

Serum-free Growth of Normal and Transformed Fibroblasts in Milk: Differential Requirements for Fibronectin

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ABSTRACT Bovine milk may be used as a supplement for the serum-free growth of certain fibroblastic cells in culture. The growth properties of three representative cell types in milk-supplemented medium were examined; fibroblastic cell strains, fibroblastic cell lines, and transformed fibroblasts. Transformed fibroblasts, which included RNA and DNA tumor virus-transformed cells and carcinogen-transformed cells, grew in milk. Instead of growing attached to the culture dishes, as they normally do in serum, transformed fibroblasts grew in milk as large clusters in suspension. In contrast, nontransformed fibroblastic cell strains and cell lines did not grow in milk-supplemented medium. Fibroblasts transformed by a temperature-sensitive transformation mutant of Rous sarcoma virus were temperature-sensitive for growth in milk. The failure of cells to adhere to the substratum in milk-supplemented medium suggested that milk might be deficient in attachment factors for fibroblasts. When the attachment of fibroblastic cells in milk-supplemented medium was facilitated by pretreating culture dishes with fibronectin, (a) transformed cells grew attached rather than in suspension, (b) normal cell lines attached and grew to confluence, and (c) normal cell strains adhered and survived but did not exhibit appreciable cell proliferation.

The physiological function of milk is to provide substances important to the nutrition, growth, and development of the newborn. In addition, mammalian milk contains viable cells (1, 2). Both human and bovine milks contain up to 4×10^6 cells/ml, primarily macrophages, T lymphocytes, and B lymphocytes. In previous studies, we demonstrated that both human (3, 4) and bovine (5) milks contain polypeptide growth factors that stimulate DNA synthesis and cell division of confluent cultures of fibroblasts. The presence of mitogens and viable cells in milk prompted us to evaluate milk as a replacement for serum in cell culture. In an initial report, we demonstrated that milk could support the long-term growth of epithelial cells in culture (6). Only colostrum, a highly enriched milk produced by mammals in the first day or two after birth of the newborn, was effective in supporting epithelial cell proliferation. In addition, colostrum was selective, supporting the growth of epithelial cells but not fibroblasts. When plated in either colostrum or regular milk, fibroblasts adhered very poorly to the substratum, suggesting that milk lacks factors that are necessary for the attachment of fibroblasts. This ob-

servation prompted us to investigate whether milk would be selective for the growth of cells that do not require attachment to the substratum to proliferate. In this report, we show that transformed fibroblasts grow readily in milk-supplemented medium as suspension cultures. On the other hand, normal fibroblasts do not grow in milk unless the attachment of cells is mediated by an adhesion-promoting factor, such as fibronectin.

MATERIALS AND METHODS

Cell Culture

Rat embryo cultures (LR1) prepared from Lewis rat embryos and three Schmidt-Ruppin D (SRD) Rous Sarcoma virus (RSV) transformants of Lewis rat embryo cells (LR3/1, LR3/2, and LR3/3) were described previously (7). F2408 cells, which are in established cell line derived from Fischer rat embryos (8), were provided by Dr. C. Basilio, New York University School of Medicine, New York. FRD-4 is an SRD transformant of F2408 cells infected and cloned as described previously (7). FR24D-1 cells were obtained by transforming F2408 cells by a subgroup D recombinant of LA24, a temperature-sensitive transformation mutant of RSV (see below). Kirsten Sarcoma virus-transformed NIH 3T3 cells (KNIH) and SV40-transformed 3T3 cells (SV29) were gifts of Dr. C. Scher,

Sidney Farber Cancer Institute, Boston, Mass. Mouse sarcoma 180 cells were purchased from American Type Culture Collection, Rockville, Md.

Avian cells and viruses were propagated by standard techniques (9). Virus strains and avian cells have been previously described (7, 10). The procedures for host range studies, interference assays, and virus cloning were as described previously (10).

The subgroup D recombinant of LA24 was constructed by infecting C/E chick embryo cells (CEC) with LA24 and the subgroup D leukosis virus, RAV-50. 48 h later, the virus produced by these cultures was harvested and used to infect C/AE CEC. Harvests from C/AE CEC were collected and cloned repeatedly on C/AE CEC. After six cycles of cloning, stocks of virus were grown up on C/E CEC and the subgroup of the virus was examined by host range and interference assays. Stocks of this recombinant, designated LA24D, exhibited only subgroup D properties. F2408 cells were transformed by LA24D, cloned, and grown into cultures by the method described previously (7).

