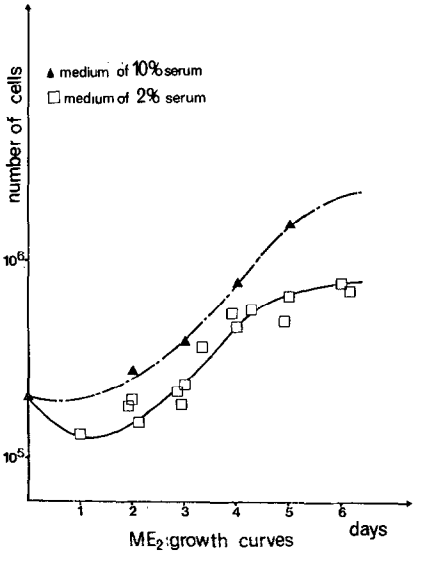


Fig. 2 :



Purity of plasma membranes.

Enzyme activity was checked in two or three experiments for each cell type : in each experiment measurement was made in duplicate or in triplicate. MF_2 cells in monolayer had

TABLE I

SPECIFIC ENZYMATIC MARKERS ACTIVITIES OF PLASMA MEMBRANES IN THE DIFFERENT FRACTIONS OF THE LAST SUCROSE GRADIENT.

CELL LINE	DENSITIES CELL NUMBER /cm^2	ENZYME ACTIVITIES	HOMOGENATE	SUCROSE DENSITIES					FINAL YIELD ooo
				1.12	1.14	1.16	1.18	1.20	
ME_2	4.10^4	AMP°ase	1.2	3.7	3.7.	1.2	1.2	1.2	25 %
		ATP°°ase	1.8	3	12.6	0	0	0	25 %
ME_2	8.10^4	AMPase	0.1	0.3	0.18	0	0.1	0	25 %
		ATPase	0.1	0.1	0	0	0	0	
MF_2	10^5	AMPase	220	940	610	15	15	1	25 %
		ATPase	2	6.8	14	0	0	0	25 %

° AMPase : 5' NUCLEOTIDASE : ADENOSINE MONO PHOSPHATASE

°° ATPase : K^+, Na^+, Mg^{++} DEPENDENT ADENOSINE TRIPHOSPHATASE.

THE ENZYME ACTIVITIES ARE EXPRESSED IN TERMS OF μM OF PHOSPHRUS LIBERATED PER HOUR PER mg OF PROTEIN.

ooo TOTAL ACTIVITY IN THE GRADIENT AND TOTAL ACTIVITY IN THE ORIGINAL HOMOGENATE.

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very high enzymatic activities : 610 μM/h/mg of proteins for the 5'Nucleotidase, 14 for the K^+ Na^+ Mg^{++} ATPase. ME_2 cells in growth phase had lower titers : 3.7 and 12.6 respectively for these two enzymes (Table 1).

We evaluated the purity of PM in term of enzymatic activities in the different layers of the last sucrose gradient. The recovered activity was expressed by the ratio of the total enzymatic activity in the gradient versus the total activity in the homogenate for a given enzyme. It can be seen (Table 2) that the specific activity of ATPase is seven times higher in the gradient than in the homogenate.

Conversely, we evaluated the contamination of PM by titration of the Alkaline Phosphatase. In MF_2 cells, this activity is mostly found with the ER membranes and only 9% of the total activity in the gradient is recovered in the lightest layers

Table 2.

Plasma Membrane Purity.

	ME_2	MF_2
Purification = $\frac{S.A.^{x} \text{ of } PM^{xx} \text{ in the } A^{\circ} \text{ layer}}{S.A. \text{ in the homogenate}}$	7	7
Contamination = $\frac{T.A.^{xxx} \text{ of } ER^{o} \text{ in the A layer}}{T.A. \text{ of ER in all layers}}$	60%	9%

$S.A.^{x}$ = Specific activity expressed in μM of Pi/h/mg of protein (ATPase Na^{+} K^{+} Mg^{++}) or in μM of PNP/h/mg of protein (Alkaline Phosphatase).
PM^{xx} = Plasma Membrane.
$T.A.^{xxx}$ = Total activity for the same enzymes.
ER^{o} = Endoplasmic Reticulum.
A° = Layer of density 1.12 - 1.14 .

Table 3.

Specific enzyme activity of plasma membranes isolated from growing and contact-inhibited ME_2 cells.

	5'Nucleotidase	K^{+} Na^{+} Mg^{++}ATPase
Growing cells	3.7	12.6
Contact-inhibited cells	0.18	0.1

Activities are expressed in μM of Pi/h/mg of protein.

(1.12 - 1.14) corresponding to the PM densities. In ME_2 cells harvested during the growth phase, the same calculation for the Alkaline Phosphatase led to 60% contamination of the PM by the ER.

Specific enzyme activity of plasma membranes isolated from growing or contact-inhibited ME_2 cells.

ME_2 cells showing contact inhibition demonstrated little PM enzymatic activity (0.3 for the 5'Nucleotidase and less than 0.I for K^{+} Na^{+} Mg^{++} ATPase) whereas the same cells taken during the growth phase had much higher titers.
The enzymatic activities dropped from 100% to 1 to 5% when the number of cells per cm^2 increased from 4.10^4 to 8.10^4 (Table 3). The same observation was made when ME_2 cells were grown in a 10% serum medium.

DISCUSSION

Growth curves showed significant differences between both cells : MF_2 cells entered an exponential growth phase with a 12 hours doubling time while ME_2 cells had a much longer doubling time of about 24 hours without a clear exponential growth phase. This absence of apparent exponential phase might result from cell contact or cell vicinity occuring soon after seeding and in proportion to the cell concentration at a given time. In this case, the experimental curve might suggest that the division time is, in fact, much less than 24 hours.

The density gradient profiles of the PM and the ER are different in the two phenotypic expressions of the MOPC 173 plasmocytoma cells : as we found that in the ME_2 cells, the purified PM contain a far larger amount of ER than do the purified PM of the MF_2 cells. It is possible that this distribution arises from the topological location of these organells as we have observed by electron microscopy : in the ME_2 cells ER is present in small amounts scattered in the cytoplasm whereas in the fibroblast we found the ER in dense aggregation around the nucleus (12).

Finally we have shown that epitheloid cells which spontaneously expressed contact inhibition had a rapid drop in their PM enzymatic activities when cells became confluent. This decrease in activity can conceivably be attributed to any of several different phenomena : to loss of the enzyme molecules; to their presence but in an inactive form ; to a modification in the membrane structure which results in a "masking" of the active sites. Along these lines, Burger (17) has shown that by addition of small amounts of different proteases to 3T3 confluent cultures, most cells will escape contact inhibition of growth and the reactivating effect of the proteolytic enzymes favors a "masking" hypothesis. Na^+ K^+ Mg^{++} ATPase activity in membranes derived from LK sheep red cells is stimulated by the formation of a complex between the L antigen and its antibody (18, 19). In addition, pretreatement with trypsin of LK cells, showing low Na^+ K^+ Mg^{++} ATPase activity, renders these cells insensitive to the K^+ pump stimulating antibody. We can imagine local modifications in the cell surface architecture such that these structural changes "mask" or inactivate the enzyme.

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