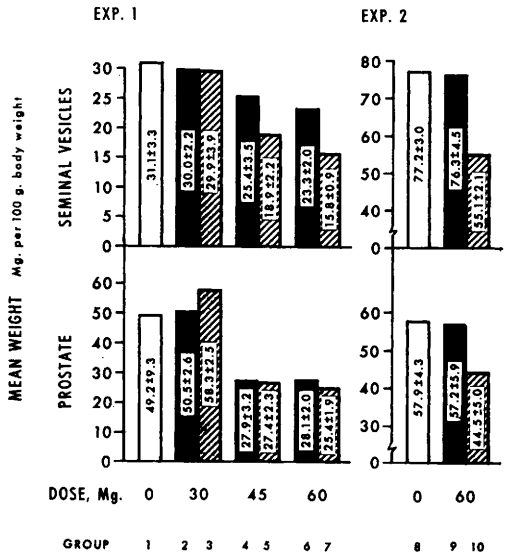

FIG. 1. Comparative inhibition of androgenic stimulation by splenic and subcutaneous depots of cyproterone acetate. □—splenic cholesterol and subcutaneous cholesterol; ■—splenic Cyp A and subcutaneous cholesterol; crosshatched area—splenic cholesterol and subcutaneous Cyp A. (Mean ± standard error)

seminal vesicles, although fewer of these tendencies were statistically significant.

Cyp A was markedly anti-androgenic. Absorption of .6 to .8 mg per day from subcutaneous pellets reduced by 30 to 50% the weight of seminal vesicles in rats receiving daily injections of .4 mg of testosterone propionate.

Summary and conclusions. The effects of splenic *vs.* subcutaneous depots of pellets of an anti-androgen, cyproterone acetate (Cyp A), were studied in castrate rats receiving daily injections of .1 mg of testosterone propionate per 100 g body weight. Mean loss of weight of pellets was similar in both sites, indicating comparable absorption. Inhibition of growth of seminal vesicles was significantly less with splenic than with subcutaneous depots of Cyp A ($P<.05$, Exp 1; $P<.001$, Exp 2). These data provide evidence that (a) this compound does not exert its anti-androgenic effects by increasing the inactivation of testosterone propionate in the liver, and that (b) Cyp A can be destroyed in the liver before producing systemic effects as an anti-androgen. The effectiveness of Cyp A as an anti-androgen was shown by a 40% reduction in androgenic response in studies employing a ratio of 2 parts of Cyp A (supplied by pellets) to 1 part of testosterone propionate (supplied in oil).

1. Junkmann, K., Neumann, F., Acta Endocrinol., 1964, Suppl. 90, 139.

2. Lerner, L. J., Recent Progr. Hormone Res., 1964, v20, 435.

3. Bridge, R. W., Scott, W. W., Invest. Urol., 1964, v2, 99.

4. Kuntzman, R., Sanaur, M., Conney, A. H., Endocrinology, 1965, v77, 952.

5. Conney, A. H., Klutch, A., J. Biol. Chem., 1963, v238, 1611.

6. Biskind, G. R., Mark, J., Bull. Hopkins Hosp., 1939, v65, 212.

7. Bernstorf, E. C., Proc. Soc. Exp. Biol. and Med., 1948, v69, 447.

8. Dunnett, C. W., J. Am. Statist. Assn., 1955, v50, 1096.

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Tissue Culture Characteristics and Oncogenicity of Three SV40-Induced Hamster Tumors. (31342)