Stock cultures of all cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM, Grand Island Biological Company [GIBCO], Grand Island, N. Y.) containing glucose (4.5 g/liter), penicillin (50 U/ml), and streptomycin (50 µg/ml) and supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.). Cells were subcultured after treatment with 0.25% trypsin, 0.2% EDTA (TE, GIBCO). Transformed cells were subcultured twice weekly and normal cells (both strains and lines) every 3–4 d as needed, with care to see that the cultures never grew to confluence. Stocks of the temperature-sensitive transformed cell line (FR24D-1) were cultured at the permissive temperature (35°C). All of the other cells were routinely grown at 37°C.

Preparation of Milk for Cell Culture

Milk and colostrum were prepared for use as a medium supplement for cell culture as described previously (5, 6). Colostrum was obtained within 24 h after birth of the calf, whereas regular milk was obtained randomly at various stages in the lactation period. For cell culture, fresh bovine milk was obtained immediately after milking. Within several hours, the milk was centrifuged at 12,000 g for 30 min to remove cellular debris (pellet) and fat (floating on the top). The defatted milk was frozen at –20°C. For cell culture, milk was thawed, diluted in DMEM (20% vol/vol, or less), and filtered through 0.45-µm Nalgene filter units (Nalge Co., Nalgene Labware Division, Rochester, N. Y.) for sterilization.

Growth Studies

Cells were detached from culture dishes by incubation with 1 ml of trypsin-EDTA (TE). An equal volume of soybean trypsin inhibitor (Type I-S, Sigma Chemical Co., St. Louis, Mo., 0.25% in phosphate-buffered saline) was added to stop the reaction. The cells were diluted to 10 ml in un-supplemented DMEM, and the cell suspension was centrifuged at 800 rpm in an International IEC centrifuge (International Equipment Co., Needham Heights, Mass.) at room temperature. The pellet was resuspended in DMEM, and the cell concentration was determined with a Coulter model Zf electronic particle counter (Coulter Electronics, Inc., Hialeah, Fla.). Aliquots of cell suspension were added directly to medium containing the desired supplement (milk, colostrum, serum, or un-supplemented) to give a final cell concentration of 2×10^4 cells/ml. The cell suspension (0.5 ml) was placed into 24-well microtiter plates (16 mm diameter, Costar, Data Packaging, Cambridge, Mass.) to give an initial cell density of 1×10^4 cells/well (5×10^3 cells/cm²). Because cells remained in suspension in milk, cultures were fed (every 3–4 d) by adding 0.5 ml of fresh medium to each well without replacing the supernates, except where indicated otherwise.

For cell counting, the culture supernates were transferred to Coulter counting vials containing an equal volume of TE. To each well, 1 ml of TE was added to dissociate the remaining cells in the well. The plates and the counting vials were incubated at 37°C for ~10 min. At the end of the incubation period, 0.5 ml of calf serum was added to each well, the cell suspension was vigorously pipetted with a Pasteur pipette, and the suspension pooled with the supernate-TE mixture in the Coulter vials. The cell suspension was diluted to 10 ml with Isoton II (Curtin Matheson Scientific Inc., Woburn, Mass.), and cell counts were determined by Coulter counting.

Passaging of Cells in Milk

Transformed cells growing in suspension in milk-supplemented medium were mixed with an equal volume of TE and incubated at 37°C for 15–20 min. Single-cell suspensions were obtained by vigorously pipetting the cultures with a Pasteur pipette. The cells were centrifuged from this suspension and resuspended in medium supplemented with 10% milk or 10% calf serum to give a final concentration of 1×10^4 cells/well in Costar 24-well microtiter plates.

Fibronectin Treatment of Culture Dishes

Human plasma fibronectin (Collaborative Research, Inc., Waltham, Mass.) was suspended in DMEM (25 µg/ml) and 0.4 ml was added to each well of 24-well microtiter plates (final concentration ~5 µg/cm²). The plates were incubated at room temperature for a minimum of 30 min, the supernates were aspirated, and the cell suspension was added immediately.

Histology and Photography

Clusters of cells growing in milk were fixed in glutaraldehyde (2%), postfixed in OsO₄, and embedded in Epon-Araldite resin with dimethylaminomethyl phenol-30 (Ladd Research Industries, Inc., Burlington, Vt.). Thick sections were cut and stained with Azure II-methylene blue.

Cells were photographed under phase in a Nikon model MS inverted-phase microscope.

