GROWTH CHARACTERISTICS OF VIRUS-TRANSFORMED CELLS

Maximum Population Density, Inhibition by Normal Cells, Serum Requirement, Growth in Soft Agar, and Xenogeneic Transplantability*

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It has been shown that strains of normal cells which attain relatively low population densities in culture (contact inhibition of growth) inhibit each other in mixed culture (1). This interstrain inhibition is not species-specific (2). Cultured cancer cells, as well as heteroploid cells which arise "spontaneously" in culture, have in varying degree escaped from contact inhibition of growth, and under identical growth conditions attain a higher population density than their diploid counterparts. Such heteroploid cells are similarly insensitive to the growth inhibitory effects of other cell types, and the degree to which they have escaped from growth inhibition in such mixed cultures usually (but not invariably) parallels their maximum growth potential in pure culture (2).

Most virus-transformed cells resemble natural cancer cells and spontaneous heteroploid transformants in that they have in varying degrees escaped from contact inhibition of growth, and attain higher population densities than the parent cell; some have an enhanced capacity to form colonies in soft agar (3, 4); many are tumorigenic; and chick embryo cells transformed by Rous sarcoma

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virus (5), and mouse 3T3 cells transformed by SV-40 (6), have been shown to have a decreased serum requirement for growth.

The present paper describes the degree of correlation between these multiple changes in a number of human, monkey, mouse, and hamster cells transformed by either SV-40, polyoma, or adenovirus.

Materials and Methods

Cell Propagation.—Stock cultures growing on a glass surface were subdivided at approximately weekly intervals. The cultures were drained, overlaid for 20–60 sec in a minimal growth medium (MEM)[1] (7) lacking calcium, magnesium, and serum, but containing 0.25% Difco 1:250 trypsin and 0.01% Versene. After standing for 1–4 min, the rounded cells were dispersed in growth medium supplemented with nonessential amino acids and 10% serum (5% calf serum plus 5% fetal calf serum), and divided 2- to 100-fold, depending on the growth potential of the cell. Cultures were fed every other day, and on the day before division. In the experiments to determine maximum population density (Table I), cultures were fed daily after the 2nd day.

Cell Strains Used.—The normal human, monkey, mouse, or rabbit cells used as substrates for superinoculation by virus-transformants were all strains which, under the conditions of these experiments, became essentially saturated at relatively low population densities (2). The virus-transformants used in this study are listed in Table I.

Test for Inhibition of Virus-transformants by Normal Cells. To determine the degree to which the growth of virus-transformants was inhibited by contact with normal cells, fully grown ^{3}H-thymidine-labeled cultures of the latter (0.05 μCi of ^{3}H-thymidine added to the growth medium for 3–5 days prior to the experiment) were superinoculated with ^{14}C-thymidine-labeled virus-transformed cells at 0.3–0.6 $\times$ 10^{4} cells/cm^{2}. As previously described (2), the radioactive labels served to control both the degree to which the superinoculated cell adhered to the cell substrate, and the persistence of both the substrate and superinoculum in the following period of incubation. The combined cultures and the controls were fed daily with medium supplemented with 5% calf serum and 5% fetal calf serum. The growth of the superinoculated cell has been expressed in Table II as a percentage of the growth observed in a control pure culture, calculated as previously described (2), and averaging the results based on cell count and cell protein determinations.

Serum Requirement.—To determine the serum requirement of the normal and virus-transformed cells, cultures were planted in growth medium supplemented either with 2% or 0.2% serum (half calf and half fetal calf), depending on the serum requirement of the specific cell line. 24 hr later, after the cells had adhered to the glass and flattened, they were fed with medium containing varying concentrations of serum, refed after 2 days, and daily thereafter. The degree of growth was measured both in terms of protein content (9) and cell number, as previously described (2).

Ability of Cells to Grow in Soft Agar.—Log-phase cultures were dispersed with 0.25% trypsin, washed with growth medium, counted, and appropriate dilutions inoculated in four replicate soft agar cultures. Semisolid agar medium (3) was prepared by mixing equal portions of 1% purified agar (Difco) in glass-distilled water and double strength basal medium (BME) (10) containing tryptose phosphate broth (20%) and calf serum (20%). A base layer of 7 ml was poured into 60 mm Petri dishes and allowed to harden, and then overlaid with 1.5 ml of the same medium containing the desired number of cells. Cultures were incubated at 37°C

[1] *Abbreviations used in this paper:* ALS, anti-lymphocyte serum; BME, basal medium (10); MEM, minimal growth medium (7).

in a humidified atmosphere of 5% CO_2 in air, and colonies more than 0.7 mm in diameter were counted after 10–20 days.

