Studies on the Variability of ABH Blood Group Antigens on Cells in Primary Culture* (33805)

WILLIAM J. KUHNS, YVONNE FAUR, SHARON BRAMSON, AND FRANCES FRIEDHOFF

Department of Pathology, New York University School of Medicine; and Immunohematology Laboratory, Bellevue Hospital, New York, New York

Earlier studies of blood groups on primary amnion cells showed that losses of ABH and Tj^a antigens occurred over a period of three or four transfers, during which time other characteristics of cell growth were becoming progressively impaired (1). The possibility that such losses of blood group activity reflect underlying metabolic disturbances has been considered. One obvious change in primary cultures of advanced age is a decrease in the rate of cell division. Among the processes which may be affected during this change are those responsible for structural determinants such as blood groups.

In the event of an association between numerical growth and serological reactivity, one would anticipate fluctuations of blood groups as reflected by mixed agglutination to be rather closely related to the division rate of the cells being tested. Such a correlation was observed in regard to A B O (H) groups in five primary amnion cell cultures.

Materials and Methods. The preparation of human amnion cell suspensions, culture procedures and the mixed agglutination test were carried out as described elsewhere (1, 3, 4). Suspensions of cells derived from amnions consisted of approximately 95% epithelial cells judged by morphological criteria, and the cellular population remained essentially epithelial during the total period of study of each preparation. The ABH blood group of each cell suspension at the time of original culture was correlated with the group on erythrocytes from the corresponding donor. Subsequent tests for blood group by the method of mixed agglutination (4) were carried out at times of cell transfer and periodically for

TABLE I. ABH Blood Groups in Amnion Cells in Primary Culture.

No.	Blood group		Total period in culture (days)	No. of passages	Last day mitotic figures observed	Appearance of later growth histology of cells
	RBC	Amnion cell				
1	O	O (H)	135	5	55	Cells grew in small clones and vacuolated cells were noted after 3 weeks; predominantly epithelial
2	O	O (H)	60	3	60	Sparse, many vacuolated cells; all appeared to be epithelial
3	B	B	155	3	36	Many vacuolated cells starting at 30 days; all appeared to be epithelial
4	A	A	83	2		Very slow growth, many separated clones, and areas of vacuolated cells; predominantly epithelial
5	A	A	120	2	65	Monolayer appeared healthy at 60 days; sparse growth at 90 days, few vacuolated cells at 120 days; all appeared to be epithelial

* Supported by United States Public Health Research Training Grant A1-00247-05.

as long as attachment of cells to culture bottles could be demonstrated. Mitotic indices were noted with cells fixed by air drying and stained with orcein—attached to cover slips from which medium had been removed. Cells arrested in metaphase were enumerated and the percentage of total cells (usually one thousand cells counted) was calculated. Cell counts obtained at times of transfer were compared with original counts or with counts obtained at the time of the previous transfer and the magnitude of the differences was used in relation to blood group activity as reflected by mixed agglutination. In instances when transfers were discontinued, cells were enumerated in demarcated areas of cover slip preparations at times of medium changes in order to provide comparisons of cell density up to the termination of experiments. Mixed agglutination tests were carried out at times on persistent but apparently nondividing cells. Parameters of cell degeneration were noted, such as vacuolization, and changes in membrane configuration. Alterations in membrane permeability during the period of culture were tested by the trypan blue dye method (5). No transformations were observed in any of the cultures grown in this series.

Results. Experiences with five amnion cell

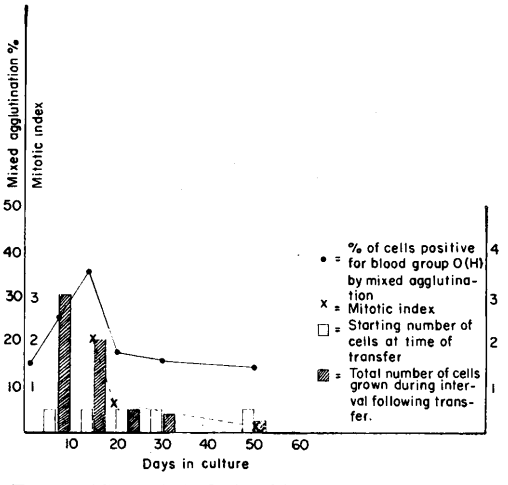

FIG. 1. (Case 1) Relationship of mixed agglutination for blood group O (H) to growth rate of human cells as reflected by mitotic index and numerical cellular increase and decrease.

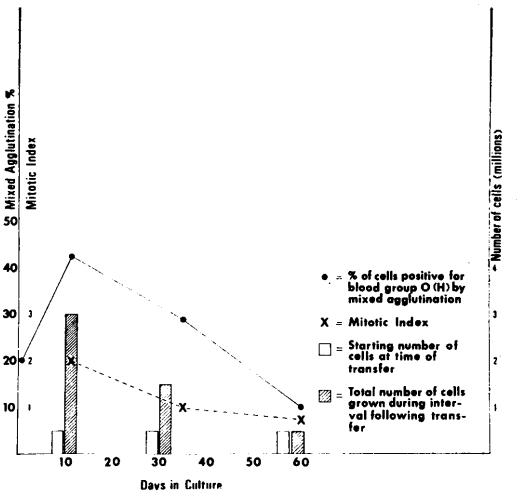

FIG. 2. (Case 2) Relationship same as Fig. 1.

preparations are summarized in Table I. Of these, two corresponded serologically to blood group O, two to group A, and one to group B. The characteristic serological course of these cells in culture is shown in Figs. 1 and 2. A rise and fall in blood group activity was observed early in the course of culture. The total periods of cells in culture ranged from 60 to 155 days. The initial period in culture was marked by evidences of vigorous cell division, but after 2–5 passages, cell division decreased markedly as judged by decrease in net cellular gain between transfers. This was accompanied by increases in cell loss and a decrease in the density of monolayers. Mitotic figures could be demonstrated in early cultures (Table I), but decreased in numbers along with an increase in age of cultured cells. When the mitotic index was carried out at times of cell transfer during the total study period (cases 1 and 2), an inverse relationship was observed between mitotic indices and period of time of cells in culture (Figs. 1 and 2). Many cells which persisted in culture changed in appearance and behavior as signified by the presence of vacuoles and susceptibility to the vital stain, trypan blue. The sparse population of cells in long-term culture allowed for repeated observations and for blood group determinations on individual cells which had ceased to divide. In this way it could be shown that some

TABLE II. Correlation between Mixed Agglutination and Rates of Division in Cultured Primary Amnion Cells.

