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Alterations of Surface Properties of Chick Embryo Fibroblasts Infected with Four Rous Sarcoma Viruses Producing Distinctive Cell Transformation[1] (38262)

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Previous investigations (1) demonstrated that activities of the UDP-galactose and GDP-mannose glycosyl transferase ectoenzyme systems on the external plasma membranes of chick embryo fibroblasts (CEF) were markedly elevated when these cells were transformed by the Schmidt-Ruppin strain of Rous sarcoma virus (RSV-SR) in comparison to normal CEF. Such cells also showed a significant elevation of their electrophoretic mobility. Since a number of strains of RSV exist which produce distinctive types of cellular transformation (2), it was of interest to determine if these alterations in the external plasma membranes were similar in colonies of transformed cells with different morphologies and variations in the degree of loss of contact inhibition induced with different cloned strains of virus (2).

Materials and Methods. The Schmidt-Ruppin strain (1) (RSV-SR), Bryan strain (RSV-Br), the morph r (RSV-Br-mr), and morph f (RSV-Br-mf) derived from RSV-Br (2) and the Rous associated virus (RAV-1) derived from RSV-Br all belonging to subgroup A were employed. The RSV viruses were cloned by cultivating the progeny obtained from single well isolated foci of infected cells under agar overlay.

Primary cultures of chick embryo cells were derived from eggs of a Gs antigen negative, leukosis free flock, tested for uniform susceptibility to RSV-subgroup A viruses, maintained in F-12 medium, and infected with the various viruses and incubated at 39° in forced air-CO_2 incubators. The cultures were split on the 4th day and used on the 6th day for biochemical studies when over 75% of the cells appeared visibly transformed (1). All cells were harvested at the point where control and infected cells had formed an almost complete monolayer on the surface of the Petri dish in which they were cultivated so the effects of cell contacts would be the same in all cultures. Coverslips were placed in similar cultures, removed, stained and examined to record the morphology of the transformed cells.

UDP-[^{14}C] galactose (240 Ci/mole) and GDP-[^{14}C] mannose (241 Ci/mole) were purchased from New England Nuclear Corp. Using these compounds, assays for the cell surface glycosyl transferases were performed as previously described (1).

The electrophoretic mobilities of aliquots of the same cells were measured in a horizontal chamber of small volume equipped with reversible blacked platinum electrodes as previously described (1). The mobilities of the particles were calculated in μm/s/V/cm; each value was obtained by timing the movement of at least 20 particles with reversal of polarity after each measurement (1).

Results. The strains of RSV each produced characteristic transformation in the CEF marked by contrast with the control cells (Fig. 1-A). The areas of cell transfor-

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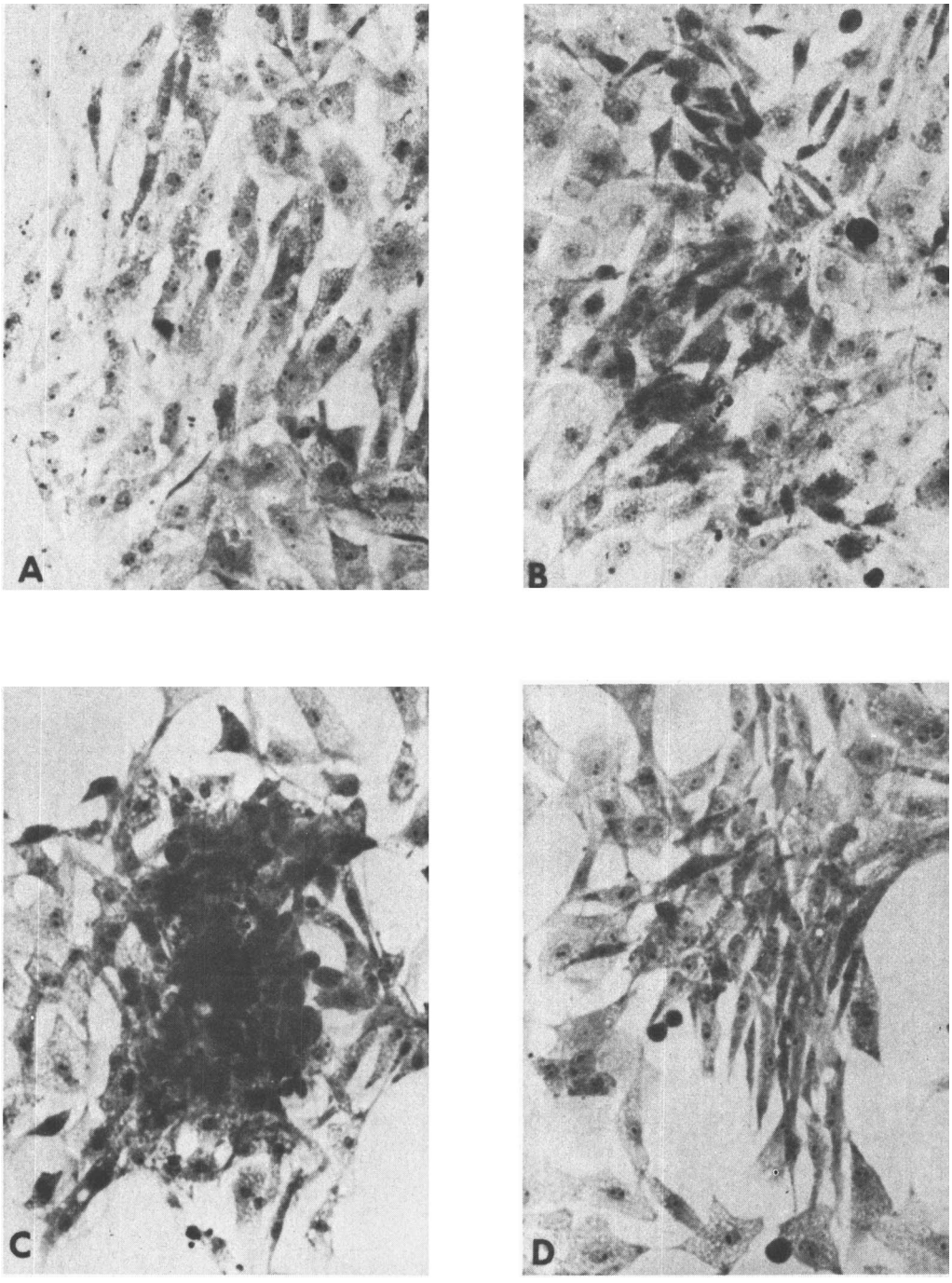