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In vitro transformation by simian virus 40 (SV40) of fibroblast cultures of hamster lung or liver has resulted in altered growth characteristics of these cells and their production of undifferentiated sarcomas or fibrosarcomas on injection into hamsters(1). Serially cultured human fibroblasts, infected with SV40, have changed morphologically into epithelial-like cells(2,3), and hamster renal cultures have been transformed by SV40

into epithelioid cells which produced adenocarcinomas *in vivo*(4), or into mixed outgrowths of epithelial and fibroblastic cells which produced either pure sarcomas or carcinosarcomas(5-7). Transplantation of cloned lines from the mixed cultures has indicated that a single epithelioid cell is capable of differentiating into tumors composed of tubular epithelium as well as sarcomatous area(8). Tissue cultures derived from a single SV40-induced primary hamster sarcoma have given rise to tumorigenic sublines of both fibroblast and epithelial morphology(9). These results suggest, as Black has postulated(8), that differentiation mechanisms are disturbed, and that the transformed newborn hamster cell propagated *in vitro* has an increased potential for change.

This report describes establishment *in vitro* of 3 spindle cell sarcomas, induced in hamsters by neonatal inoculation with SV40, which differed in morphology and growth characteristics in culture. These were stable properties of the tumors and were not correlated with malignant capacity *in vivo*.

Materials and methods. Virus. The SV40 strain has been described previously(10).

Tumor induction, transplantation, tests for oncogenicity. Syrian hamsters, random bred for approximately 10 years in these laboratories, were originally obtained from the Lakeview Hamster Colony, Newfield, N. J. Animals were inoculated in the dorsal subcutaneous tissues when approximately 24 hours old with 0.2 ml of SV40.

Tumors were removed aseptically and transplanted by either of two methods. 1) They were cut into 1.0-2.0 mm fragments in LAPAGT medium(11) containing horse serum 2%, penicillin 100 μ, streptomycin 50 γ, and neomycin 20 γ/ml (LAPAGT-HS), and 2-6 fragments were transplanted by trocar into the dorsal subcutaneous tissues of weanling 21-30 day-old-hamsters. 2) Cells were liberated from tumor fragments by dispersal with 0.25% trypsin in phosphate buffered saline (PBS)(12) containing 0.001% desoxyribonuclease,* washed with PBS, and resuspended in LAPAGT-HS. Cell concentrations were determined by direct count in a hemocytometer and 10^3-10^5 cells were inoculated subcutaneously into suckling (1-4-day-old) or weanling hamsters.

In experiments to determine oncogenicity, cell suspensions from hamster-passed tumors were diluted serially in LAPAGT-HS and inoculated in 0.2 ml amounts subcutaneously into the flanks of weanling hamsters. Animals were observed twice weekly for tumors at the site of inoculation during 3 months; they were autopsied when tumors were large, and some tumors were removed for histological examination. Tissue cultured tumor cells were tested in the same way, except that crystalline trypsin 0.01% in Hanks' balanced salt solution(13) at pH 8.5 was used to dissociate cells from culture bottles.

Tissue cultures. To establish *in vitro* lines of tumors, 12 or 15 tumor fragments were explanted into 8 oz Neutraglas bottles† onto a thin layer of coagulum composed of 2 parts chicken plasma‡ and 1 part chick embryo extract prepared by Earle's procedure(14). Fifteen ml of LAPAGT medium containing cow serum 10% and aureomycin 50 γ/ml (LAPAGT-CS) was added to each bottle; the bottles were stoppered, incubated at 37°C, and replenished with fresh medium each 2 or 3 days. As cells migrated from the fragments, the plasma in these areas became liquefied and confluent cell sheets were subsequently established on glass in most primary cultures. These were subcultured thereafter by treatment with 5 ml of crystalline trypsin 0.01% in Hanks' balanced salt solution. The dissociated cells were diluted with LAPAGT-CS to desired cell concentrations and inoculated into other bottles. For cytological study, coverslip cultures were prepared as reported earlier(10). CMK cultures were prepared as previously described(10) from trypsinized, frozen kidney cells which had been pretested for SV40 and other simian viruses.

Hamster kidney cells BHK 21(15) used as control antigens in complement fixation (CF) tests were obtained from the Registry of

* Pancreatic desoxyribonuclease—Calbiochem Co., Los Angeles.