Colonies growing in soft agar occasionally were examined for heterogeneity with respect to plating efficiency and heterotransplantability (see below). Monolayer cultures derived from single colonies in soft agar were replated, and the plating efficiency in soft agar determined for three successive clones. Subcultures from the third cloning were reimplanted in the hamster cheek pouch for comparison with the original culture. No significant differences were seen between the parent colonies and their derived clones, and they are not distinguished in the tables.

Tumorigenic Activity.—

Hamster cheek pouch: As originally reported by Patt, Handler et al. (11, 12), the cheek pouch of the Syrian hamster (*Mesocricetus auratus*) accepts xenogeneic transplants of some neoplastic tissues, but with few exceptions rejects xenogeneic transplants of normal tissue. This provided a useful transplantation system for the assay of the "malignant potential" of cultured cells (13). The cell lines listed in Table I were implanted into the cheek pouches of Syrian hamsters according to methods described elsewhere (13). All manipulations were done in complete growth medium (BME) to preserve the physiologic integrity of the cells. Log phase cultures of the parent cell lines, or of cultures derived from single colonies, were dispersed with trypsin pooled, sedimented at 50–70 *g*, washed three times with BME, and diluted with BME so that the desired inoculum was contained in 0.1 ml of the final suspension. The total preparation time was less than 2 hr, and more than 90–95% of the cells implanted excluded trypan blue. Both cheek pouches of each of six hamsters were everted under light Nembutal anesthesia, and the inocula were implanted into the connective tissue as an "intracutaneous" bleb. There hamsters in each group were "conditioned" with cortisone acetate (saline suspension obtained from Merck, Sharp & Dohme, West Point, Pa.), administered subcutaneously in doses of 2.5 mg twice weekly. The cheek pouches were observed once or twice weekly for at least 60 days postimplantation, and evaluated as described elsewhere (13).

Baby hamster: Since human cancer cells of lymphoid origin can produce tumors in normal or anti-lymphocytic serum (ALS)-treated neonatal hamsters (15, 16), the virus-transformants were similarly tested. Each animal in two litters of newborn Syrian hamsters (less than 24 hr of age, with 8–10 animals per litter) received an intraperitoneal implant of at least 10^7 packed cells in a volume of 0.1 ml. One litter was treated with ALS administered intraperitoneally 3 times weekly in doses of 0.05 ml, beginning at the time of implantation. The animals were observed for tumor formation and sacrificed after 60–90 days.

Mouse inoculation: Swiss female mice, 8–10 wk of age, were injected subcutaneously into the flank with cells prepared as for hamster cheek pouch inoculation. Mice were observed for tumor formation twice weekly for 9 months. In addition, newborn mice were injected intracerebrally with 0.01 ml of a similar cell suspension, and observed for signs of cerebral involvement. When mice died after inoculation, brain tissue was examined histologically for presence of tumor tissue; and survivors were sacrificed for similar examination after 2 months.

RESULTS

Growth Capacity of Virus-Transformants.—As seen in the last two columns of Table I and in Fig. 1, although transformation by viruses usually caused a significant increase in the maximum cell population achieved under the conditions of the present experiments, the magnitude of that increase varied widely. Marked increases were observed in six different lines after SV-40 transformation; but an adenovirus-transformed monkey cell showed only slightly en-

TABLE I

Virus-Transformed Cells Used for Superinoculation, and Their Growth Potential Relative to Parent Cell

Transforming virus	Parent cell				Virus-transformed cell					
	Species	Strain	Maximum population density*		Designation of transformant	Date of transformation	Maximum population density		Maximum growth relative to that of parent cell as 1	
			Protein	Cells			Protein	Cells	Protein	Cells
			$\mu g/cm^2$	10^4 per cm^2			$\mu g/cm^2$	10^4 per cm^2		
SV-40	Human	W 98	—	—	W 98-Va-E	April 1963	219	59	2.4‡	4.2‡
	"	W 98 (adult skin)	—	—	W 98-Va-H	April 1963	175	59	1.9‡	4.2‡
	"	W-18 (adult buccal)	—	—	W-18-Va2	Sept. 1962	239	70	2.5‡	5.0‡
	"	Wi-26 (embryonic lung)	68		Wi-26-Va4	April 1963	359	93	5.3	6.6
	Monkey	AGMK§ (kidney)	49	8	GMK-EVa	Feb. 1966	244	52	5.0	6.5
	Mouse	3T3 (skin)	59	10	SV-3T3		163	49	2.8	4.9
Adeno 7	Monkey	AGMK§ (kidney)	49	8	GMK-adeno	August 1965	140	47	2.8	5.9
Adeno 7 SV-40 hybrid	Monkey	AGMK§ (kidney)	49	8	GMK-LL-E-46	April 1966	96	26	1.9	3.2
Polyoma	Hamster‖	Baby hamster kidney BHK 21-E	245	80	BHK-Py	Sept. 1962	236	80	0.97	1.0
		‖	195	37						

