dergo subsequent necessary metabolic steps. It is unlikely that the non-antigenicity of the succinylated BPO-PLL conjugates is due to an inability of these conjugates to be phagocytized by macrophages present in immunological tissues. Both succinylated and non-succinylated H^3-tagged DNP-PLL conjugates appear in draining lymph node macrophages in comparable amounts following their injection into the foot-pad(18).

The known requirements of macromolecular substances for antigenicity are "foreignness" of structural configurations and *in vivo* degradability. These present results suggest an additional requirement, *ie.*, that degradation products of antigen must be capable of undergoing subsequent, as yet undefined, specific metabolic steps. In view of what is known of this system(2,3,17), one possibility is that such a step may be the coupling of antigenic fragments to RNA, which would require an activating enzyme specific for the lysine side chain.

Summary. The antigenicities of various benzylpenicilloyl-poly-L-lysine (BPO-PLL) conjugates were compared in random bred and in strain 2 guinea pigs. Lightly coupled BPO-PLL conjugates were antigenic. Lightly coupled BPO-PLL conjugates whose ε-amino groups had been exhaustively succinylated with succinic anhydride were non-antigenic. A heavily coupled BPO-PLL conjugate was non-antigenic. These findings are interpreted with regard to the metabolism of antigens.

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Virus-Induced Intranuclear Antigen in Cells Transformed by Papovavirus SV40.* (29472)

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Transformation of hamster cells by papovavirus SV40 is accompanied by induction of new cellular antigen(s). The specificity of this antigen can be measured by complement-fixation tests(1) as well as by *in vivo* immunity tests(2-5). Thus, SV40-transformed cells fail to produce tumors when introduced into hamsters previously inoculated with the virus; prior inoculation of other papovaviruses has not conferred significant protection against cells transformed by SV40. The

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methods used to detect these antigens do not yield information concerning the intracellular sites of antigen production nor the proportion of transformed cells per culture or per tumor producing this antigen.

The present report describes the immunofluorescent detection and localization of new antigens in cells transformed *in vitro* as well as *in vivo* by SV40. The method has also been adapted to detect antibodies against the SV40-induced antigens. Preliminary information on the properties of the antigens is also reported.

Materials and methods. Cells. H-50 cell line. These cells were originally derived from a hamster tumor induced by SV40(6) and have undergone 20 passages in tissue culture. Hamsters inoculated with SV40 are resistant to challenge with these cells(2) as measured by ability of the cells to induce tumors. The H-50 cell line is known to contain the complement-fixing antigen induced by SV40(1), but numerous attempts to isolate infectious virus from these cells as well as from cell-induced tumors have been negative(7). The cells are grown in 16 oz bottles in fluid nutrient composed of 90% Eagle's basal medium and 10% calf serum; 100 u penicillin and 100 μg streptomycin are added per ml. The pH is routinely adjusted to 7.4-7.6 with 7.5% $NaHCO_3$. Cells are dispersed with trypsin and grown on round 15 mm cover glasses for immunofluorescent assay.

2X-10 cell line. These cells were derived from hamster embryonic cells transformed *in vitro* by SV40(6). They have been maintained in tissue culture through 29 passages and are routinely handled like the H-50 cells. Tumor induction by these cells can also be prevented by prior inoculation of hamsters with SV40(2), and the cells have also been found to contain the same complement-fixing antigen present in the H-50 line (unpublished results).

Other cells. A cell line, X-10, derived from human embryonic cells transformed by SV40 (6) was also included in this study. Transformation of the cultures had been accompanied by changes in morphology but introduction of the cells into weanling hamsters did not result in tumors(6).

Normal hamster embryonic cells and a line of the hamster BHK21 cells(8) were used as cells not transformed by SV40. The BHK21 cell line used was found to be resistant to induction of new cellular antigens by SV40 as measured by inability of SV40 to protect hamsters against subsequent challenge with the BHK21 cells previously exposed to SV40 (9). The cells are, however, capable of inducing tumors in hamsters(10).