† Kimble Glass Co. #1425.

‡ Difco Laboratories, Detroit, Mich.

Certified Cell Cultures of the American Type Culture Collection, Washington, D. C. A culture received in 52nd passage was further propagated here in Eagle's medium(16) fortified with 10% cow serum and 10% tryptose phosphate broth.

Tests for infectious virus. Tests for SV40 were made by inoculating 0.5 ml of undiluted tissue culture fluid or whole culture homogenates into 4 to 8 CMK cultures and observing these over a 5-week period. Tests were also made with the Eddy-Gerber(17,18) overlay method, by inoculating viable tumor cells directly onto CMK monolayers; and also by inoculating hamster passage tumor cells, disrupted by freezing and thawing, into CMK cultures.

Test for CF tumor antigen and SV40 neutralizing antibody. The microtechnique of Sever(19) as described by Huebner(20) using 2 exact units of complement was employed for CF testing. Antigens were 20% homogenates of tumors or normal hamster muscle, clarified by centrifugation at 2500 rpm for 20 minutes, and 10% suspensions of tissue culture cells scraped from the glass, frozen-thawed and homogenized in a Ten Broeck grinder. All antigens were prepared cold in veronal buffered diluent(21) and stored at —70°C until used.

Sera, collected by cardiac puncture from normal or tumor-bearing hamsters which had been starved for 18-24 hours, were heated at 56°C for 30 minutes. Sera were tested for CF tumor antibody, using 4-8 units of SV40 hamster tumor antigen, pool 3-835, received from Dr. R. J. Huebner, National Institutes of Health. A pool of positive, high-titered sera was used at a dilution containing 8 units of antibody for antigen titrations.

In tests for SV40 neutralizing antibody, serial 2-fold dilutions of hamster sera were mixed in 0.2 ml volumes with 0.2 ml PBS containing SV40 at 200-300 $TCID_{50}$/0.1 ml, or control PBS. Mixtures were incubated 2 hours at room temperature and then inoculated in 0.1 ml volumes to CMK cultures. Cultures were observed 3 weeks for viral cytopathic effect.

Plating efficiency, growth studies on cultured tumor cells. Cell suspensions were plated without feeders by the techniques of Puck(22) as described previously(11). In experiments to determine growth rates, 16 × 150 mm tubes were seeded with 25,000 to 50,000 cells in LAPAGT-CS supplemented with sodium bicarbonate to a final concentration of 2.2 g/liter. Cultures were held at 37°C in an humidified incubator, continuously gassed with 5% CO_2 in air. Fresh medium was added each 2 or 3 days. Replicate cultures were removed on days 3, 6, 8, 10, and 12 for estimates of total cell numbers by enumeration of cell nuclei(23). Individual counts on 3 or 4 tubes were averaged for each determination.

Results. The primary tumors from which transplantable lines SVH1, SVH2 and SVH3 were established developed near the site of inoculation after 4-5 months. SVH1 and SVH2 developed in males; SVH3 in a female hamster. All tumors remained localized and when removed were well-encapsulated. Each tumor was transplanted to suckling hamsters for 2 or 3 passages and thereafter to weanling hamsters. On histological examination at the 4th, 12th and 20th passages, all tumors were found to be spindle cell sarcomas, well differentiated, with numerous giant cells and frequent mitotic figures. SVH3 tumors were composed of spindle shaped cells arranged in fascicular pattern.

All transplanted tumors were highly tumorigenic after 5, 12 and 20 hamster passages, an inoculum of less than 50 cells producing tumors in more than 50% of the animals. In one instance, a 5th passage SVH3 tumor which had been frozen and stored in liquid nitrogen was similar to a 20th passage SVH3 tumor in tumorigenicity. No sex difference in hamster susceptibility to transplants was observed. Transplanted tumors were soft, often with much central necrosis, and tended to invade the underlying musculature. No difference in virulence was detected among the 3 tumors; all of them grew rapidly and killed the animal within 1-2 months. Gross metastases were not observed, with one exception: an SVH3 tumor in 20th passage invaded the body wall, and foci of tumor growth were found around the small intestine, in the liver and in heart blood.

