takes place in nature, polymyxin-resistant *V. cholerae* strains may be isolated, as well as polymyxin-sensitive *V. el tor* strains.

Of the available tests for differentiating *V. cholerae* and *V. el tor* strains phage-typing(3) has been found to yield the most consistent results. However, this test requires a somewhat specialized technique and is therefore not suitable for all laboratories. Polymyxin-sensitivity test by the plate method appears to be the next best among available methods and may be adopted either alone or as a confirmatory test with the phage-typing procedure.

The turbidimetric method of testing polymyxin sensitivity showed up interesting differences in the growth patterns of the two types of vibrios in the presence of the antibiotic. The results will be published later.

Summary. A comparative study has been made of 4 methods, namely, disc, well, plate and turbidimetric methods for testing polymyxin-sensitivity of the choleragenic vibrios. The plate method using 15 μg/ml of polymyxin B in agar medium is recommended as the method of choice for routine screening tests for differentiating *V. cholerae* and *V. el tor* strains. By this method all the 502 strains of classical cholera vibrios tested were found sensitive and 99.5% of the 544 strains of El Tor vibrios completely or partially resistant to polymyxin B. With the turbidimetric method, resting cells of both *V. cholerae* and *V. el tor* showed evidence of progressive lysis in the presence of polymyxin. But when vibrios were tested in their growing phase *V. cholerae* strains appeared sensitive to polymyxin while the *V. el tor* cultures proved resistant. El Tor strains, however, showed an initial phase of growth inhibition before their normal growth pattern was restored.

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New Surface Antigen in Cells Transformed by Simian Papovavirus SV40.* (30330)

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Transformation of hamster cells *in vivo* and *in vitro* by papovavirus SV40 results in the appearance of new cellular antigens. The specificity of these antigens has been demonstrated by transplantation rejection procedures(1-4). A new antigen in these cells can also be detected by complement-fixation tests (5,6) and by immunofluorescent methods(7, 8) using sera from hamsters bearing large tumors induced by SV40-transformed cells; the latter method has located this "tumor" or T antigen in the nucleus. Since antibodies against the intranuclear T antigen cannot be detected in hamsters immunized with live SV40 and proven resistant to SV40-transformed cells, it is unlikely that the T antibody is involved in rejection of the tumor cells. This paper describes the presence of new and specific antigens at the surface of SV40-transformed hamster cells and the synthesis in hamsters of antibody against the antigens.

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Materials and methods. Cell lines. The H-50 cells were originally derived from a

hamster tumor induced by SV40(9) and have undergone 90 tissue culture passages. The cells contain the specific intranuclear T antigen detected by immunofluorescent methods (8) but are free of infectious SV40 virus(10). The cells were grown in 16-oz prescription bottles in Eagle's medium supplemented with 10% heat inactivated calf serum, 100 units of penicillin and 100 μg of streptomycin per ml. Cells to be inoculated into animals were dispersed with trypsin, centrifuged and resuspended in Eagle's medium. Cell viability was determined by the dye exclusion test using trypan blue.

H-50 cells were prepared for extraction by harvesting cells from 12 bottles (16 oz) with the help of a rubber policeman. The cells were washed with Tris buffer (pH 7.4), sedimented by centrifugation, and resuspended in 12 ml of the buffer. The cells in the suspension were ruptured by sonication for 30 seconds at 10 KC/second and passed through a 450 mμ Millipore filter.

The BHK21 cell line, derived from baby hamster kidneys by Macpherson and Stoker (11), had been maintained in serial tissue culture passages in Eagle's medium with 10% calf serum. The cells are oncogenic in hamsters but do not synthesize SV40 tumor antigen(12).

Hamster embryo fibroblasts, primary green monkey kidney cells, rabbit kidney cells and human embryonic lung cells were grown as previously described(1,9).

Animal experiments. Three-week-old hamsters were vaccinated at weekly intervals with 3 subcutaneous injections of SV40 using 6 $\times$ 10^6 plaque forming units per inoculation. One week after the last injection, the animals were challenged subcutaneously with 10^5 viable H-50 cells. Those hamsters which were resistant and did not develop tumors within 12 weeks of this challenge were bled and rechallenged subcutaneously with 10^5 viable H-50 or other cells. All animals were bled out 3 weeks after the final inoculation. The sera were examined in immunofluorescent tests for the presence of antibody reacting with H-50 or other cells.