* Highest value obtained in 2–9 experiments under conditions of present experiments (daily feeding with medium containing 10% serum; see p. 864).

‡ Parent cell not available for study. Results with virus transformant were compared with average value obtained with nine normal human diploid fibroblasts (90 μg protein and 14×10^4 cells/cm^2).

§ Early passage, presumably diploid at time of viral transformation.

‖ Cell line isolated by Stoker and Macpherson (8). Two "control" sublines were used, one obtained from the American Type Culture Collection, Rockville Md., and one a spontaneous transformant passaged in this laboratory (BHK 21-E).

hanced growth capacity. A monkey cell transformed by an adeno-SV-40 hybrid showed the small increase in maximum population density seen in the adenovirus transformant, rather than the marked increase caused by SV-40 transformation alone. Finally, although the polyoma-transformed hamster kidney cell attained an extremely high population density, the growth capacity of that virus-transformant was no greater than that of a cell (BHK-E) which had transformed "spontaneously" in culture.

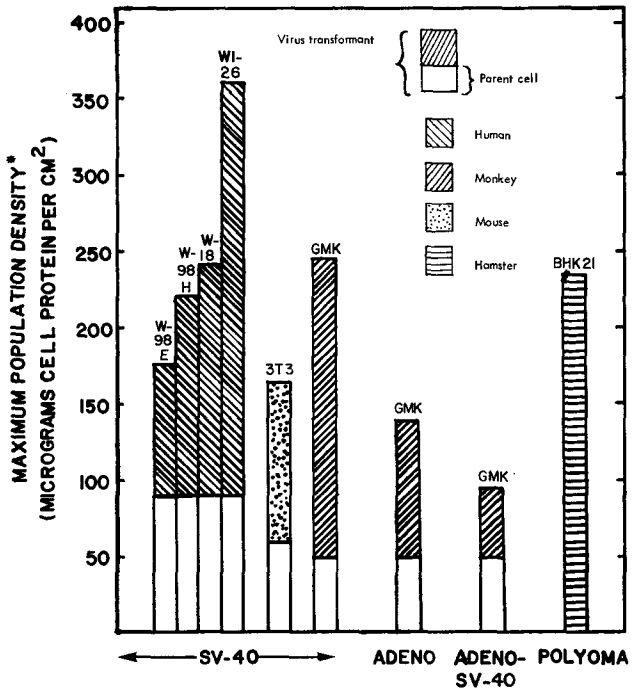

FIG. 1. The effect of virus transformation on the maximum population density achieved under standardized conditions of growth. (For human cell controls, see footnote ‡, Table I).

It is of interest that the virus-transformed cells were usually smaller than the parent cells. The increase in cell population effected by transformation of human, monkey, and mouse cells regularly exceeded the increase in cell protein, the amount of protein per cell in the transformants varying between 48–77% of that of the parent cell (cf. last two columns of Table I).

Susceptibility of Virus-transformed Cells to Growth Inhibition by Normal Cells.—With a wide spectrum of normal and cancer cells, it had been found (2) that when small numbers of cells were superinoculated onto an essentially saturated monolayer of human or mouse cells, the ability of the superinoculated

TABLE II

Growth of Virus-Transformed Cells when Superinoculated onto Essentially Saturated Cultures of Normal Human, Monkey, Mouse and Rabbit Cells