Hamsters bearing primary tumors and 12 hamsters with large transplantable tumors were bled and the sera tested for SV40 neutralizing antibody. The serum of one hamster with primary tumor SVH1 was positive with a titer of 16 for viral antibody; all other sera were negative. Infectivity tests have been uniformly negative for SV40 by direct overlay of 10^4 to 2×10^6 viable or disrupted hamster passage tumor cells onto CMK monolayers.

Growth rate, morphology in tissue culture. Tissue cultures were prepared from each of the transplanted tumors in their 4th or 5th hamster passage. All tumors grew readily without a growth lag, and *in vitro* cell lines were derived from them with ease. Such lines produced rapid, marked acidification of the medium, and differences in morphology and growth characteristics of the 3 tumors were noted early after explantation.

SVH1 and SVH2 grew more rapidly than SVH3, establishing confluent cultures in shorter periods with smaller inocula. As confluency approached, the growth rate of SVH3 decreased as cultures reached cell concentrations near 6×10^5/ml, whereas populations of SVH1 and SVH2 continued rapid multiplication to saturation densities around 1.2×10^6 for SVH1 and $1.7\text{-}2.0 \times 10^6$ for SVH2.

Giant multinucleated cells and cells with lobated or fragmented nuclei, which have been observed in tumors and cells transformed *in vitro* by SV40(2,7,10) were a prominent feature of all cell lines. SVH1 cultures were composed of a network of spindle or pleomorphic cells, loosely connected by cytoplasmic processes. These cells grew at random in disorganized fashion over the glass, and were seen as widely scattered, radially migrating cells from colony edges. These morphological characteristics have been maintained through 36 *in vitro* passages. (Fig. 1 — A and B).

In earlier culture passages, SVH2 consisted of spindle-shaped cells growing in parallel orientation, or as random networks in other areas of the same culture. In later passages, the cells were more plump and triangle-shaped, appeared closely joined in compact patches, and had blunt, ruffled membranes rather than elongated cytoplasmic processes. Margins of colonies tended to be smoother, giving the cultures a pseudo-epithelioid appearance. The cells have retained this appearance through the 38th tissue culture passage (Fig. 1 — C and D).

SVH3 cells have been maintained through 34 culture passages. Cultures from the outset have consisted of spindle or oval cells which multiplied in ropelike patterns and whorls, indicating some inhibition of free cell movement by cell-to-cell contact(24). Colonies of SVH3 have remained feathery-edged, comprised of cells growing with oriented direction, similar in morphology to those of normal fibroblasts. In some cultures of SVH3, a small number of cell variants has been observed which produce colonies of randomly growing cells, similar to those seen in SVH1 cultures. These variants do not appear to be increasing and no attempt has been made to isolate them. The morphology of SVH3 cells in culture is shown in Fig. 1 — E and F.

Tumors SVH1 and SVH2 were also explanted to bottles after 12, and SVH3 after 19, hamster passages. Each culture line had the same morphology as corresponding cultures established after 4-5 hamster passages. Later passage hamster tumors also retained the growth rates and plating efficiencies *in vitro* which were characteristic of the cultured lines from earlier hamster passage tumors (Table I).

Tests for infective SV40 at various passage levels on all cultured tumor lines have been negative.

Plating efficiency, oncogenicity of cultured tumor lines. Colony formation from small numbers of cells without feeders ranged from 1.7 to 8.3% for cultured tumor line SVH1, and from 1.3 to 9.2% for SVH2. Cultured tumor SVH3 regularly showed low plating ability of less than 1.0%, comparable in our experience to that of normal human or hamster fibroblasts. Plating efficiencies of cultured lines SVH1 and SVH2 increased with *in vitro* passage, while SVH3 continued to show poor ability to grow from small inocula (Table I).