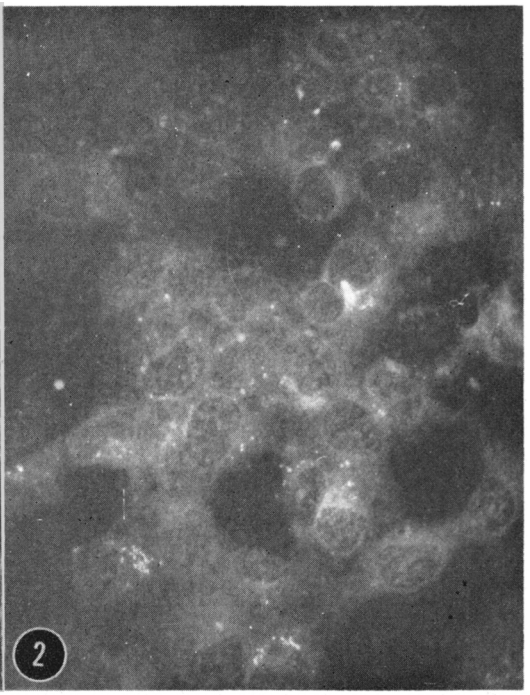
Immunofluorescence technic. Cells from 16-oz bottles were dispersed with trypsin and 2×10^4 cells were seeded on 15 mm diameter coverglasses. After incubation for 4 hours at 37°C in 5% CO_2, the cells were flooded with 5 ml (per 60 mm petri dish) of medium (see below). After a monolayer had formed, the cells were washed 3 times with Tris buffer, pH 7.4, air-dried and fixed for 1 minute with acetone at room temperature. The fixed cells were reacted for 25 minutes, with the hamster serum under test, washed 3 times with Tris buffer, and reacted with anti-hamster globulin rabbit globulin labeled with fluorescein isothiocyanate. The labeled globulin had been adsorbed twice with mouse liver powder (100 mg/ml) and was the same as that used in previous studies(8,13). After washing and air-drying, the cells were mounted in Elvanol on slides and were examined with a Zeiss fluorescence microscope. Cells in suspension (unfixed) were also reacted with the same reagents and viewed as above.

Results. Sera from hamsters that were immunized with SV40 and found resistant to 2 challenges with cells transformed by the virus reacted specifically with the H-50 cells in the indirect immunofluorescent test. The reaction was localized at the cell surface (Fig. 1). The internal cytoplasm and the nucleus did not show any fluorescent staining (Fig. 1). Sera from the hamsters prior to the second challenge did not react with the H-50 cells (Fig. 2). Cells reacted in suspension with the positive immuno-reagents exhibited a ring around the cells often resembling a "necklace" (Fig. 3) but careful inspection of these cells revealed that reactions had also occurred on the surface of other areas of the cytoplasm. Antibody to the surface antigens of H-50 cells could not be detected in sera from hamsters bearing large subcutaneous tumors induced by SV40 transformed cells. These sera, however, possessed antibodies against the virus-induced intranuclear antigens of H-50 cells.

The positive hamster serum reacting with the surface antigens of H-50 cells failed to react in the immunofluorescent test with normal hamster embryo fibroblasts, the hamster cell line BHK21, rabbit kidney cells, primary green monkey kidney cells, primary human

lung cells, and cells transformed by adenovirus type 12 (Table I). However, the positive sera did react with the 2X-10 cell line which are progeny of cells transformed *in vitro* by SV40(9).

Growth of all non-SV40 transformed cells proceeded normally in these sera (at concentrations of 10-30%). However, the H-50 cells multiplied poorly in the sera possessing antibody to the surface antigens; they usually