RESULTS

Growth of Cells in Milk

The growth properties of a fibroblastic cell strain (LR1), a fibroblastic cell line (F2408), and a Rous sarcoma virus-transformed fibroblastic cell line (LR3/1) in medium supplemented with various concentrations (0–20%) of milk, colostrum, and serum were examined (Fig. 1). RSV-transformed cells grew in all three media. The cell density at the optimal concentration of colostrum was ~30% of that observed in serum. In milk, the transformed cells grew to a density ~12% of that achieved in serum. Furthermore, the final cell number in colostrum represented a 50-fold increase and in milk represented a 20-fold increase over the initial plating value. On the other hand, the cell strain and cell line grew in serum but not in milk or colostrum at any of the concentrations tested. The approximate generation times for RSV-transformed cells growing in milk, colostrum, and serum were 31, 22, and 14 h, respectively (not shown).

Growth Properties of Temperature-sensitive RSV-transformed Cells in Milk

In general, cells transformed by temperature-sensitive transformation mutants of RSV are temperature-sensitive for a

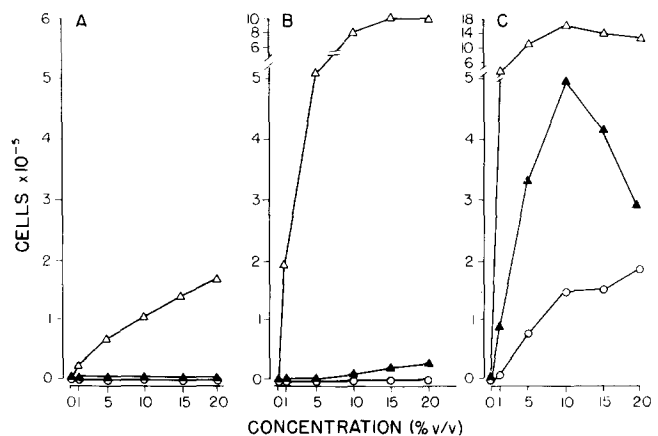


FIGURE 1 Growth response of normal and transformed fibroblasts in various concentrations of milk, colostrum, or serum. The cells were plated (1×10^4 /well) in 24-well microtiter plates. Each point represents the average of duplicate cell counts determined on day 10. A, Cell strain (rat embryo cells: LR1); B, cell line (rat fibroblast cell line: F2408); C, transformed fibroblasts (RSV-transformed rat embryo cells: LR3/1). ○, milk; ▲, colostrum; Δ, serum.

variety of properties considered to be *in vitro* markers for cellular transformation (11). If growth in milk and colostrum is a phenotype associated with cellular transformation, then it should also be temperature-sensitive. F2408 cells transformed by a subgroup D recombinant of a temperature-sensitive transformation mutant of Rous sarcoma virus were cultured in various concentrations of milk, colostrum, and serum at 35°C (permissive temperature) and 39°C (nonpermissive temperature) (Fig. 2). At 35°C, the temperature-sensitive cells (FR24D-1) grew well in all three media, consistent with the growth response observed for wild type virus-transformed cells. At 39°C, the temperature-sensitive transformed cells exhibited growth behavior similar, but not identical, to that of the normal parental cell line (F2408). They grew well in serum, not at all in milk, but, unlike the parental cells, they showed some growth in the higher concentrations of colostrum at 39°C.

Growth curves of the temperature-sensitive cells in 10% milk, colostrum, and serum are shown in Fig. 3. These cells grew well at the permissive temperature in serum, colostrum, and milk. The generation times at 35°C in serum, colostrum, and milk were 15, 22, and 29 h, respectively. At 39°C, these cells grew in serum with a doubling time of 18 h but did not grow at all in colostrum or milk. The lack of growth in milk at 39°C was not caused by cell death. Cultures incubated at 39°C in milk for various periods of time, up to 7 d, were shifted down to 35°C. In every case, growth resumed following a lag period of several days at a rate comparable to that observed with cultures incubated at 35°C for the entire experiment. In contrast, temperature-sensitive cells incubated in unsupplemented medium at 39°C failed to survive (data not shown).

Long-Term Passage of Transformed Cells in Milk

The temperature-sensitive transformed cells can be subcultured at the permissive temperature in medium supplemented with milk. In one series of experiments, cells were subcultured 12 times over 5 mo (~60 population doublings) before the