Virus-transformed cell superinoculated onto monolayer	Virus	SV-40						Adeno	SV 40-adeno hybrid	Polyoma
	Cell species	Human				Monkey	Mouse	Monkey		Hamster
	Transformed cell line	W 98-E	W 98-H	W 18-VA2	Wi26-VA4	GMK-EVA	SV-3T3	GMK-adeno	GMK-LL	BHK-PY
Cell in substrate monolayer		Growth of superinoculated virus-transformant (% of growth observed in pure culture)*								
Human diploid fibroblasts‡	Median, % of control	34	36	39	41	48	8	45	50	54
	Range, % of control	(11–45)	(28–51)	(4–79)	(0–75)	(45–67)	(0–12)	(30–71)	(20–76)	(26–73)
African green monkey kidney‖	Median	—	—	—	—	32	25	3	17	—
	Range	(5, 13)	(19, 22)	(10, 22)	(3, 8)	(0–103)	(24–100)	(0–7)	(0–59)	(61, 70)
Mouse skin fibroblasts (3T3)	Median					7	11			—
	Range	(38)	(74)	(0, 5)	(56)	(2–44)	(0–52)	(13, 14)	(17, 50)	(43, 100)
Rabbit lens¶ Diploid epithelial strain	Range	(59)	(31, 60)		(20, 30)	(48)	(15, 30)	(20)	(10)	(100)
Heteroploid fibroblastic strain	Range	(21)		(28)		(56)	(0, 36)	(0, 48)	(1)	(82)

* It is to be noted that e.g. a 50% inhibition in cell growth after a period of 6 days in fact represents a relatively minor inhibition of the rate of cellular replication. If the cell number had increased e.g. 32-fold in that 6-day period, 50% inhibition means that the average cell doubling time had increased from 1.2 to 1.5 days.

‡ Nine different strains of human diploid fibroblasts (BAL, Detroit 510 and 551, E 699, HG 46, Wi 26, Wi 38, and embryonic skin).

§ 34 = median; 11–45 = range, % of growth observed in pure culture (single no. indicates only one experiment).

‖ Three lines of African green monkey cells (AGMK, CP, CV).

¶ See (14).

cell to grow was usually correlated with its inherent capacity for growth: the higher the population density attained by a given cell type in pure culture, the less was its susceptibility to inhibition by other cell types. When virus-transformed cells were similarly superinoculated, the results were extremely variable (Table II), and there was no such clear relationship between growth capacity

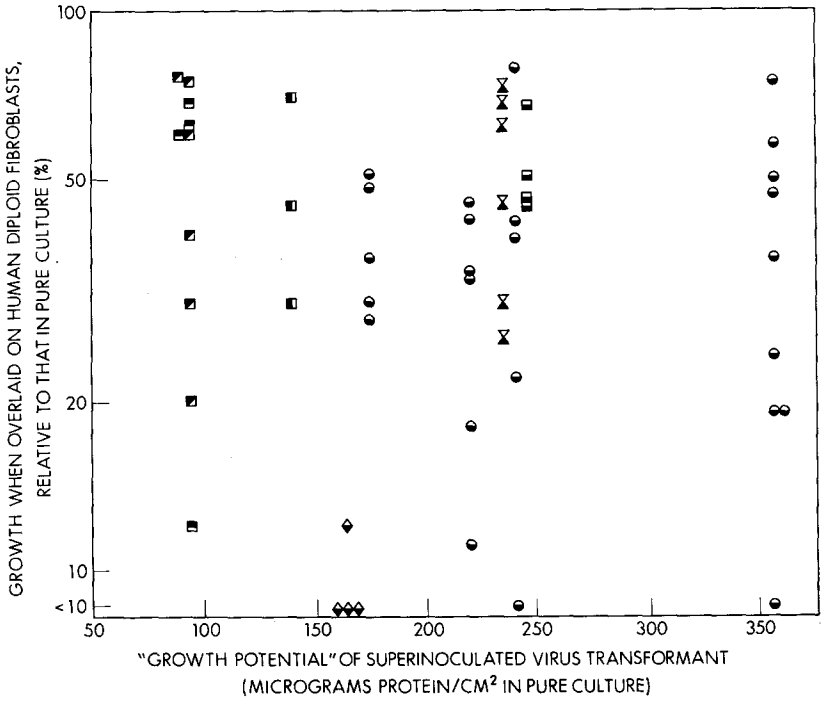

FIG. 2. Growth of virus-transformed cells when superinoculated onto essentially saturated monolayers of human diploid fibroblasts. Human cells transformed by SV-40, ◒; monkey cells transformed by SV-40 ◘; by adeno, ◧; by adeno–SV-40, ◩; mouse cells transformed by SV-40 ⧫, ; hamster cells transformed by polyoma, ⧗.

and susceptibility to inhibition (Fig. 2). Some of the virus-transformants, including a few with a high capacity for growth, were markedly inhibited (cf. inhibition of SV-40-transformed mouse and human cells by human diploid fibroblasts (⧫ and ◒ in Fig. 2), of GMK-EVa and W 18-Va by mouse fibroblasts (Table II), and of GMK-adeno by both the parent cell and by rabbit lens cells). At the other extreme, some virus-transformants, like most naturally occurring cancer cells (2), were not appreciably inhibited by contact with a saturated cell substrate (cf. polyoma-transformed BHK cell overlaid onto any other cell type: last column of Table II, and ⧫ in Fig. 2). In most of the other

cell combinations, the transformants were partially but significantly inhibited, although the results in repeat experiments sometimes varied widely (cf. Table II). As with the normal and cancer cells previously studied (2), the intercell inhibitions observed were not species-specific.