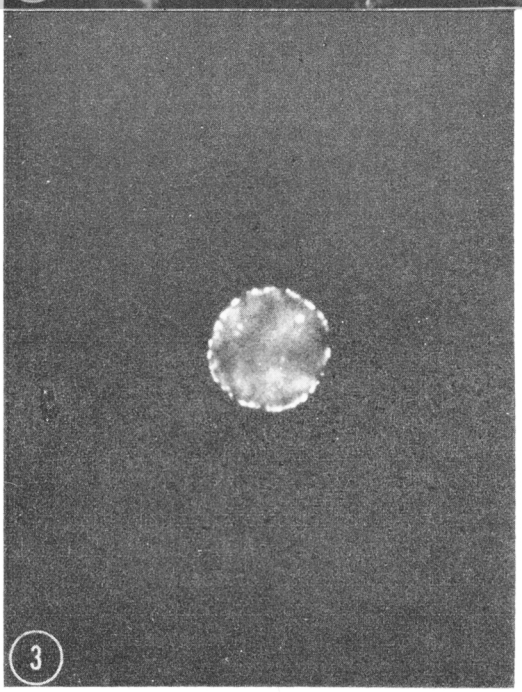


FIG. 1. Immunofluorescence photomicrograph of H-50 cells reacted with serum from protected hamsters inoculated twice with the homologous cells. × 300.

FIG. 2. Immunofluorescence photomicrograph of H-50 cells reacted with serum from protected hamsters prior to rechallenge. × 300.

FIG. 3. Immunofluorescence photomicrograph of H-50 cell reacted in suspension with serum from protected hamsters inoculated twice with H-50 cells. × 300.

TABLE I. Immunofluorescent Results with Various Cells Tested Against Positive Sera Reacting with Surface Antigens of H-50 Cells.

Cells	Immunofluorescent results with positive serum
H-50	Positive
2X-10	"
Normal hamster fibroblasts	Negative
Hamster cell line, BHK21	"
Rabbit kidney cells	"
Primary green monkey kidney cells	"
Primary human lung cells	"
Hamster cells transformed by adenovirus type 12	"

rounded and did not flatten out in typical fashion.

Table II summarizes results of tests to determine the specificity of antibody against the surface antigens of H-50 cells. On rechallenge with H-50 cells, 100% of the protected hamsters developed antibodies, which were not evident at all before the rechallenge. Challenge with ruptured H-50 or infectious SV40 converted 3 of 5 hamsters. None of the animals inoculated with type 7 adenovirus showed any positive responses. Inoculation of inactivated SV40, HEF or BHK21 cells yielded only 1 hamster per group capable of reacting with H-50 cells.

Animals challenged first with H-50 cells and then with BHK21 cells developed tumors within 3 weeks. However, the sera from these animals failed to react either with H-50 or with BHK21 cells in the immunofluorescence test. Sera from hamsters given HEF cells as their second challenge also failed to react with the homologous cells or with H-50 cells.

Absorption of the positive serum reacting with the surface antigens of H-50 cells with HEF cells did not remove the antibody reacting with the surface antigens (Table III).

TABLE III. Reaction of Tumor Specific Surface Antigens of H-50 Cells with Various Sera in Immunofluorescent Tests.

Serum	Reaction
Serum from normal hamsters	Negative
" " tumor-bearing hamsters	"
" " protected hamsters*	Positive
" " protected hamsters adsorbed with HEF	"
" " rabbits injected with sheep red blood cells	Negative

* After 2 challenges with H-50 cells.
HEF = hamster embryonic fibroblasts.

Antibody against sheep red blood cells failed to react with the H-50 cells in the indirect immunofluorescence test (Table III).

The surface antigens of H-50 cells were sensitive to the action of absolute methanol and 95% ethanol for 3 minutes. The antigens were also destroyed by acetone fixation for 5 minutes. One minute fixation with acetone was found to be optimal for demonstration of the antigens by the immunofluorescent technic.

Discussion. There is increasing evidence that neoplasms induced by viruses possess tumor specific antigens but, with the exception of the intranuclear T antigen, the location of these antigens in the tumor cells is still largely unknown. The results in this

TABLE II. Antibody Response in Protected Hamsters* to Inoculation of Various Antigens.

Antigens used to rechallenge	Antigen dose	Hamsters developing tumors†	Hamsters developing antibody against surface antigens of H-50 cells‡	% Positive
H-50 cells	1×10^5 cells	0/15	15/15	100
Ruptured H-50 cells	1×10^6 cells	0/5	3/5	60
SV40 virus (live)	6×10^6 PFU	0/5	3/5	60
" " (inactivated)	—	0/9	1/9	11
Adenovirus type 7	1×10^4 PFU	0/7	0/7	0
HEF	1×10^5 cells	0/11	1/11	9
BHK21 cell line	1×10^5 cells	6/6	1/6	17

* Prechallenge sera were negative for antibody against surface antigens.
† Numerator = No. of animals developing tumors; denominator = No. of animals inoculated.
‡ Numerator = No. of animals showing antibody to surface antigens; denominator = No. of animals inoculated.
PFU = plaque-forming units.

study reveal the presence of new specific antigens at the surface of cells transformed by papovavirus SV40.

