AN ENZYMATIC FUNCTION ASSOCIATED WITH TRANSFORMATION OF FIBROBLASTS BY ONCOGENIC VIRUSES*

II. Mammalian Fibroblast Cultures Transformed by DNA and RNA Tumor Viruses

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In view of the preceding results[1] obtained with chick fibroblasts and avian viruses, it appeared desirable to study the properties of other cells transformed by different viruses. This is important in order to provide additional information bearing both on the generality and on the determinants of specificity of the observed phenomena.

Materials and Methods

The materials and methods are exactly as described for the analogous experimental systems examined in the accompanying report.[1]

RESULTS

Hamster Embryo Cells Transformed by SV-40.—

Transformed hamster fibrinolysin: cell factor: serum factor: serum specificity: The second system that was investigated consisted of primary cultures of hamster embryo cells, some of which had been transformed by SV-40 virus. A clone was isolated (Methods) and was judged to be transformed by the following criteria: positive for SV-40 nuclear T-antigen using fluorescent antibody staining, disordered cell orientation, multi-layered growth, and growth in soft agar.

When plated on Petri dishes coated with [^{125}I]fibrin films, SV-40-transformed hamster cultures exhibit fibrinolytic activity, whereas cultures of normal cells do not; this is shown by the data in Table I and the serum specificity for this activity is presented in Table II. To test for the elaboration of a specific cell factor comparable to that produced by transformed chick cells, carefully washed monolayers were incubated overnight with serum-free medium and the resulting supernatant fluids were tested for fibrinolytic activity both alone and after addi-

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[1] Part I, the accompanying paper (*J. Exp. Med.* **137**:85).

TABLE I

Fibrinolytic Activity of Some Normal and Transformed Mammalian Fibroblasts

	Radioactivity released in medium (percent of total)
	%
Control (no cells)	1.2
Normal hamster	1.4
SV-40-transformed hamster	31
Normal mouse C57BL	1.65
MSV-transformed C57BL	9.6

Petri dishes (60 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; total radioactivity 10^5 cpm) were prepared as described in Methods. Cells were plated at 2×10^5 cells per dish in F10 medium supplemented with 10% fetal bovine serum. Medium was removed for radioactivity assay at daily intervals and replaced with fresh medium. The values given in the table represent the total radioactivity accumulated in the medium between 24 and 48 h after plating.

TABLE II

Fibrinolytic Activity of Hamster Embryo Fibroblasts Transformed by SV-40: Effects of Different Sera

Test sera Serum concentration (vol/vol):	Radioactivity released into growth medium (percent of total)	
	1%	10%
	%	%
Fetal bovine	1.3	1.2
Bovine	1.4	1.3
Turkey	1.4	1.2
Chicken	1.5	1.3
Guinea pig	1.7	2.2
African green monkey	2.5	25
Dog	3.5	18
Hamster	2.2	25

Petri dishes (60 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; total radioactivity 10^6 cpm per dish) were prepared as described in Methods. Hamster cells transformed by SV-40 were plated in medium containing 10% of fetal bovine serum at a density of 2.2×10^5 cells per dish. After 24 h of incubation the cells were washed with TD and changed to medium with one of the indicated sera. The medium was removed and assayed for radioactivity after 16 h.

tion of a variety of sera. As seen in Table III, neither transformed culture HF[2] alone nor an Eagle's serum-free medium contains any detectable fibrinolytic activity; however when the HF from transformed cultures is mixed with an appropriate serum, enzymatic activity is produced. As is the case with normal

[2] *Abbreviations used in this paper:* HF, cell-free and serum-free culture supernatant fluid obtained by incubating monolayer cultures with Eagle's medium (see Materials and Methods, part I); MSV, mouse sarcoma virus; NPGB, nitrophenyl-*p*-guanidinobenzoate (19); RAV, Rous-associated virus; RSV, Rous sarcoma virus; SR-RSV, Schmidt-Ruppin strain of Rous sarcoma virus; STI, soybean trypsin inhibitor, fraction VI (29); TS-5-SR-RSV, temperature-sensitive mutant of SR-RSV.