Immunofluorescent technic. The details of the method used have been described(11,12). Cells on cover glasses were air-dried and fixed for varying periods in acetone at room temperature. They were then allowed to react with hamster sera (diluted 1:2) obtained from animals being studied in various experimental systems. The cover glasses were then washed and exposed to anti-hamster globulin rabbit globulin labeled with fluorescein isothiocyanate by the method of Marshall *et al* (13). The labeled reagent had been previously adsorbed twice with mouse liver powder. All tests included cells that reacted only with the labeled reagents, and both positive and negative sera were included in each experiment. Observations were made using a Zeiss fluorescence microscope employing a dark field condenser. The light source and filters for this system have been described (11).

Results. Sera from hamsters bearing large tumors induced by cells transformed by SV40 *in vitro* or *in vivo* reacted with antigen in such SV40-transformed cells (Table I).

TABLE I. Sera from Hamsters* Yielding Positive Immunofluorescent Results When Tested Against H-50 Cells.

Challenge cells	No. cells inoculated	Tumor size in mm
H-50	10^4	20 × 20
"	10^4	10 × 10
"	10^3	8 × 8
"	10^3	12 × 12
"	10^2	10 × 10
"	10^2	10 × 10
"	10^2	12 × 12
"	10^2	10 × 10
"	10^2	6 × 8
2X-10	10^3	10 × 10
"	10^3	15 × 15
"	10^3	20 × 20

* Bled 3 mo after inoculation of challenge cells.

TABLE II. Sera from Hamsters* Yielding Negative Immunofluorescent Results When Tested Against H-50 Cells.

Challenge cells	No. cells inoculated	Tumor size in mm
H-50	10^4	6 × 6
"	10^3	negative
"	10^3	"
"	10^2	8 × 8
"	10^2	negative
"	10^2	"
"	10^2	4 × 4

* Bled 3 mo after inoculation of challenge cells.

Most of the positive sera had been obtained from hamsters bearing tumors that measured 10 × 10 mm or larger and all hamsters had been bled 3 months after challenge with either H-50 or 2X-10 cells. The number of cells used to challenge the animals did not seem to influence the results.

Sera obtained from hamsters bearing tumors smaller than 10 × 10 mm or bearing no tumors were negative (Table II). The largest tumor seen in this negative group measured 8 × 8 mm. However, sera from some animals bearing tumors in this size range were positive (Table I) but the reaction tended to be less strong than that exhibited by sera from hamsters bearing the larger tumors. In no instance did serum from hamsters without a tumor react with the antigen measured by immunofluorescence. This was true with sera from normal hamsters as well as with sera from hamsters immunized with live SV40 and proven to be resistant to the SV40 transformed cells.

A number of cells were then tested with a positive and a negative hamster serum. Both sera had been obtained from hamsters inoculated with H-50 cells. The positive serum had been obtained from a hamster developing a tumor after challenge with 100 H-50 cells; the negative hamster failed to develop a tumor in spite of challenge with 1000 H-50 cells. The results are summarized in Table III. The 3 cell lines originating from cells transformed by SV40 (hamster H-50 and 2X-10, and human X-10) reacted with the positive, but not with the negative, serum. The normal embryonic hamster cells and the hamster BHK21 cell line failed to react with either sera. These results have been confirmed with other sera from single hamsters as well as with positive and negative hamster serum pools.

The localization of the antigen was similar in the 3 positive cell lines. Specific fluorescence was seen only in the nucleus (Fig. 1, 2); the reaction was particulate. The nucleoli did not react (Fig. 1, 2). The nucleus of every cell in the H-50 and in the 2X-10 cultures reacted with the positive sera. Negative sera failed to react with any of the nuclei (Fig. 3). Cells in the final stages of mitosis contained the antigen in both daughter nuclei apparently in equal amounts (Fig. 2). Occasional nuclei reacted at the site of of the nuclear membrane (Fig. 4); this additional reaction seemed to be more pronounced with the human X-10 cells.