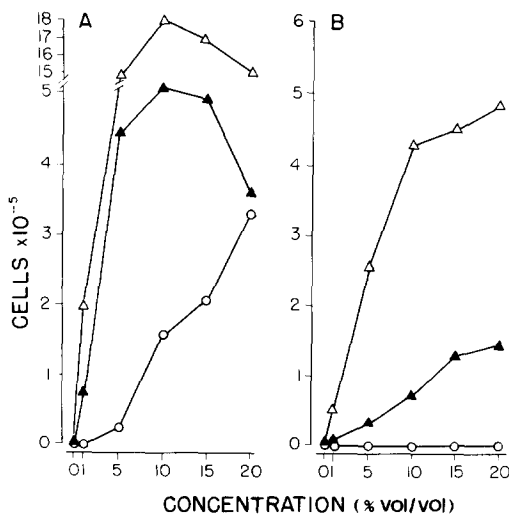


FIGURE 2 Growth response of temperature-sensitive RSV-transformed fibroblasts in various concentrations of milk, colostrum, or serum at permissive and nonpermissive temperatures. FR24D-1 cells (1×10^4 /well) were plated in 24 well microtiter plates. The plates were incubated at 35°C (permissive temperature) or 39°C (nonpermissive temperature). Each point represents the average of duplicate cell counts determined on day 10. A, 35°C; B, 39°C. O, milk; ▲, colostrum; Δ, serum.

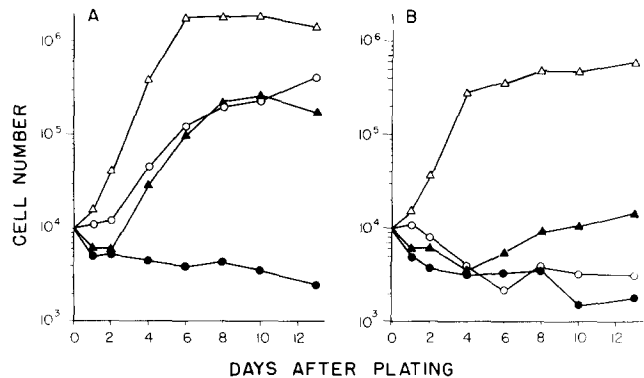


FIGURE 3 Growth curves of temperature-sensitive RSV-transformed fibroblasts in medium supplemented with milk, colostrum, or serum at permissive and nonpermissive temperatures. FR24D-1 cells (1×10^4 /well) were plated in each well of 24 well microtiter plates. Duplicate cell counts were made every 2–3 d. A, 35°C; B, 39°C. O, milk; ▲, colostrum; Δ, serum; ●, unsupplemented.

study was terminated. Growth kinetics were examined periodically and the generation times in milk remained constant (~30 h). In addition, these cells remained temperature-sensitive for growth in milk and displayed the characteristic temperature-sensitive properties when transferred to medium containing serum (not shown).

Morphology of Transformed Cells Growing in Colostrum and Milk

Phase contrast photomicrographs of the growth patterns of RSV-transformed cells in serum, milk, and colostrum are presented in Fig. 4. In serum-supplemented medium, transformed cells grew attached to the dish (Fig. 4a). However, in colostrum (Fig. 4b) and milk (Fig. 4c and d), transformed cells grew as large clusters in suspension. There was no attachment of transformed cells to the culture dishes in regular milk at concentrations ranging from 0 to 30%. In colostrum, there was no attachment of transformed cells at concentrations from 0 to 10% but some attachment (<10% of the cells) occurred at higher concentrations. In milk-supplemented medium, the size of the cell clusters increased with time in culture. Relatively small clusters were seen in the first 5 d (Fig. 4c). By 10 d, some of the clusters approached 1 mm in diameter (Fig. 4d). When serum was added to the transformed cells growing in milk, the clusters attached to the dish, spread, and the cells grew out from the clusters to form confluent monolayers (Fig. 4e). There was no evidence of necrosis at the center of large clusters, as shown in the histological section (Fig. 4f).

Growth of Other Transformed Cells in Milk

A comparison of the growth responses of a variety of transformed cells in medium supplemented with serum, colostrum, and milk are presented in Table I. Cell lines transformed by RSV, Kirsten sarcoma virus, SV40, and by a carcinogen were tested. Transformed cells all grew well in colostrum, reaching saturation densities ranging from 15 to 50% of that attained in serum. These transformed cells also grew in milk, but not so well as in colostrum. There was no growth of any of these cell lines in unsupplemented medium. In milk and colostrum, all of these transformed cell lines grew in suspension as large cell aggregates, but in serum they grew attached to the dish.