TABLE III

Fibrinolytic Activity of HF From Cultures of Hamster Cells Transformed by SV-40 Virus: Effects of Different Sera

Test fluid	Serum	Radioactivity released into solution (percent of total)
		%
Medium	None	0.6
Medium	Dog	0.5
Normal hamster HF	Dog	0.6
Hamster—SV-40 HF	None	0.6
" " "	Dog	41.5
" " "	Hamster	35.1
" " "	African green monkey	17.5
" " "	Human	8.6
" " "	Chicken	1.2
" " "	Calf	0.9
" " "	Fetal bovine	0.6

Petri dishes (35 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; total radioactivity 5×10^4 cpm) were prepared as described in Methods. The appropriate serum was added to HF or medium to yield a final serum concentration of 2.5% (vol/vol) in a final volume of 1 ml. Incubation was for 5 h at 36°C.

chick cells, HF from normal hamster embryo cultures shows very little fibrinolytic activity. Thus SV-40 hamster cultures, like Rous sarcoma virus (RSV)-transformed chick fibroblasts, produce a factor that must interact with a second protein from suitable sera to generate fibrinolysin.

It is of interest to compare the findings in Table III with the previous results given by chick cell HF (Table VI of part I[1]). In the case of chick cell HF, normal chicken and monkey sera yield the highest rates of fibrinolysis. The fibrinolytic activity with SV-40 hamster HF differs from that found with chick cell HF: with SV-40 hamster HF the most efficient activating sera are those of dog, hamster, and monkey. As shown previously for chick cell HF, some of the "nonactivating" sera behave as though they contain inhibitors. Fetal bovine serum, which belonged in this category for the chick system, is inhibitory with the SV-40 hamster system as well (Table IV). The fibrinolytic activity derived from hamster cell cultures is also inhibited by nitrophenyl-*p*-guanidinobenzoate (NPGB) and by the same soybean trypsin inhibitor (STI) that blocks the action of chick enzyme (Table IV).

Parallel changes in morphology and fibrinolytic activity: Since the hamster cells transformed by SV-40 have been cloned, the cell morphology is rather uniform under any given set of culture conditions. This provides the opportunity for correlating modifications in cell morphology both with changes in serum supplements and with the accompanying fibrinolytic activity. Correlations of this type are illustrated in Figs. 1, 2, and 3.

In Fig. 1 are shown representative fields from cultures of normal and SV-40-

TABLE IV

Fibrinolysis by HF from Hamster Cells Transformed by SV-40: Effect of Various Inhibitors

Dog serum (2.5%)	Inhibitor	Radioactivity released into solution (percent of total)	Percent inhibition
		%	%
+	None	41.6	
+	Fetal bovine serum (2.5%)	0.6	98.6
+	Fetal bovine serum (0.6%)	0.6	98.6
+	Fetal bovine serum (0.3%)	4.5	90.0
−	Fetal bovine serum (2.5%)	0.6	
−	None	0.7	
+	STI* 100 μg/ml	0.6	98.6
+	STI 20 μg/ml	0.6	98.6
+	STI 10 μg/ml	1.1	97.4
+	STI 1 μg/ml	26.0	37.5
+	NPGB 40 μg/ml	0.6	98.6

Petri dishes (35 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; total radioactivity 5×10^4 cpm) were prepared as described in Methods. The inhibitor was added to HF from SV-40-transformed hamster fibroblast cultures at the desired concentration, followed by dog serum at a final concentration of 2.5% (vol/vol). The degree of inhibition is based on the level of fibrinolysis observed with dog serum in absence of inhibitor (top line).

* Soybean trypsin inhibitor, fraction VI.

transformed hamster cells under various conditions. All of these cultures were grown in Dulbecco modified Eagle's medium containing either nonactivating fetal bovine serum (10% vol/vol), or dog serum (10% vol/vol), which yields maximal fibrinolytic activity. The growth pattern (or morphology) of normal hamster cells is similar in the two tested sera (Fig. 1 *a*, 1 *b*). The SV-40-transformed hamster cells grown in fetal bovine serum (Fig. 1 *c*) form a confluent layer resembling that of normal cells; however, transformed cells grown in dog serum (Fig. 1 *d*) show a striking change in morphology: they are highly refractile, clumped, and retracted from the monolayer. When maintained in media supplemented with dog serum the transformed cells retain viability, although they ultimately detach from the surface of the culture and continue to multiply "in suspension." The clumps formed in this way may be harvested by centrifugation at low speeds and transferred to fresh media. Upon transfer to media containing fetal bovine serum the clumps settle and the constituent cells reattach to the surface and regain the original morphology (Fig. 2 *a*). In contrast, when returned to media supplemented with dog serum, the transformed cells persist as large clumps (Fig. 2 *c*). The soybean trypsin inhibitor that blocks fibrinolytic activity generated in dog serum medium also prevents the morphology that develops with dog serum (Fig. 2 *b*).