	Blood group	Interval between cell transfer (days)	Magnitude of increase or decrease in no. of cells between times of transfer	Mitotic index	Percentage of cells positive for blood group by mixed agglutination
1.	O (H)	0– 7	6		25
		7–14	8	2	35
		14–20	4	0.6	18
		20–30	1		15
		30–50	0.5	0.1	14
2.	O (H)	0–12	6	2	42
		12–35	3	1	30
		35–60	1	0.7	10
3.	B	0–10	12		80
		10–30	8	ND[a]	60
		30–70	4		50
4.	A	0– 8	8	ND	70
		8–25	2		25
5.	A	0–10	4	ND	2
		10–20	12		40

[a] ND = Not done during this period.

persistent cells retained blood group activity since they reacted positively in the mixed agglutination test. Other cells were not reactive, possibly due to the phase in the cell cycle at which metabolic change occurred.

A positive correlation was demonstrated between rates of cell division and A, B, O (H) blood group activity as judged by the magnitude of increase in cells in comparison with results of mixed agglutination on suspensions tested at times of transfer (Figs. 1 and 2, Table II). Thus with rare exception increases or decreases in the extent of mixed agglutination appeared to reflect corresponding changes in the rate of cell division. A plateau effect observed with the passage of time in some cultures (Fig. 1) could be accounted for by persistence on monolayers of nondividing blood group active cells.

Discussion. In accordance with earlier findings, the present results indicated losses of A, B, and H blood group activity as progressive deterioration of other cultural characteristics occurred (1, 6). It was of interest that a period of increased blood group activity was closely correlated with the rate of cell division as judged by the magnitude of increase in cell numbers between transfers. Correlations between different amnion cell preparations were generally similar, except that group A and B preparations gave higher degrees of mixed agglutination than did O suspensions, thus confirming previous results (1). In experiments such as these, contamination of epithelial cell preparations with fibroblasts may present difficulties of interpretation, since fibroblasts considered to be unreactive for blood groups may grow at a disproportionate rate to epithelial cells contained in the same culture. However, cellular configuration and morphology was predominantly or entirely epithelial in type during the period of study of each culture.

The parallelism between rate of cell division and serological activity may indicate a higher rate of production of intermediates responsible for expression of the latter function but the possibility must be considered that the relationship of cell division to blood group activity was fortuitous. For example, the cell doubling rate in some cells may have

been matched in others by synthesis of new blood group material which was not necessarily reflected by the mixed agglutination test. In this circumstance alternate methods for blood group determination would be necessary to provide a more complete picture. On the basis of mixed agglutination carried out on synchronized HeLa cells, mitoitc cells appeared to exhibit more intense degrees of blood group surface activity than did cells in interphase (2). In the present experiments, changes in blood group activity could be explained on an increase in mitotic cells during periods when the cell generation times were the shortest. This was reflected by changes in the mitotic index which tended to parallel population changes.

A peculiar finding was the presence of blood group active cells for prolonged periods, *i.e.*, 4–6 months, at times when they were no longer functional as dividing cells, and were structurally impaired as judged by reactivity in the presence of vital stain. A similar result was obtained on trypan blue positive transformed cells in previous studies of ours (3). From these results it would seem that A–B–O determinants may remain serologically active for as long as intactness of membranes can be demonstrated, despite impairment of vital cell functions. Variability in this regard was observed, but this, as well as persistence of such cells on monolayers, may be related to the phase in the cell cycle at which cellular impairment occurred, and resistance of the impaired attached cells to manipulations associated with medium changes.

Summary. ABH blood group activity was demonstrated on primary amnion cells in culture for periods up to 6 months and beyond the time when cell division could be demonstrated. Blood group reactive and nonreactive populations were observed in each culture, a finding in accord with those seen in early primary cultures and in established lines. During the time that cell division was observed, the rate of division was correlated with numbers of blood group positive cells as judged by mixed agglutination. Serological activity may be retained in cells with membrane defects which have lost the capacity for further division. Such activity is variable from cell to cell and may depend upon the time that impairment of reproductive capacity occurs in the cell cycle, and subsequent retention of such cells on the monolayer.

The authors wish to thank Dr. Burton Goldberg of the Department of Pathology, New York University, School of Medicine, for reviewing this manuscript.

1. Friedhoff, F. and Kuhs, W., Transfusion 8, 244 (1968).

2. Kuhns, W. and Bramson, S., Nature **219**, 938 (1968).

3. Chessin, L., Bramson, S., Kuhns, W., and Hirschhorn K., Blood **25**, 944 (1964).

4. Kelus, A., Gurner, B. W., and Coombs, R. R. A., Immunology **2**, 262 (1959).

5. Parker, R. C., "Methods of Tissue Culture," 3rd ed. Harper (Hoeber), New York (1961).

6. Högman, C., Exptl. Cell Res. **24**, 137 (1960).

Received Sept. 25, 1968. P.S.E.B.M., 1969, Vol. 131.