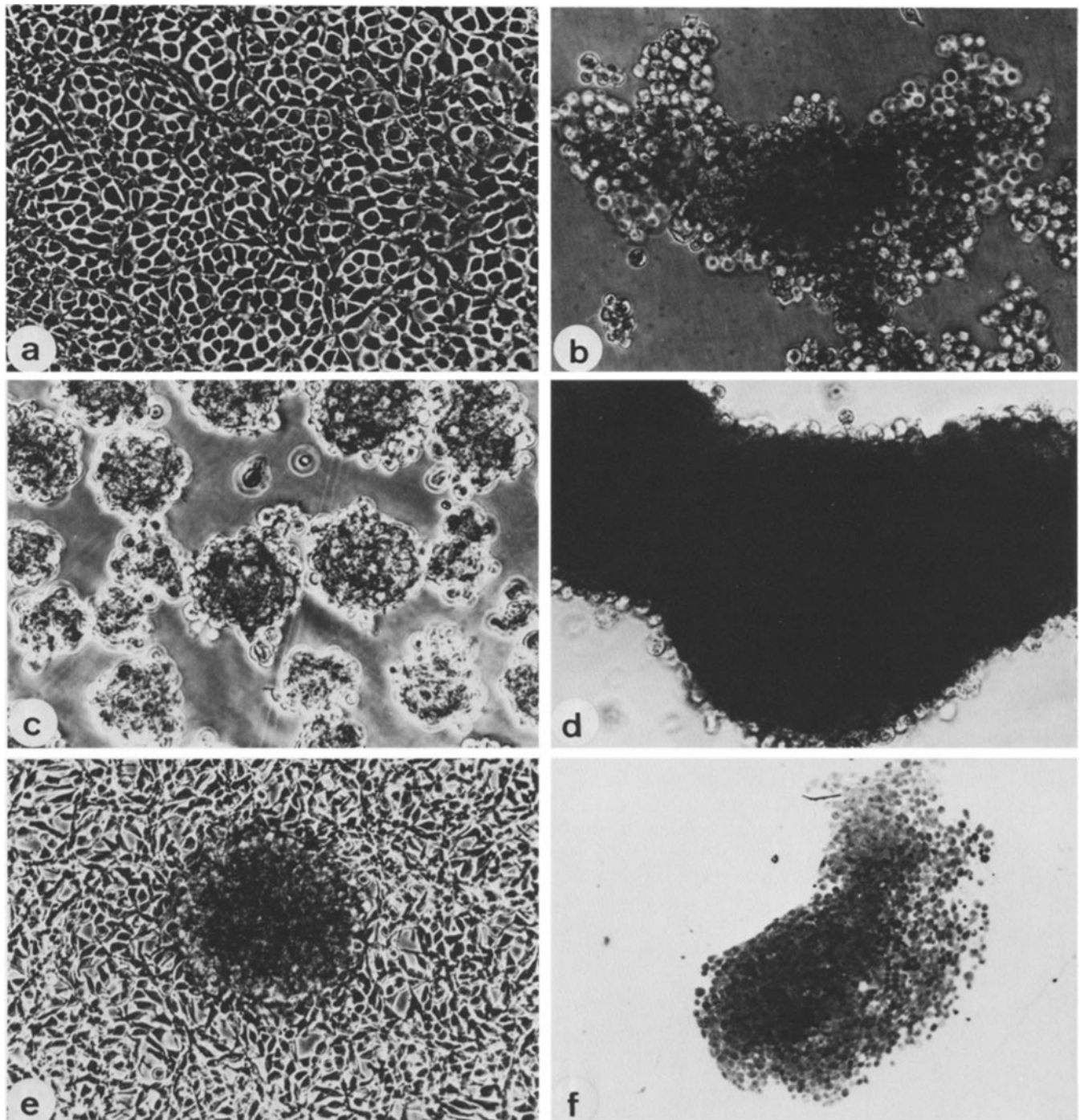


FIGURE 4 Comparison of the morphology of RSV-transformed cells growing in serum, colostrum, and milk. RSV-transformed cells growing in the various media were photographed after 10 d in culture, except where indicated otherwise. a, 10% calf serum (LR3/1); b, 10% colostrum (LR3/1); c, 10% milk, day 5 (LR3/1); d, 10% milk (LR3/1); e, FR24D-1 cells were grown at 35°C in 10% milk for 7 d followed by the addition of calf serum and photographed 3 d later; f, histology of a cluster of LR3/1 cells growing in 10% milk.

Effects of Fibronectin on the Growth of Cells in Milk

Transformed cells grow in suspension in medium supplemented with milk or colostrum but grow attached to the substratum when cultured in medium supplemented with serum. This may be caused by the presence in serum of attachment factors that are absent from milk. One factor present in serum that has been found to mediate the adhesion of a variety

of cell types to either plastic or collagen substrata is fibronectin (12, 13). The fibronectin content of milk and colostrum was analyzed. Fibronectin like material was detected in colostrum but not in older milk. However, the amount of fibronectin in colostrum was considerably less than in serum (Steimer et al., manuscript in preparation). The apparent lack of fibronectin in milk prompted us to test the effects of adding plasma fibronectin to milk and colostrum-supplemented cultures.