By cultivating SV-40-transformed hamster cells with various serum supple-

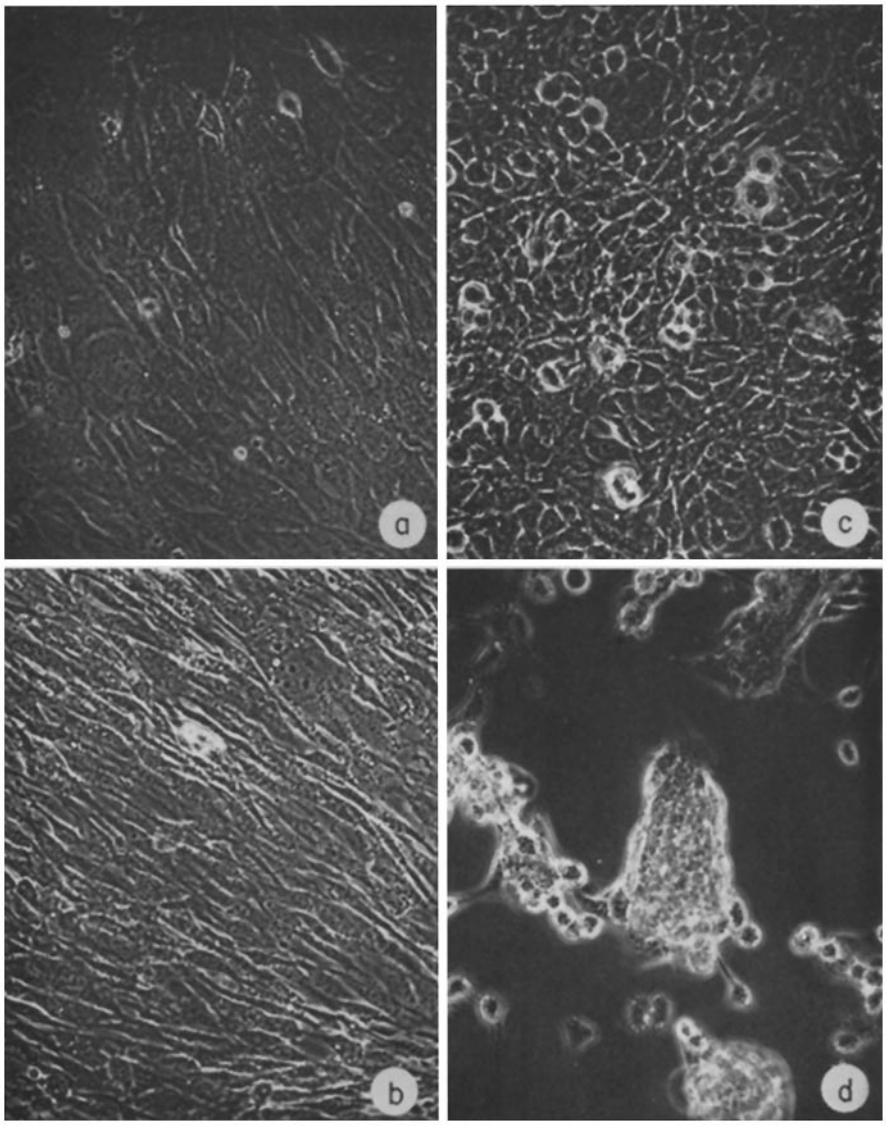

FIG. 1. Morphology of normal and SV-40-transformed hamster cells: response to different sera. Primary hamster embryo fibroblasts and hamster SV-40-transformed fibroblasts were plated at 2×10^5 cells per 60 mm dish in Dulbecco modified Eagle's medium with 10% of fetal bovine serum. After 16 h the cultures were washed twice with medium and incubated for 52 h in Dulbecco medium with fetal bovine or dog serum (10% final concentration). (*a*) Normal cells in fetal bovine serum; (*b*) normal cells in dog serum, (*c*) transformed cells in fetal bovine serum; (*d*) transformed cells in dog serum. × 230.