Tests for oncogenicity in weanling hamsters revealed the cultured tumor lines to be as

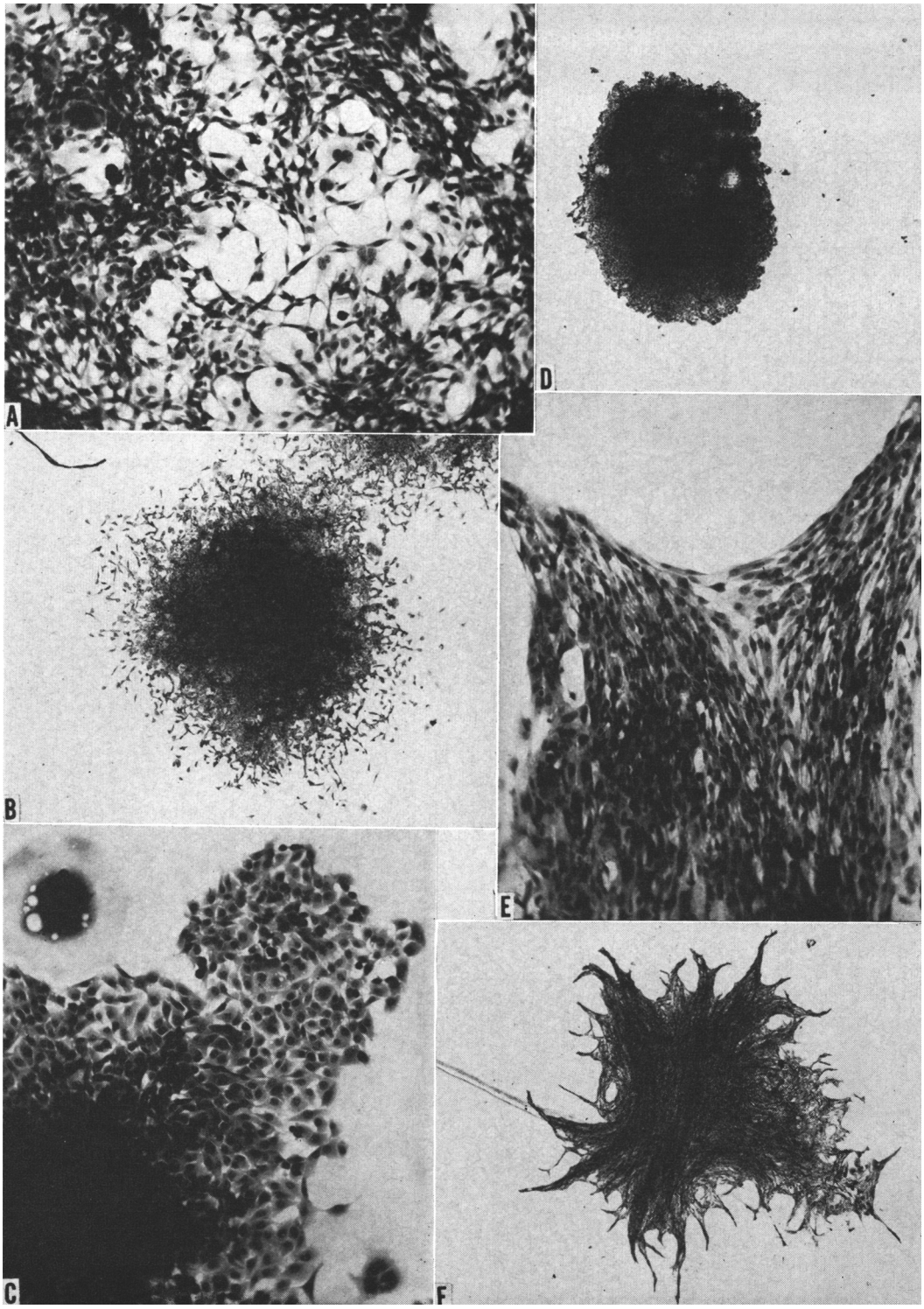

FIG. 1. A. SVH1 cultured hamster tumor line—28th passage *in vitro*. B. SVH1 cultured