Tissue culture dishes were coated with 5 $\mu\text{g}/\text{cm}^2$ of human

plasma fibronectin, also referred to as cold-insoluble globulin (CIG) (14, 15). Temperature-sensitive RSV-transformed cells were plated at the permissive and nonpermissive temperature in the presence and absence of CIG. In serum, these cells grew attached to the dish at both temperatures (Fig. 5 *a* and *b*). The coating of culture dishes with CIG had a dramatic effect on the morphology and growth response of cells in milk-supplemented medium. At 35°C in the absence of CIG, these cells grew in suspension and formed clusters (Fig. 5 *c*). At 39°C, the temperature-sensitive cells remained in suspension without growing (Fig. 5 *d*). When plasma fibronectin was added to the temperature-sensitive transformed cells cultured in milk at 35°C, the cells grew attached to the substratum rather than in suspension (Fig. 5 *e*) and assumed a transformed morphology similar to that in serum. At 39°C, CIG promoted the attachment of the temperature-sensitive transformed cells in milk (Fig. 5 *f*). The cultures grew to confluence and the morphology of the cells was similar to that of normal cells growing in serum.

The results of growth studies of the temperature-sensitive transformed cells in the presence and absence of CIG are shown in Table II. In serum, the temperature-sensitive transformed cells grew at both temperatures. The addition of CIG had no effect on cell growth. In milk, there was growth at 35°C in either the presence or absence of CIG. At 39°C, there was growth only when CIG was present. There was some attachment of the temperature-sensitive transformed cells at 39°C in colostrum without exogenous CIG. These attached cells proliferated, resulting in a final cell number ~10% of that found in colostrum in the presence of CIG. Fibronectin alone was not sufficient for cell growth because cells did not grow in unsupplemented medium on CIG precoated dishes.

Normal cell lines and cell strains did not grow in medium supplemented with milk (Fig. 1). The growth behavior of these cells in milk with CIG was examined. The cell line F2408 and the cell strain LR1 were cultured in the presence and absence of CIG in unsupplemented medium and medium supplemented with either serum, milk, or colostrum (Table III). In serum, both cell types grew equally well whether or not CIG was added. However, in milk- or in colostrum-supplemented medium, the normal cells grew only in the presence of CIG. The addition of CIG increased the saturation density of F2408

cells by 75–80-fold in either milk or colostrum. In the presence of CIG, F2408 cells grew with a generation time of 29 h in milk as compared with a generation time of 24 h in serum (not shown). Although LR1 cells required fibronectin to attach and survive in milk or colostrum, growth was minimal (Table III). The cell number, as compared with the initial plating density, represented only 40 and 60% increase in colostrum and milk, respectively.

DISCUSSION

Milk is a source of growth factor activity (3–5) and can be used as a supplement for growing certain cell types in culture (6). However, the growth response of a variety of cell types is much different from that observed in serum. The growth properties of rat cells representing three cell types were examined: fibroblastic cell strains, fibroblastic cell lines, and transformed fibroblasts. There were several outstanding differences between the growth behavior of these cells in milk- and that in serum-supplemented medium. (*a*) Unlike serum, milk is selective and only allows transformed cells to grow. (*b*) In milk, transformed cells grow in suspension rather than attached to the culture dish as they do in serum. (*c*) Normal cells grow in milk only when attachment factors are provided.

The differences in the growth behavior of fibroblasts in milk and in serum may, in part, be attributed to differences in fibronectin content. Fibronectin like material can be detected in colostrum but not in older milk. However, the amount of fibronectin in colostrum is only 4–5% of that found in serum (Steimer et al., manuscript in preparation). This deficiency of fibronectin does not prevent the growth of transformed cells. These cells will grow in milk as suspension cultures. However, normal cells must attach to the substratum to proliferate. Attachment factors, such as fibronectin, must be added for normal fibroblasts to grow in milk-supplemented medium. Milk and fibronectin are all that are required to replace serum for the growth of a normal fibroblast cell line. However, fibroblast cell strains are more fastidious and require additional factors for growth that are absent from milk. Experiments in progress indicate that the addition of various factors, such as transferrin, epidermal growth factor, or insulin, will improve the growth of cell strains in milk.