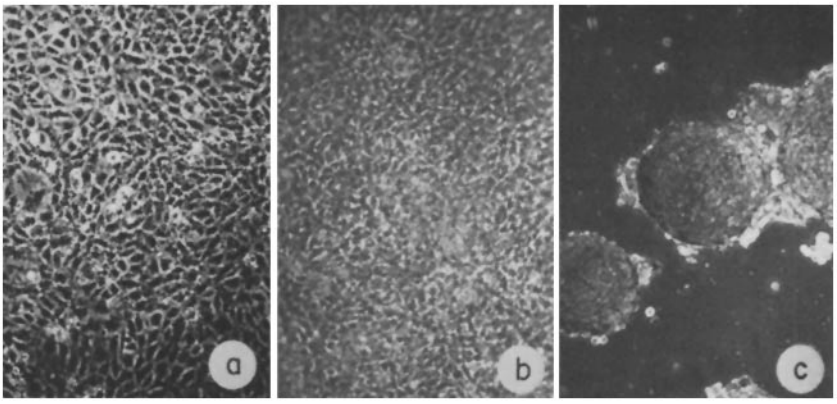

FIG. 2. Morphology of hamster embryo cells transformed by SV-40 virus: effects of some sera and of soybean trypsin inhibitor. Hamster embryo fibroblasts transformed by SV-40 were grown for 3 days in medium containing 10% of dog serum. The clumps of growing cells which were largely detached from the monolayer were collected by centrifugation and resuspended and plated in fresh medium supplemented either with dog serum (10%) or with fetal bovine serum (10%). One culture growing in dog serum also received soybean trypsin inhibitor (fraction VI) at a concentration of 50 μg/ml. The photomicrographs were taken 72 h after plating. (*a*) SV-40 hamster cells in fetal bovine serum medium; (*b*) SV-40 hamster cells in dog serum medium with soybean trypsin inhibitor; (*c*) SV-40 hamster cells in dog serum medium. × 66.

ments we have found additional correlations between fibrinolytic activity and cell morphology. Thus, SV-40 hamster cells grown in media containing chicken serum, a nonactivating serum for fibrinolysis, show the same morphology (Fig. 3 *a*) as do cells in fetal bovine serum (Fig. 2 *a*). Monkey serum, an activating serum, has the same effect on morphology (Fig. 3 *b*) as dog serum (Fig. 3 *c*). A further instance of a parallel change involving fibrinolytic activity and another parameter of transformation is shown by the effect of soybean trypsin inhibitor on the ability of transformed cells to form colonies in soft agar. Although this inhibitor does not influence the growth rate of transformed cells in liquid medium, it reduces the production of colonies in agar by more than 75% (Table V).

Mouse Cells Transformed by Mouse Sarcoma Virus or SV-40 Virus.—

Mouse sarcoma virus (MSV): To obtain additional information about the possible general association of fibrinolytic activity with viral sarcomas we have examined the properties of murine embryo cells transformed by one of several types of virus. In these systems we have investigated the properties of some normal and transformed cells on radioactive fibrin films, while others have been compared with respect to the activities of the serum-free supernatant fluids, or HF, from the respective cultures.

The fibrinolytic activity of normal and MSV-transformed mouse embryo

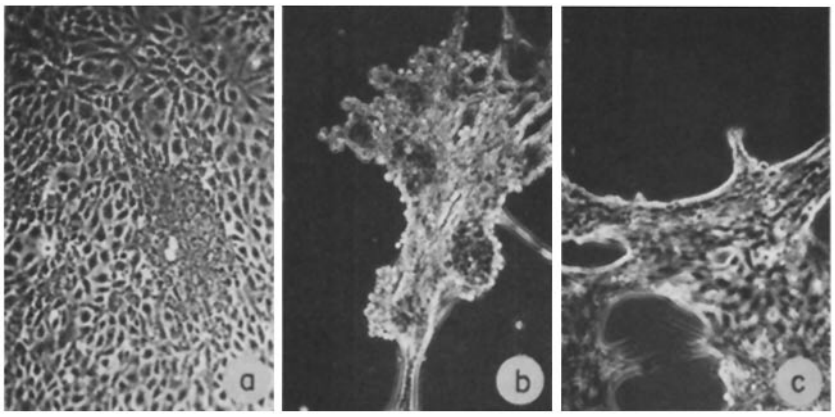

FIG. 3. Morphology of SV-40-transformed hamster embryo cells: effects of additional sera. Hamster embryo fibroblasts transformed by SV-40 were plated at 2.5×10^5 cell per 60 mm dish in Dulbecco modified Eagle's medium containing 10% fetal bovine serum. After 16 h of incubation the cells were washed twice with the same medium containing 2% of the appropriate serum and incubated for 72 h in medium containing 10% of the same serum. (*a*) SV-40 hamster cells in chicken serum medium; (*b*) SV-40 hamster cells in monkey serum medium; (*c*) SV-40 hamster cells in dog serum medium. × 66.