The varying susceptibility of virus-transformants to inhibition by contact with normal cells, and the variability in the results obtained in replicate experiments with the same cell lines (Table II) are consistent with the widely vary-

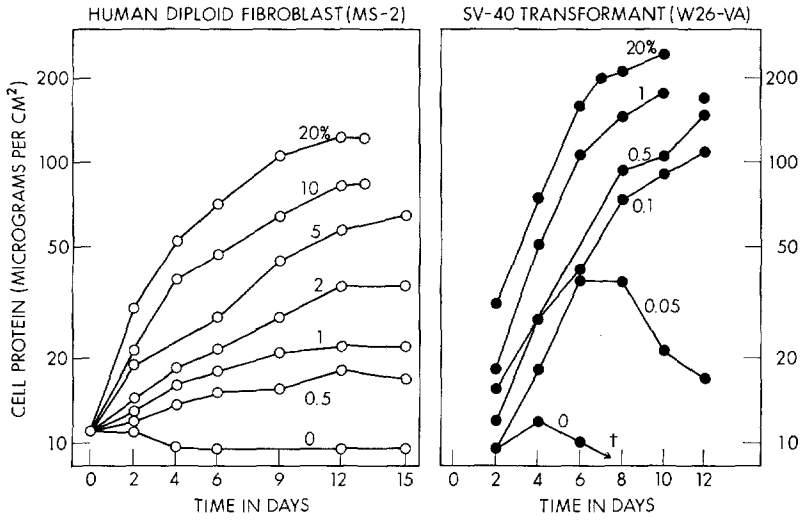

FIG. 3. The growth of normal and SV-40-transformed human fibroblasts as a function of the serum concentration (per cent in medium).

ing results previously reported, ranging from marked inhibition of growth (17–19) to essentially total escape from inhibition (20, 21).

The Serum Requirement of Normal and Virus-Transformed Cells.—In confirmation of the results of Temin (5) with chick embryo cells transformed by Rous virus, and of Holley (6) with the mouse 3T3 and its SV-40 transformant, significantly lower concentrations of serum were required for the sustained growth of most virus-transformants than for the parent cells. Although both cell types grew at essentially the same rate in high concentrations of serum (20%), the virus-transformants generally attained higher population densities. A typical experiment is shown in Fig. 3.

Fig. 4 contrasts the effect of serum concentration on the population density achieved by a number of normal and SV-40-transformed human cells; and Fig. 5 similarly contrasts the difference in their response to serum, measured in

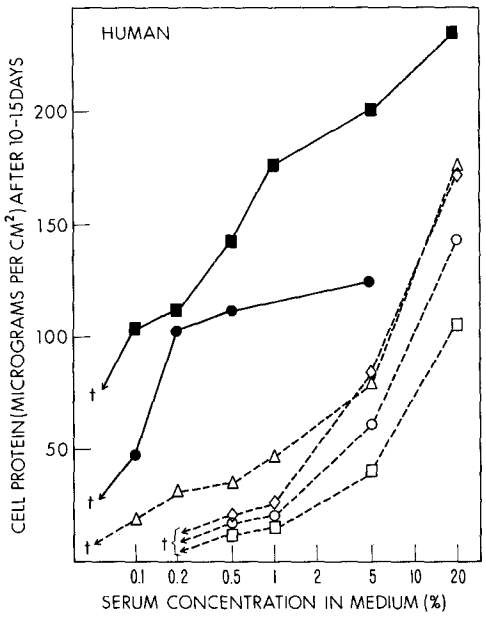

FIG. 4. The effect of serum concentration on the population density achieved by normal and SV-40-transformed fibroblasts. Normal strains: MS 2A, ○; Penny, ◇; Wi 38, △; Detroit, □. SV-40-transformed strains: W98-H, ●; W26-VA, ■.