hamster tumor colony—26th passage *in vitro*. C. SVH2 cultured hamster tumor line—22nd passage *in vitro*. D. SVH2 cultured hamster tumor colony—28th passage *in vitro*. E. SVH3 cultured hamster tumor line—11th passage *in vitro*. F. SVH3 cultured hamster tumor colony—21st passage *in vitro*.

fully oncogenic as the tumors in parallel passage in hamsters. Each of the cultured lines consistently produced tumors in 50% of the inoculated animals with less than 50 cells, when tested around the 12th, 20th and 27th tissue culture passages. In several tests, each of the cultured lines has produced tumors in 50-100% of the inoculated animals with 10 cells.

No change in oncogenicity with passage *in vitro* could be demonstrated, and although the 3 cultured tumors manifested distinct differences in morphology, plating efficiency and growth rate *in vitro,* no significant difference in their malignant potential *in vivo* was observed (Table II).

Tumors produced from cultured tumor cells grew rapidly to massive size, usually larger than the hamster, and killed the animal in about 2 months. Gross metastases were not observed. Histologically, the tumors induced by inoculation of the cultured tumor cells were all classified as very pleomorphic, completely undifferentiated sarcomas.

CF test for SV40 tumor antigen. CF antigens were demonstrated in homogenates of all 3 transplanted tumors when reacted with hamster sera positive for SV40 tumor CF

TABLE I. Plating Efficiency of Tissue Cultured Lines of Hamster Tumors SVH1, SVH2 and SVH3.

Cultured tumor line	Tissue culture passage	Cells plated (thousands)	Avg No. of microscopic colonies/plate	Plating efficiency*
SVH1	12	10	175	1.7
SVH2	14	10	136	1.4
SVH3	12	10	45	.4
SVH1	19	1	35	3.5
SVH2	21	1	70	7.0
SVH3	19	1	3	.3
SVH1	24	1	83	8.3
SVH2	26	1	92	9.2
SVH3	20	3	18	.6
SVH1 HP 12†	6	10	180	1.8
SVH3 HP 19‡	8	10	52	.5

* $\frac{\text{Avg No. colonies/plate} \times 100}{\text{No. cells plated}}$

† Explanted to tissue culture after 12 hamster passages.
‡ Explanted to tissue culture after 19 hamster passages.

TABLE II. Oncogenicity for Weanling Hamsters of Cultured Hamster Tumor Lines. SVH1, SVH2 and SVH3.

Tumor line	No. of passages *in vitro*	No. of cells inoculated	No. of hamsters with tumors/No. of hamsters inoculated — Days after inoculation					
			16	20	23	29	41	60
SVH1	12	1000	1/6	4/6	6/6			
		500	0/6	3/6	5/6	5/6	6/6	
		50	0/6	1/6	2/6	3/6	5/6	5/6
SVH2	14	1000	0/6	1/6	6/6			
		500	0/6	2/6	4/6	5/6	5/6	6/6
		50	0/6	0/6	1/6	3/6	4/6	4/6
SVH3	12	1000	1/6	5/6	6/6			
		500	1/6	3/6	4/6	4/6	6/6	
		50	0/6	2/6	6/6			

TABLE III. SV40 Tumor CF Antigen in SV40-Induced Tumor Lines Maintained *in vivo* and *in vitro*.