TABLE V

The Effect of Soybean Trypsin Inhibitor on Hamster SV-40-Transformed Cells Growing in Liquid Medium and in Agar

Days after plating	No. of cells per dish × 10^6					Plating efficiency in agar
	1	2	3	4	5	
						%
+ Soybean trypsin inhibitor fraction VI	0.16	0.52	3.9	6.0	5.1	3.9
No inhibitor	0.16	0.63	3.6	6.1	4.0	17.0

Hamster SV-40-transformed cells were plated at 1×10^5 cells per 60 mm dish in medium containing 10% of fetal bovine serum. One group of cultures received 10 μg/ml of soybean trypsin inhibitor every day after plating; another group served as controls. Every day one control and one treated culture were trypsinized and the cells counted. On day 4 the trypsinized cells were plated in 0.33% agar (28) and the colonies that formed were counted after 10 days. The inhibitor reduces growth of transformed cells in agar but not in liquid medium.

cells is shown in Table I. These results are consistent with those of experiments in which HF's from the corresponding cultures are compared: as seen in Table VI, the HF from mouse cells transformed by MSV gives rise to fibrinolytic activity when mixed with the appropriate serum; the HF from normal cells does not. In this respect, therefore, mouse cell cultures resemble those obtained from hamster and chick embryos. Morover, the mouse system also shows a distinctive spectrum of activating sera; some of the sera that activate fibrinolysis with

TABLE VI

Fibrinolytic Activity of HF from Mouse Embryo Fibroblasts Transformed by Mouse Sarcoma Virus: Serum Specificity

Test fluid	Serum	Radioactivity released into solution (percent of total)
		%
Medium (serum free)		0.6
Medium	Dog	0.6
Normal mouse culture HF	Dog	0.8
MSV-transformed culture HF	Dog	70.0
" " " "	Monkey	79.1
" " " "	Human	44.3
" " " "	Mouse	9.6
" " " "	Hamster	2.6
" " " "	Rat	0.6
" " " "	Pig	0.7
" " " "	Fetal pig	0.6
" " " "	Calf	0.5
" " " "	Fetal bovine	0.5

Petri dishes (35 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; 5×10^4 cpm total radioactivity) were prepared as described in Methods. Serum-free medium or HF (1 ml) was supplemented with the appropriate serum at a final concentration of 2.5% and the mixture incubated for 5 h at 37°C.

hamster HF and chick HF behave similarly with mouse HF, whereas others do not (Table VI). Again, fetal bovine serum and chicken serum are inhibitory when added to media containing activating sera, and soybean trypsin inhibitor and NPGB block the enzyme derived from mouse cultures just as they do those originating in transformed hamster and chick cells (Table VII).

Because dog serum effectively activates fibrinolysis with either hamster or mouse HF, whereas hamster serum does not, it appears likely that the pattern of specificity of activating sera is determined by some properties associated with the cell factor in the HF. Further evidence in support of this view is presented below.

Mouse cells, 3T3, transformed by SV-40: Since mouse cells may be transformed by both MSV and SV-40, a comparison of such transformed cultures might provide some preliminary indication regarding the possible viral or cellular determination of the fibrinolytic enzyme. Similar information might emerge from a comparison of the enzymes derived from two cell types, mouse and hamster, transformed by a single virus, SV-40. In common with all of the cell types described above, transformed SV-40-3T3 cells produce a protein factor that generates fibrinolytic activity on interaction with appropriate sera; normal, untransformed 3T3 cells do not. This is shown in Table VIII, where the spectrum of activating sera is seen to be identical with that obtained with mouse embryo cells transformed by MSV. Also noteworthy is the failure of hamster

serum to activate fibrinolysis with SV-40-3T3 cell factor (HF). Both of these facts suggest that the cell-derived factor is cell specific and cell determined, rather than virus specific and/or virus determined.