FIG. 1. Cell cultures of normal cells (A), and of cells infected with RSV-SR (B), RSV-Br or RSV-Br-mr (C) and RSV-Br-mf (D). Giemsa stain × 320.

mation produced by RSV-SR contained both fusiform and rounded cells with some overlapping due to loss of contact inhibition but no dense concentration of piled cells (Fig. 1-B). The RAV infected cells appeared similar to control cells but showed more vacuoles. The parent RSV-Br and its derived RSV-Br-mr produced the same marked rounding of cells with piling into dense foci indicating a marked loss of contact inhibition (Fig. 1-C). The RSV-Br-mf produced a marked fusiform character in the cells with significant overlapping of cells due to some loss of contact inhibition but no piling into dense foci (Fig. 1-D).

The galactose and mannose glycosyl transferase activities were markedly elevated in all cells infected with RSV as shown by the average values of three experiments presented in Table I in comparison to the values obtained with control or RAV-1 infected cells. The electrophoretic mobilities of cells infected with the various strains of RSV were significantly increased over those obtained with control or RAV-1 infected cells (Table I).

Discussion. The availability of cloned strains of RSV producing a variety of characteristic morphological alterations and changes in social behavior of cells makes it possible to correlate various properties of these cells with cell morphology and the degree of loss of contact inhibition. The use of RAV-1 infected cells permits the separation of the role of morphological transformation of the cell from virus replication which includes budding of the virus from a cell membrane into which new virus antigens have been introduced. Using these strains, no difference in the activities of plasma membrane UDP-galactose and GDP-mannose glycosyl transferase ectoenzyme systems were found. Likewise the elevation in electrophoretic mobility of CEF infected with the various RSV was similar. Thus RSV-Br and RSV-Br-mr which produce the most striking morphological alterations and loss of contact inhibition have the same effects on these ectoenzyme changes and electrophoretic mobilities as the RSV-Br-mf and RSV-SR which produce less marked changes in these two characteristics of RSV transformed cells. This is interesting in light of the suggestion that these enzymes and acceptors may mediate cell: cell contact phenomena (4, 5).

The changes at the plasma membrane as detected by measurement of these glycosyl transferases and the electrophoretic mobilities of these RSV infected cells are however clearly associated with virus induced transformation of the cells and are not due to replication of the virus since infection with RAV-1 which multiplies efficiently in these cells does not induce significant changes.

Summary. Four cloned strains of RSV producing different morphological changes and varying degrees of loss of contact inhibition of chick embryo fibroblasts on infection and transformation of these cells *in vitro* induce similar marked elevations in the activities of the UDP-galactose and GDP-mannose glycosyl transferase ectoenzyme systems when compared to normal or

TABLE I. Cell Surface Glycosyl Transferase Activity and Electrophoretic Mobility of CEF Infected with RSV and RAV.

Virus	^{14}C-labeled precursor[a]		Electrophoretic mobility (μm/s/V/cm)
	UDP-galactose	GDP-mannose	
RSV-SR	1228	2020	-2.45 ± 0.03
RSV-Br	2042	1928	-2.51 ± 0.02
RSV-Br-mr	2989	3853	-2.49 ± 0.05
RSV-Br-mf	2040	2389	-2.41 ± 0.04
RAV-1	229	269	-2.01 ± 0.01
None	302	314	-1.97 ± 0.03

[a] Expressed as cpm per 10^6 cells.

RAV-1 infected cells. Such transformed cells also exhibit significant elevations in their electrophoretic mobilities when compared to control or RAV-1 infected cells. Thus the type of morphological change or the degree of loss of contact inhibition is not related to these cell surface changes in RSV transformed cells.

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