Fixation for 10 minutes with either acetone or methanol often caused a diminution or even complete loss of the reaction. Although staining of unfixed air-dried preparations solved this problem, it occasionally caused loss of the cells or poor preservation of cellular architecture. Five minutes of fixation with acetone following air drying of the cells has been found to yield the most reproducible system.

Discussion. The presence of new cellular antigens following transformation of cells by papovaviruses is considered indicative of the integration of at least part of the viral genome with that of the host cell. This is particularly true with the H-50 and 2X-10 cells

TABLE III. Immunofluorescent Results with Various Cells Tested Against Positive and Negative Hamster Sera.

Cell	Immunofluorescent results with hamster sera	
	Positive serum*	Negative serum†
SV40-transformed hamster H-50	Pos	Neg
" " " 2X-10	"	"
" " human X-10	"	"
Hamster cell line BHK21	Neg	"
Normal hamster primary cell culture	"	"

* Obtained from hamster bearing a tumor induced with 100 H-50 cells.

† Obtained from hamster challenged with 1000 H-50 cells but which failed to develop a tumor.

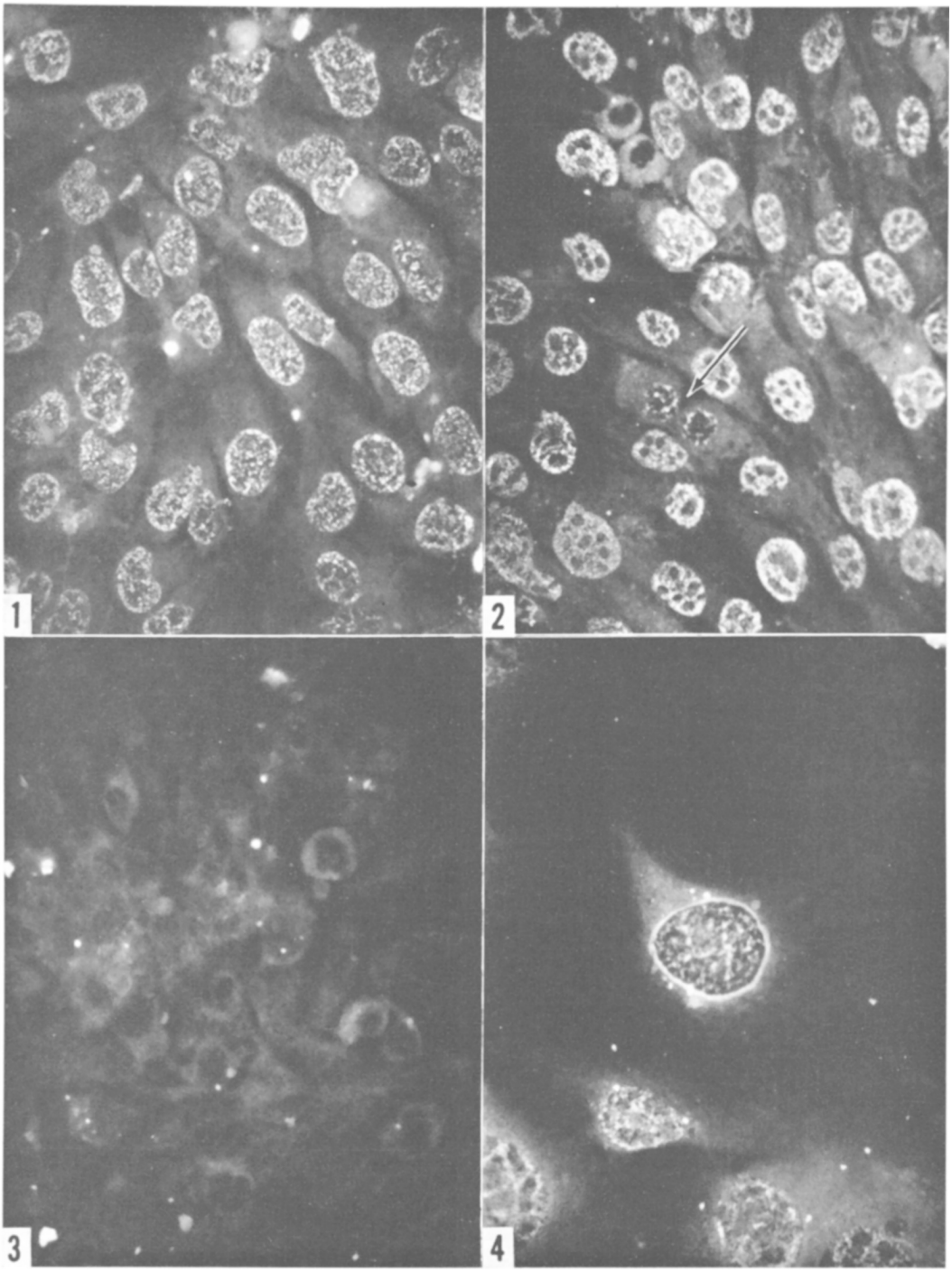

FIG. 1. Immunofluorescent photomicrograph of H-50 cells after exposure to serum from tumor-bearing hamster. Note white (reacting) nuclei. × 400

FIG. 2. Immunofluorescent photomicrograph of 2X-10 cells after exposure to serum from tumor-bearing hamster. Note white (reacting) nuclei. Dark, non-reacting nucleoli are prominent. Arrow points to cell in late stage of division, with antigen present in both daughter nuclei. × 400

FIG. 3. Immunofluorescent photomicrograph of H-50 cells after exposure to serum from non-

tumor bearing hamster. Nuclei are negative. × 400

FIG. 4. Immunofluorescent photomicrograph of H-50 cells after exposure to serum from tumor-bearing hamster. Specific staining of intranuclear material and reaction at site of nuclear membrane is evident. × 640

which are free of infectious virus. Attempts to induce these cells to produce infectious virus have not been successful. Detection of new complement-fixing antigens has not resolved the problem of the proportion of cells in the transformed cultures involved in production of such antigens.