Rat Embryo Cells Transformed by B-77 Virus: Comparison with Transformed

TABLE VII

Fibrinolysis by HF from MSV-Transformed Mouse Embryo Cells: Effects of Inhibitors

	Serum	Inhibitor	Radioactivity released into solution (percent of total)	Percent inhibition
			%	%
A	Dog	None	26.0	
	Dog	Fetal bovine serum, 5%	0.8	97.0
	Dog	Fetal bovine serum, 2.5%	5.6	88.5
	Dog	Fetal bovine serum, 1.0%	8.7	66.6
	Dog	Fetal bovine serum, 0.1%	19.9	24.5
	None	Fetal bovine serum, 1.0%	0.7	
	Dog	Chicken serum, 5%	8.0	69.3
	Dog	Chicken serum, 2.5%	12.4	52.3
	Dog	Chicken serum, 1.0%	15.9	28.9
	Dog	Chicken serum, 0.1%	39.8	0
	None	Chicken serum, 1.0%	0.7	
B	Dog	None	63.0	
	None	None	1.0	
	Dog	STI 100 μg/ml	0.5	99
	Dog	STI 10 μg/ml	4.8	92
	Dog	STI 1 μg/ml	56.1	11
	Dog	NPGB 40 μg/ml	0.5	99

Petri dishes (35 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; total radioactivity 5×10^4 cpm) were prepared as described in Methods. The inhibitors were added at the desired final concentration to 1 ml of HF and the mixture supplemented, where indicated, with dog serum at a final concentration of: A, 1% (vol/vol), or B, 2.5%. The plates were then incubated for 3 h at 37°C and the radioactivity of the solution was assayed.

TABLE VIII

Fibrinolysis by Different HF's: Serum Specificity

Cell origin of HF	Radioactivity released into solution (percent of total)			
	No serum	Dog serum	Mouse serum	Hamster serum
	%	%	%	%
3T3—Normal	1.9	2.5	0.9	NT*
3T3—MSV	2.5	18.0	12.5	3.9
3T3—SV-40	2.0	67.0	23.4	5.5
Hamster—SV-40	2.7	63.2	35	60.2

Petri dishes (35 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2; total radioactivity 4×10^4 cpm) were prepared as described in Methods. The appropriate sera were added to 1 ml of the respective HF's at a final concentration of 2.5% (vol/vol) and the plates incubated for 6 h at 37°C, after which the solution was assayed for radioactivity.

* Not tested.

Chick Cells.—Normal rat embryo cells do not produce fibrinolytic activity when plated onto [^{125}I]fibrin films, nor do they yield HF which generates such activity on interaction with serum-supplemented media. However, rat embryo cells transformed by B-77 virus (an avian sarcoma virus) do produce fibrinolysin under both sets of conditions. The titer of enzyme is lower than obtained with hamster embryo or chick embryo fibroblasts transformed by SV-40 and RSV, respectively. However, as seen in Table IX, the activity is clearly present in HF from transformed rat cultures, and the spectrum of activating sera for rat-B77-transformed cells resembles that obtained for transformed mouse HF.

Also presented in Table IX is the spectrum of activating sera for chick embryo fibroblast cultures transformed by the same strain of B-77 virus as that used to transform rat embryo cells. The behavior of the two types of cells with the same set of sera is different, although transformation was produced by the same virus in both; this is a further indication that the properties of the cell factor in HF from transformed cultures is largely, and perhaps exclusively, determined by the cell type that releases it into the medium, rather than by the transforming virus. In a preliminary experiment rat-B77-transformed cells were cocultivated with chick embryo cells, and new chick cells were infected with the virus released from the mixed cultures. The serum specificity for fibri-

TABLE IX

Fibrinolytic Activity of Rat and Chick Cells and HF from Cultures Transformed by B77: Serum Specificity

Serum	Radioactivity released into supernatant fluid (percent of total)			
	A: Transformed cells		B: HF	
	Chick-RSV B77	Rat-RSV B77	Chick-RSV B77	Rat-RSV B77
	%	%	%	%
Dog	4.7	98.1	2.3	27.9
Monkey	4.1	45.3	NT*	NT
Chicken	32.7	3.0	NT	NT
Fetal bovine	3.0	3.0	NT	NT
No serum	3.0	3.0	1.1	4.6