Colostrum is a specialized milk produced in the first days after birth. Colostrum is highly enriched in protein and fat (16). In addition, the growth factor activity is much greater than that of milk produced later in the lactation period. For example, colostrum at a concentration of 2% contains more growth factor activity than regular milk at a concentration of 20% (5). In general, colostrum is a better growth supplement for cell culture than regular milk. Transformed cells grow at a faster rate and reach a higher density in colostrum-supplemented medium. In addition, normal cell lines cultured on fibronectin-coated dishes in colostrum-supplemented medium reach a higher saturation density than in milk. Colostrum contains a small, but detectable amount of fibronectinlike protein. The presence of fibronectin may explain why there is a limited amount of attachment and growth of normal fibroblast lines in colostrum in the absence of exogenous fibronectin.

The fibronectin-free property of milk may provide several advantages for studying the growth of cells in culture. Milk as a supplement may be useful for assaying the ability of attachment factors to promote adhesion of various cell types. For example, the effects of fibronectin on the adhesion and proliferation of nonfibroblastic cells, such as epithelial cells, may be

TABLE I
The Growth Response of Various Transformed Cells in
Medium Supplemented with Milk, Colostrum, and Serum

Cell line	Cells/well × 10 ⁻³			
	Serum*	Milk‡	Colostrum§	Unsupplemented
RSV rat (LR3/1)	2,100	180	700	1.5
RSV rat (LR3/2)	2,280	90	792	3.5
RSV rat (LR5/1)	2,280	161	316	1.2
RSV rat (FRD-4)	1,610	187	210	3.1
KNIH	1,120	55	248	1.8
Mouse sarcoma	1,410	284	ND	3.7
SV29	934	19	452	2.0

Cells of each cell type (1×10^4) were plated in 24 well microtiter plates in medium supplemented with 5, 10, or 20% of either milk, serum, or colostrum. The cultures were incubated for 10 d with feeding every 3 d. On day 10, all cultures were counted. Each value represents the average of duplicate wells. ND, Not determined.

* The optimal serum concentration was 10%.

‡ The optimal milk concentration was 20%, except SV29 which required 30%.

§ The optimal colostrum concentration was 10%, except LR5/1 and KNIH which required 5%.