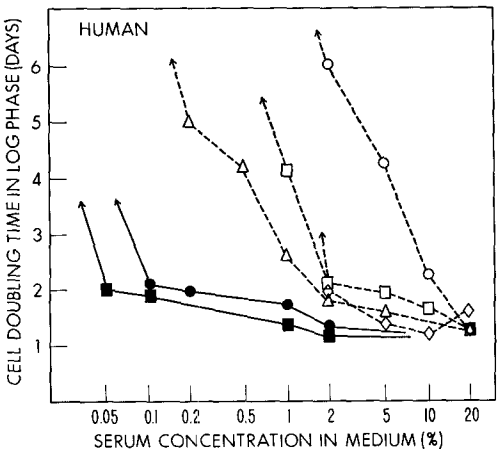

FIG. 5. The serum requirement of normal and SV-40-transformed human cells, measured by the average doubling time for cell protein during the logarithmic phase of growth. (For symbols, cf. Fig. 4).

terms of doubling time for cell protein in log phase. Not all the virus-transformants, however, showed as striking an effect as the SV-40-transformed human lines. In Table III, the serum response of the virus-transformants has been expressed as the concentration which permits protein synthesis in log phase at half the rate observed in 20% serum. As there seen, neither the SV-transformed mouse fibroblast nor the polyoma-transformed hamster kidney cell differed significantly from the parent with respect to their serum require-

TABLE III

Concentration of Serum which Permits Cell Multiplication at 50% of the Rate Observed in 20% Serum*

Species	Cell strain	Virus	Transformant‡	"50% effective" serum concentration	
				Normal	Virus transformant‡
Human	MS 2	SV-40	98H	10	0.1, <0.1
	Penny	"	18Va	3, 1–2	1
	Wi 38	"	26Va	1, 1	<0.2, <0.05, 0.4, <0.1
	Det	"		1.7	
Monkey	GMK	Adeno	GMK-adeno	1, 2±	0.8, 0.5
	"	SV-40	EVa		0.5, 0.6
	"	Adeno–SV40 hybrid	LL		<0.2, <0.2
Mouse	3T3	SV-40	SV-3T3	2, 2, 3.4	2.5
Hamster	BHK 21	Polyoma			1.5
"		Spontaneous transformant			1–2

* Based on time required for doubling of cell protein in logarithmic phase of growth under conditions of present experiments.

‡ See footnote‡, Table I.

ment. In both cases, however, the parent cell used for comparison was a line which had transformed "spontaneously" in culture and had developed at least some of the properties of virus-transformants. Subcultures of the same line obtained from other laboratories had significantly higher serum requirements than that here studied. The monkey kidney adeno- and SV-40-transformants showed a small but significant decrease in serum requirement; and that requirement was greatly reduced in the cell transformed by the hybrid SV-40–adenovirus.

Colony Formation in Soft Agar.—It has been found in a number of laboratories

(4) that virus-transformation often results in increased plating efficiency in soft agar. With the virus-transformants here studied, the number of colonies formed per 10^5 cells varied widely, from zero in the case of the GMK-adeno-transformant to 1565 for the hamster polyoma-transformant (Table IV). Surprisingly, two sublines of the same hamster kidney culture (cf. Table I, footnote[11]), both of which were tumorigenic, gave wholly disparate results on inoculation into soft agar. One yielded an average of 690 colonies per 10^5 cells inoculated,

TABLE IV

Plating Efficiency of Virus-Transformants in Soft Agar

Cell		Transforming virus	Strain designation	Cell inoculum × 10^4	Plating efficiency (colonies/10^5 cells)	
Species	Strain				Range	Mean
Human		None	Variety of human diploid fibroblasts	−100	<0.1	0
	W-98	SV-40	W-98-Va-E	2.5–10	40–44	42
			W-98-Va-H	0.5–8	80–153	111
	W-18		W-18-Va2	0.125–4	165–240	191
	Wi-26		Wi-26-Va4	5–20	10–12	11
Mouse	3T3	None	3T3	10–100	1.8–2.4	2
		SV-40	3T3-SV40	2.5–10	28–30	29
Monkey	AGMK	None	AGMK	6.75–100	<0.1	0
		SV-40	AGMK-EVa	0.625–10	302–432	363
		Adeno 7	AGMK-adeno	5–100	0	0
		Adeno 7-SV 40	AGMK-LLE46	5–40	13.5–20	15.7
Hamster	BHK	None (spontaneous transformant)	BHK-21	5–100	<0.1!	<0.1!
			BHK-21-E	0.675–1.25	550–770!	690!
		Polyoma	BHK-PY	0.25–2	1480–1520	1540

and the other gave none with 10^6 cells. Except for the mouse 3T3 cell, none of the normal cells tested produced colonies, even with inocula of 10^6 cells.