Comparison of chick embryo and rat embryo cells transformed by B77 virus: Petri dishes (60 mm diameter) containing [^{125}I]fibrin (10 μg/cm^2: total radioactivity 1.8×10^6 cpm) were prepared as described in Methods. The dishes were inoculated with 8×10^5 cells per plate in each case and incubated for 16 h with medium containing fetal bovine serum 10% (vol/vol). After 16 h the medium was removed, the monolayer washed with TD, and fresh medium, containing the indicated serum at a final concentration of 5% was added. The cultures were incubated for a further 24 h and the medium was then assayed for radioactivity. Comparison of HF's from chick and rat embryo cultures transformed by B77: Petri dishes (35 mm diameter) contained [^{125}I]fibrin (10 μg/cm^2; total radioactivity 3×10^4 cpm). The respective HF's (1 ml) were supplemented with the various sera (final concentration 2.5% vol/vol) and incubated at 37°C for 6 h (transformed chick cells) or 17 h (transformed rat cells), after which the fluids were assayed for radioactivity.

* Not tested.

nolytic activity generated by these cells was similar to that of chick cells infected with B-77 propagated in chick cells.

Human Sera, Human Serum Factor, and Human Cells.—Because the extension and application of the preceding findings to human neoplasia is of obvious interest, we have begun to survery human sera and to examine primary cultures of human tumors for properties analogous to those described above for the other cell types. The findings with human material will be presented in detail in a separate communication, but they are summarized here briefly as follows.

(*a*) Most human sera behave, like monkey serum, as activating sera with HF from various cells, although the efficiency of "activation" is not uniform with different transformed cells and their respective HF's.

(*b*) In purification procedures the human serum factor that participates in fibrinolysis fractionates in a manner similar to the chicken serum factor.

(*c*) Certain human sera, including some from tumor-bearing patients, are nonactivating; these are in fact inhibitory, in analogy with the sera of tumor-bearing chickens.

(*d*) In preliminary experiments a single human carcinoma has so far been maintained and slowly propagated in primary culture. This tumor shows fibrinolytic activity when the cells are in contact with [^{125}I]fibrin films. Primary cultures of human embryo fibroblasts produce no detectable fibrinolysis under identical conditions.

DISCUSSION AND CONCLUSIONS

The preceding results support the following tentative conclusions.

(*a*) Virus-transformed cells derived from at least four species, chicken, hamster, mouse, and rat, give rise to fibrinolytic activity when the growth media are supplemented with appropriate sera. Cultures of normal, untransformed cells do not produce any comparable enzymatic activity, and this fibrinolysin therefore represents an important and qualitative enzymatic difference between normal and virus-transformed cells.

(*b*) The fibrinolysin is generated by the interaction of two protein factors, neither of which shows activity alone. One of these is a protein that is released into culture media by transformed cells and by primary cultures of chicken sarcomas, and the second is a protein that is found in sera of numerous vertebrate species, including man. The difference in fibrinolytic activity between normal and transformed cultures is based on the fact that transformed cells release a cell factor that is not released, or is released in much lower concentrations by normal cultures. Quantitative estimates, derived from titrations of HF from both normal and RSV-transformed chick cultures, show that normal HF contains less than 5% of the cell factor found in the transformed HF.

The following tentative nomenclature is proposed, pending elucidation of the enzymatic mechanism and biological role of the two protein factors: for the overall enzymatic activity, the designation fibrinolysin T; for the factor pro-

duced by transformed cells, fibrinolysin T^C; and for the serum factor, fibrinolysin T_S. An enzymatic activity produced by the interaction of transformed chick HF and monkey serum (or the respective purified factors) would be designated fibrinolysin $T_{S;\,Monkey}^{C;\,Chick}$.

(*c*) The production of the cell factor, and therefore fibrinolytic activity, is closely correlated with morphological evidence of transformation as shown by the behavior of cultures infected with a mutant of RSV that is temperature-sensitive for cell transformation. The emergence of fibrinolytic activity requires the synthesis of RNA and protein after transfer of such cultures to permissive conditions. Moreover, fibrinolytic activity is also correlated with a change in morphology of hamster cultures transformed by SV-40 virus. The role of the enzyme in the development of the transformed state therefore deserves careful study.