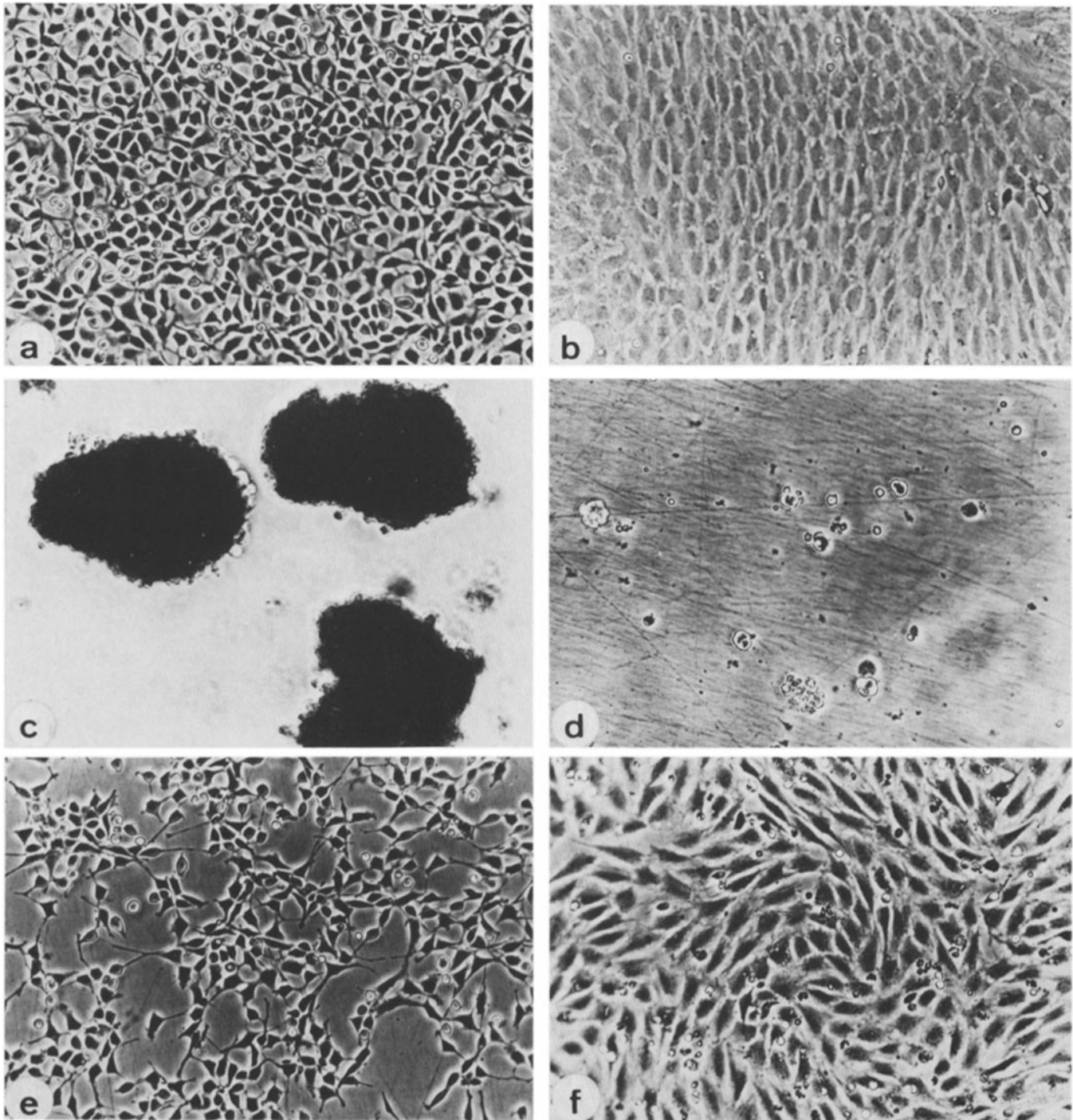


FIGURE 5 Effects of fibronectin on the morphology of temperature-sensitive RSV-transformed cells cultured in medium supplemented with milk at the permissive and nonpermissive temperature. FR24D-1 cells (1×10^4) were plated in 24 well microtiter plates. The plates were incubated at 35° or 39°C and photographed on day seven. a, 10% calf serum at 35°C; b, 10% calf serum at 39°C; c, 10% milk at 35°C; d, 10% milk at 39°C; e, 10% milk + plasma fibronectin ($5 \mu\text{g}/\text{cm}^2$) at 35°C; f, 10% milk + plasma fibronectin ($5 \mu\text{g}/\text{cm}^2$) at 39°C.

studied. In addition, the effectiveness of other attachment factors, such as chondronectin (17) or laminin (18), on the attachment and proliferation of homologous and heterologous cell types can be assayed. Such experiments cannot be done with serum supplementation without first removing the fibronectin. The fibronectin-free characteristic of milk may be used to select for the growth of certain cells in culture. For example, transformed fibroblasts that are anchorage independent (19)

grow in milk but normal cells that are anchorage dependent fail to grow. In a mixed population of normal and transformed cells, it may be possible with the use of milk to preferentially select for the transformed cells. In addition, milk may be useful for culturing other anchorage-independent cell types, such as chondrocytes (20). Milk is an inexpensive, readily obtainable growth supplement for cells in culture whose potential uses should be explored.

TABLE II
Growth of Temperature-sensitive RSV-transformed Cells in the Presence and Absence of Fibronectin *

Medium supplement	Temperature °C	Cell no. × 10 ⁻³	
		+C1G	-C1G
10% Serum	35	1,600	1,600
	39	340	300
10% Milk	35	280	190
	39	50	1.8
10% Colostrum	35	600	200
	39	320	37
Unsupplemented	35	5.7	3.9
	39	2.3	0.8

Cells (1×10^4 /well) in 0.5 ml of DMEM, supplemented as indicated in column one, were added to each well. One set of cultures were incubated at 35°C and one set at 39°C. Cell counts were made on day seven and the values reported represent the average of duplicate cultures.

* Microtiter plates (24 well) were coated with 5 $\mu\text{g}/\text{cm}^2$ of human fibronectin or left uncoated.

TABLE III
Effects of Fibronectin on the Growth of Normal Rat Fibroblastic Cell Strain and Cell Line *

Cell type	Medium supplement	Cell no. × 10 ⁻³	
		+C1G	-C1G
Cell strain (LR1)	10% Serum	40	40
	10% Milk	16	0.8
	10% Colostrum	14	3.5
	Unsupplemented	0.5	0.8
Cell line (F2408)	10% Serum	360	430
	10% Milk	120	1.6
	10% Colostrum	200	2.5
	Unsupplemented	12	4.2

1×10^4 cells in 0.5 ml of DMEM, supplemented as indicated in column two, were added to each well. Cell counts were made on day 7 and the values reported represented the average of duplicate assays.

* 24 well microtiter plates were treated with 5 $\mu\text{g}/\text{cm}^2$ of human fibronectin or left untreated.

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