Tumorigenic Activity.—

Hamster cheek pouch: As indicated in the first two columns of Table V, of the nine virus-transformed cell lines here studied, only the polyoma-transformed hamster kidney cell (3) was tumorigenic when implanted in the cheek pouch of the Syrian hamster. In this case, however, the parent culture was just as tumorigenic as the virus transformant. The two sublines of the parent cell used in these experiments, both of which had transformed spontaneously in terms of

maximum population density and escape from contact inhibition by normal cells, were equally tumorigenic, despite the fact that one of these sublines did not grow in soft agar.

Baby hamsters and mice: Similar results were obtained when the virus-trans-

TABLE V

*Degree of Correlation in the Altered Properties of Viral Transformants**

Viral transformant			Ability to produce tumor‡			Escape from contact inhibition					Decreased serum requirement (Table III)	Greater plating efficiency in soft agar (Table IV)
			In hamster cheek pouch	In baby hamster§	In mouse brain	Increased population density under standardized conditions of growth (Table I)		Susceptibility to inhibition by normal cell monolayers (Table II)				
						Protein	Cell	Human	Monkey	Mouse		
Human	SV-40	98E	0‡	—	—	++	+++	+	+++	+		+
		H	0	0	—	+	+++	+	++	0	++	++
		18Va	0	—	—	++	++	+	++	+++	0	++
		26Va	0	0	—	+++	+++	+	+++		+++	+
		GMK-EVa	0	0	—	+++	+++	±	±	+++	+	++
Monkey	Adeno	GMK-adeno	0	—	—	++	+++	+	+++	++	+	0
	SV 40-adeno hybrid	AGMK-LL-E46	0	0	—	+	++	±	+	+	++	+
Mouse	SV-40	SV-3T3	0	0	0	++	+++	+++	+	++	0	+
Hamster kidney	Polyoma	BHK-21-Py	+(10^5)§	+(10^5)	0		—‖	±	0	0	—‖	+++

* +, ++, +++ reflect the following arbitrary weighing of data in Tables I-IV:

	0	±	+	++	+++
Growth relative to control (Table I)			>1–2	>2–4	>4–
Growth inhibition by other cells (Table II)	>60	>40–60	>20–40	>10–20	>10
Serum requirement for 50% growth (Table III)	≧1%		>0.2–1.0	>0.05–0.2	≧0.05
Colonies/10^5 cells in soft agar (Table IV)	0		1–100	>100–1000	>1000

‡ 0, no tumor produced by 10^6 cells

§ +, tumor produced by 10^5 cells, but not at lower doses. In this case, however, cultures of the parent cell were also tumorigenic, and in both the hamster cheek pouch and baby hamster.

‖ Parent (untransformed) culture not available for study.

formants were inoculated intraperitoneally into neonatal hamsters. The polyoma-transformed hamster kidney cell (BHK-21) was tumorigenic, as were "control" sublines of the parent cell, but none of the other virus-transformants studied produced tumors even with inocula of 10^6 cells (Table V). Neither the

parent BHK nor its polyoma-transformant produced tumors in newborn mice injected intracerebrally.

DISCUSSION

Virus-transformation effects a number of major changes in cell properties, in addition to the acquired oncogenicity (22); and a similar pattern of change is seen in cancer cells and in spontaneous transformants in vitro (22), as well as in cells transformed by X-ray (23) or by carcinogens (24). The degree of association among the five such properties studied in the present experiments with eight virus-transformants is indicated in Table V. Except for tumorigenicity, all the virus-transformed lines were altered with respect to most of the properties studied. That association was, however, not invariable; and there was no clear correlation in the magnitude of the changes observed. Thus, the hybrid SV-40–adenovirus–transformant of the monkey kidney line grew to smaller population densities than either of the individual transformants (Table I), but had a lower serum requirement than either (Table II), and was intermediate with respect to its capacity to grow in soft agar (Table IV).

The most prominent characteristic of the virus-transformants here studied was the failure of all but one (the polyoma-transformed baby hamster kidney cell)[2] to produce tumors on inoculation either into the cheek pouch of the hamster, into neonatal hamsters, or into the mouse brain. This may mean only that a negative result in these specific systems does not exclude "malignancy" (i.e. the ability of the transformed cell to produce a cancer) under other conditions. The fact that the transformed cells failed to produce tumors in the hamster cheek pouch or peritoneal cavity even when the immunologic mechanisms of the host had been depressed by treatment with cortisone acetate or anti-lymphocytic serum, respectively, does not wholly exclude the possibility that the generally negative results reflect heterospecific rejection of a potentially tumorigenic cell.[3] Alternatively, however, the cells may never have been malignant, or if tumorigenic when first transformed, had lost that property in the course of their serial propagation, as has been shown to occur with both naturally occurring cancer cells (25) and virus-transformants (26, 30).