Antigen	Passage No.	Antigen CF titer* Tumor-bearing hamster pool serum
SVH1 Tumor	2 hamster	>1:64
T.C.†	18 *in vitro*	1:8
Tumor	25 " " ‡	1:16
SVH2 Tumor	7 hamster	1:8
T.C.	22 *in vitro*	1:32
T.C.	29 " "	1:8
SVH3 Tumor	2 hamster	>1:64
Tumor	20 "	>1:64
T.C.	64 *in vitro*	1:32
T.C.	22 " "	<1:2
T.C.	26 " "	1:16
Tumor	20 " " ‡	>1:64
Normal hamster muscle		<1:2
BHK21 T.C.	82 *in vitro*	<1:2

* No positive CF reaction of any of the tumor antigens with normal hamster serum pool or Rhesus monkey serum pool with naturally-occurring antibodies to SV40 viral CF antigen (titer, 1:64).
† Tissue cultured cells.
‡ Passage in tissue culture when tumor reestablished in hamster.

antibody. All tissue cultured tumor cells, with the exception of one preparation, were also found positive for the tumor antigen. Control antigens prepared from normal hamster muscle and BHK 21 cells were negative. Results are summarized in Table III.

Discussion. This study shows that the differentiated spindle cell sarcomas, induced in hamsters by neonatal inoculation of SV40, have wide variations in morphology and growth properties when explanted and maintained in tissue culture. The differences appear to be relatively stable characteristics of the tumors and not the result of overgrowth of cell variants arising during cultivation *in vitro*. This is supported by the observation that a second explantation of each of the tumors after 12-19 hamster passages produced tissue culture lines with morphological and growth characteristics indistinguishable from the respective tumor of the earlier passages.

That some dedifferentiation of the tumor cells occurred during culture is indicated by the appearance of tumors produced by SVH1, SVH2 and SVH3 cells after 12 or 14 passages *in vitro*. All tumors developing from subcutaneous inoculation of these cultured tumor cells were very pleomorphic, completely undifferentiated sarcomas. The oncogenic ability of the tumors neither increased nor decreased with continuous passage *in vitro* or *in vivo*.

Each of the explanted tumors differed in morphology, growth rate, and plating efficiency *in vitro*. Tumor SVH3 presented the most extreme variation. When plated on glass, SVH3 tumors formed colonies of spindle cells oriented in bundle-like patterns suggestive of contact inhibition, whereas SVH1 and SVH2 formed colonies of cells which appeared to multiply and move at random without definite organization. The growth rate of cultured SVH3 cells was lower than that of SVH1 and SVH2, and decreased as cultures reached confluency, perhaps because of the inhibitory effect of cell crowding. Tissue cultures with a high degree of contact inhibition are known to undergo mitotic arrest and decreased macromolecular synthesis when an increasing proportion of cells comes in contact with other cells, whether these are normal diploid cells(25) or certain aneuploid lines(26). In addition, the plating efficiency of cultured SVH3 cells was consistently below that of SVH1 or SVH2 cells, usually 10-fold. However, when inoculated into weanling hamsters no significant differences in the minimum lethal cell dose or growth rates of developing tumors could be detected among the 3 lines.

The lack of correlation between morphological transformation *in vitro* and neoplastic change has been well documented(1,27,28,29). The present work further emphasizes that morphology and growth behavior of tumor cells in tissue culture are not necessarily associated with their malignant or histological characteristics *in vivo*. Contact inhibition appears to be a characteristic of certain cell strains, normal or malignant, growing in the usual type of stationary culture. The property is descriptive of cell relationships at particular substrates(30), and is not an attribute of the cell itself. A recent report(31) demonstrates the diminished role of contact inhibition in arresting cell movement and proliferation when cells are cultured in a continuous perfusion system.