(*d*) It is noteworthy that cultures transformed by DNA (SV-40) and RNA viruses (RSV, MSV, B-77) produce similar kinds of fibrinolytic activities. This raises the possibility that the virus-stimulated cellular response which culminates in transformation may involve a very small number, or even a single enzymatic pathway as an obligatory common element.

(*e*) The spectrum of sera that are effective in producing fibrinolytic activity differs according to the transformed cell type from which the cell factor is derived. This aspect of specificity therefore appears to be governed by the nature of the cell rather than by the virus. This conclusion is supported by a comparison of (*i*) rat and chick cells transformed by B-77 virus, (*ii*) mouse cells transformed by MSV or SV-40, and (*iii*) mouse and hamster cells transformed by SV-40.

(*f*) The sera of normal chickens give rise to a fibrinolysin after interaction with a factor produced by cultures of transformed chick embryo cells, whereas sera obtained from birds infected with RSV do not. The failure of sera from tumor-bearing chickens to produce fibrinolytic activity under these conditions[3] is due to the presence of an inhibitor of the overall fibrinolytic reaction. We do not know whether this inhibitor is directed against the transformed cell factor, the serum factor, the product of their interaction, or a subsequent step in the reaction sequence that ultimately yields fibrinolysis. Nevertheless, the fact that all of the tested sera from tumor-bearing fowl are inhibitory to fibrinolysis, while all of those from normal fowl are activating, suggests (*i*) that the inhibitor is formed by the host in response to the tumor, and (*ii*) that it could play an important part in the frequent regression of avian sarcomas. The presence of a similar inhibitory activity in the serum of some tumor-bearing patients is also consistent with the hypothesis that this may be the expression of a natural defense mechanism.

[3] In addition to the inhibitory protein the sera of tumor-bearing animals also contain the activating factor that is present in normal sera. The activating serum factor is readily separated from the inhibitor by ammonium sulfate fractionation.

On the basis of the preceding data we cannot know which molecule is actually responsible for fibrinolysis: it could be either the cell factor, the serum factor, a product resulting from their interaction, or another protein(s) contaminating the fibrinogen or fibrin. Moreover, it remains unclear whether fibrin is a normal substrate for the hydrolytic enzyme in question, and what is the relationship, if any, to the serum and other factors that have been investigated in other laboratories (25). Finally, the relationship of this fibrinolytic activity to that of plasmin[4] must be defined precisely; the data obtained to date with several sera indicate that there are differences in the two activities. Therefore, while the observations described in this paper invite speculation and comment, it seems appropriate to defer further discussion pending clarification of relevant questions, some of which form the subject of current experiments.

SUMMARY

Chick, hamster, mouse, and rat embryo fibroblast cultures, transformed by either DNA or RNA viruses, show fibrinolytic activity under suitable conditions of growth and in appropriate media; normal counterpart cultures do not. The fibrinolysin is produced by the interaction of two protein factors: one of these, a cell factor, is released by transformed cells and accumulates in the medium when cultures are incubated in the absence of serum. The second factor, the serum factor, is a specific protein that is present in sera of many avian and mammalian species, including man.

Not all sera yield fibrinolysin on interaction with any given transformed cell factor, and the spectrum of activating sera is distinctive for each cell factor. This pattern appears to be determined by the cell type, rather than by the transforming virus.

An important role for the fibrinolysin in oncogenic transformation is suggested by the following correlations. (*a*) The initial appearance of fibrinolysin precedes the morphological change after the transfer to permissive temperatures of chick fibroblast cultures infected with a temperature-sensitive mutant of RSV. (*b*) The initiation of fibrinolysis and of morphological change both require the synthesis of new protein, but not the synthesis of either DNA or rRNA. (*c*) The activity of the fibrinolysin is correlated with the retention of abnormal morphology in hamster cells transformed by SV-40. (*d*) The sera of normal chicks effectively activate fibrinolysis with the cell factor from transformed chick cells. In contrast the sera of chicks with RSV tumors do not; these contain an inhibitor of the fibrinolytic activity.

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[4] Defined as fibrinolytic activity activated by streptokinase.

Note Added in Proof.—Since this manuscript was submitted for publication we have found that a cell factor is also produced by (*a*) primary cultures of dimethylbenzanthracene-induced rat mammary carcinomas, (*b*) mouse fibroblasts transformed by methylcholanthrene, and (*c*) several human tumor cell lines. The properties of these cell factors closely resemble those obtained from transformed chick and hamster fibroblast cultures.

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