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[2] It is of interest that the two sublines of the BHK (hamster kidney) cell used as controls for the polyoma transformant were just as tumorigenic as the virus transformant. The possibility that these spontaneous variants may have been transformed by an unrecognized virus can of course not be excluded. The presence of virus-like particles in clones of this strain has in fact been reported from two laboratories (28, 29).

[3] It has in fact recently been found (Dr. E. Stanbridge and Dr. F. Perkins, personal communication) that an SV-40–transformed human line produced "tumor nodules" when inoculated subcutaneously into mice treated with anti-lymphocytic serum; and Dr. B. Pessac (personal communication) has similarly found that mice treated with X-ray postnatally developed tumor nodules after subcutaneous inoculation with either the parent or SV-40–transformed 3T3 lines. Those tumors, however, regressed unless the mice were treated with anti-lymphocytic serum.

Be that as it may, it is apparent that an enhanced capacity for growth, relative insusceptibility to inhibition by contact with normal cells, decreased serum requirement, and enhanced capacity to grow in soft agar, did not in and of themselves endow the cells here studied with the ability to produce a tumor in hamsters or mice. Along the same lines, Rabson et al. (31) have found that SV-40–transformed monkey cells were not tumorigenic on autologous inoculation, and widely varying results have been obtained with cloned sublines of polyoma-transformed Chinese hamster cells (30). Further, McAllister and Reed (32) have pointed out that normal and cancerous human cells cannot be distinguished on the basis of their capacity to grow in soft agar. In the present experiments also, a number of virus-transformants which grew in soft agar failed to produce tumors on inoculation; and conversely, although two spontaneous transformants of a hamster kidney line were just as malignant as the virus-transformant, as judged by animal inoculation, one failed to form colonies in soft agar even with inocula of 10^6 cells (Table IV). The dissociation between the tumorigenicity of virus-transformants and many of the other altered properties is further evidenced by the fact that at least some of these alterations may develop before the transformants become capable of producing a tumor (21). Conversely, revertants of polyoma-transformed hamster cells have been described which retain the capacity to produce a tumor although they have reverted toward normal with respect to most of the other changes associated with viral transformation (27). The possibility of course remains that altered properties of virus transformants other than those here studied (e.g. a new antigenic activity, or a specific chemical change in a cell glycolipid) (36) may be the immediate determinants of their oncogenicity.

Despite the evidence that the phenotypic consequences of viral transformation here studied are neither a necessary nor sufficient condition for the ability of the transformed cells to produce a tumor in experimental animals, the fact remains that these alterations and tumorigenicity are conjoined with high frequency, not only in virus-transformants, but in spontaneous transformants and naturally occurring cancer cells as well. Further, it has been shown by two different procedures that mouse cells selected simply on the basis of their increased susceptibility to contact inhibition of growth also showed decreased tumorigenicity (33, 34). Similarly, revertants of polyoma-transformed hamster (26) and mouse cells (31) have been described which had lost their capacity to produce a tumor and had simultaneously reverted toward normal in terms of saturation density, susceptibility to contact inhibition, and inability to clone in soft agar.

As already indicated, the fact that the phenotypic changes seen in virus transformants are not invariably associated with tumorigenicity may reflect only the inadequacy of any of the presently available animal tests of malignancy. Alternatively, however, the determinants of malignancy may be closely

linked to, rather than identical with, those of the other properties of virus-transformed cells here studied. In any case, as previously suggested by Federoff (35) and others, there as yet appear to be no generally valid in vitro criteria for predicting tumorigenicity.

SUMMARY

Virus transformants (like cancer cells, cells transformed by X-ray or carcinogens, or those which have transformed spontaneously) exhibit a number of phenotypic changes which are usually associated, and which may be lost concurrently. That association is, however, not invariable. More particularly, the altered characteristics here studied (escape from contact inhibition of growth and susceptibility to inhibition by other cells, decreased serum requirement, and ability to grow in soft agar) do not, in and of themselves, endow the cell with the capacity to produce a tumor, at least as judged by the methods of assay here used. Although the question as to whether the tumorigenicity of virus transformants is causally linked to any of these associated changes cannot be answered definitively, the evidence suggests a close linkage, rather than identity, between the determinants of oncogenicity and the other properties here studied.

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