Summary. Three spindle cell sarcomas

induced in hamsters by neonatal inoculation of SV40 were explanted and maintained as cultured cell lines *in vitro*. The tumors varied widely from each other in morphology, plating efficiency, and growth rate in tissue culture. These appeared to be stable characteristics of the tumors. On transplantation into hamsters, the 3 tumor lines demonstrated no difference in tumorigenicity or rapidity of tumor growth, indicating a lack of correlation between *in vitro* growth characteristics and malignant potential. During the period of observation, no increase or decrease in oncogenicity with continued passage *in vitro* or *in vivo* occurred, although some dedifferentiation of the tumor cells took place during passage in tissue culture. All tumors and *in vitro* lines were found to contain the SV40 tumor antigen and to be free of infectious SV40.

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1. Diamandopoulos, T., Enders, J. F., Proc. Nat. Acad. Sci., 1965, v54, 1092.

2. Ashkenazi, A., Melnick, J. L., J. Nat. Cancer Inst., 1963, v30, 1227.

3. Ponten, J., Jense, F., Koprowski, H., J. Cell. Comp. Physiol., 1963, v61, 145.

4. Shein, H. M., Enders, J. F., Levinthal, J. D., Burket, A. E., Proc. Nat. Acad. Sci., 1963, v49, 28.

5. Rabson, A. S., Kirschstein, R. L., Proc. Soc. Exp. Biol. and Med., 1962, v111, 323.

6. Black, P. H., Rowe, W. P., Proc. Nat. Acad. Sci., 1963, v50, 606.

7. Enders, J. F., Harvey Lectures, 1965, Series 59, 113.

8. Black, P. H., Rowe, W. P., Cooper, H. L., Proc. Nat. Acad. Sci., 1963, v50, 847.

9. Dubreuil, R., Lussier, G., Pavilavis, V., Marois, P., DiFranco, E., Rev. Canad. Biol., 1964, v23, 303.

10. Moyer, A. W., Wallace, R., Cox, H. R., J. Nat. Cancer Inst., 1964, v33, 227.

11. Wallace, R. E., Proc. Soc. Exp. Biol. and Med., 1964, v116, 990.

12. Dulbecco, R., Vogt, M., J. Exp. Med., 1954, v99, 167.

13. Hanks, J. H., Wallace, R. E., Proc. Soc. Exp. Biol. and Med., 1949, v71, 196.

14. Earle, W. R., J. Nat. Cancer Inst., 1943, v4, 165.

15. MacPherson, I., Stoker, M., Virology, 1962, v16, 147.

16. Eagle, H., J. Exp. Med., 1955, v102, 37.

17. Eddy, B. E., Borman, G. S., Grubbs, G. E., Young, R. D., Virology, 1962, v17, 65.

18. Gerber, P., Kirschstein, R. L., ibid., 1962, v18, 582.

19. Sever, J. L., J. Immunol., 1962, v88, 320.

20. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., Proc. Nat. Acad. Sci., 1963, v50, 379.

21. Lennette, E., Schmidt, N., Eds., In Diagnostic Procedures for Viral and Rickettsial Diseases, 1964, p295.

22. Puck, R. R., Marcus, P. I., Cieciura, S. J., J. Exp. Med., 1956, v103, 273.

23. Sanford, K. K., Earle, W. R., Evans, V. J., Waltz, H. K., Shannon, J. E., J. Nat. Cancer Inst., 1951, v11, 773.

24. Abercrombie, M., Heaysman, J. E. M., Exp. Cell Res., 1953, v5, 111.

25. Eagle, J., Science, 1965, v148, 42.

26. Todaro, G., Green, H., Goldberg, B. D., Proc. Nat. Acad. Sci., 1964, v51, 66.

27. Defendi, V., Lehman, J., Kraemer, P., Virology, 1963, v19, 592.

28. Black, P. H., Rowe, W. P., Proc. Soc. Exp. Biol. and Med., 1963, v114, 721.

29. Barski, G., Cassingena, R., J. Nat. Cancer Inst., 1963, v30, 865.

30. Carter, S. B., Nature, 1965, v208, 1183.

31. Kruse, P., Miedema, E., J. Cell. Biol., 1965, v27, 273.

Received April 25, 1966. P.S.E.B.M., 1966, v122.