Immunofluorescent detection of SV40-induced antigen in the nuclei of all cells in the SV40-transformed cultures resolves both the question of intracellular site of antigen production as well as that of the proportion of cells involved. Demonstration of this antigen in 100% of the cells is similar to the state seen in bacterial lysogeny where all bacteria carry prophage. Failure to induce the H-50 cells to produce infectious virus(7), may be simply technical (*i.e.*, the proper inducing agent has not been found) or may reflect integration of only an incomplete portion of the virus genome. The antigen produced in response to this genome, however, appears to be similar in all SV40-transformed cells since this antigen has been detected in hamster cells transformed by the virus *in vivo* (H-50), or *in vitro* (2X-10), as well as in transformed cells of human origin (X-10). Results using the immunofluorescent and complement-fixation technics appear to measure the same or similar antigens since only animals bearing large tumors have circulating antibody capable of reacting in both tests.

The localization and appearance of the SV40-induced antigen is strikingly similar to that seen when cells are reacted with sera from patients with lupus erythematosus (11). A comparison of Fig. 1 and 2 in this study with that of Fig. 4 and 7 of an earlier study(11) readily reveal the striking similarity. Failure of antigen detected in the lupus antinuclear reaction to withstand acetone and methanol(11) is also similar to results obtained with the SV40-induced antigens. Thus the antigen detected in the transformed cells might well be either nucleoprotein or DNA but definitive evidence must still be obtained. Attempts are now underway to determine whether the SV40-induced antigens are viral precursors or "new" cellular antigens.

Summary. Hamsters bearing virus-free tumors induced by cells transformed *in vitro* or *in vivo* by papovavirus SV40 produce circulating antibody capable of reacting with intranuclear antigens synthesized by SV40-transformed cells. The reaction is particulate and does not involve the nucleolus. All cells in transformed cultures, regardless of whether they are of hamster or of human origin, synthesize the antigen.

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