Failure of the sera obtained from hamsters bearing large tumors induced by cells transformed by SV40 to react with the surface of the transformed cells indicates that the antigens located at the cell surface are not antigenically related to the intranuclear T antigen. Since such sera can react with the intranuclear T antigen, these results rule out the possibility of displacement of the T antigen to the surface of the cell. The immunologic difference of the two antigens is further supported by the finding that sera from protected hamsters challenged twice with transformed cells and reacting with the surface antigens fail to react with the T antigen.

The failure of the serum containing antibodies to surface antigens to react with normal hamster cells, with the oncogenic BHK21 hamster cells, with hamster cells transformed by type 12 adenovirus, and with normal cells from other sources supports the specificity of the antigen-antibody reaction observed. Failure to remove the antibody by absorption of the sera with hamster embryonic fibroblasts further confirms that the antibody is directed specifically against surface antigens of H-50 cells. The possibility that the reaction at the cell surface could be due to Forssman type antigen was eliminated when anti-sheep red blood cell serum failed to react with the H-50 cells in the indirect immunofluorescence test.

Failure of the sera from hamsters challenged with BHK21 and HEF hamster cells to react with the homologous cells ruled out the possibility that the antibodies specific for the surface antigens of H-50 cells are directed against hamster isoantigens. Tumor specific antigens distinct from isoantigens have also been demonstrated in neoplasms induced by viruses(1-4,14) and by chemical carcinogens (15). Sabin(16) has recently described a hamster isoantigen present in some hamsters but not others. The H-50 cells do not apparently contain this isoantigen and inoculation of hamsters would therefore not stimulate antibodies against the hamster antigen.

That new antigens develop at the surface of cells transformed by SV40 is similar to the finding of new surface antigens in mouse sarcoma cells induced by methylcholanthrene (15), lymphomas induced by Moloney virus (14,17) and leukemia induced by Graffi virus (18). In these cases, antigens have been demonstrated on the cell surface by either immunofluorescence technics or by cytotoxic tests.

It is possible that the surface antigens described may be involved in the resistance of hamsters inoculated with SV40 virus against SV40-transformed cells. This hypothesis is supported by the observation that inoculation of X-irradiated SV40-transformed tumor cells into hamsters that had received SV40 at birth interferes with the development of tumors (19). Though the role of the new surface antigens of the SV40-transformed cells is not yet known, it appears certain that these antigens are not viral antigens since SV40 immune sera of high homologous titer do not react with the cells(8). This system should, therefore, be considered as a model for further study of new antigens of tumor cells free of virus. In the work with murine leukemia, the tumor cells still contain virus(14,17,18).

Summary. Hamster cells transformed by papovavirus SV40 *in vitro* and *in vivo* possess new surface antigens that can be detected by the indirect immunofluorescent technic. Antibody against the surface antigens of the SV40-transformed cells fail to react with normal cells from various species including the hamster, with the "spontaneously" oncogenic hamster BHK21 cells, or with hamster cells transformed by adenovirus type 12. Inoculation of hamsters with cells not transformed by SV40 did not elicit synthesis of an antibody capable of reacting with the surface antigens of the cells transformed by the papovavirus.

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Protective Effect of Estrogen Against Vascular Damage to the Testis Caused by Cadmium.* (30331)

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The complete necrosis of the testis induced by a single subcutaneous injection of soluble cadmium salt to rats and mice has been amply documented(1-4). It has also been shown that the primary effect of cadmium is on the vascular system of the testis and that damage to the interstitial tissue and seminiferous tubules is secondary(5-7). In unpublished observations we have confirmed the report of Parizek(8) that cadmium does not produce testicular injury in Wistar rats before the age of 9-16 days. This suggests there may be a particular hormonal balance existing in the immature animal which prevents the testicular vasculature from being damaged by cadmium. We raised the question of whether the testis of the mature animal could be protected from cadmium injury by administration of hormones. This paper presents results of experiments undertaken to determine whether androgen or estrogen would alter the vascular response of the mature mouse testis to cadmium.

Materials and methods. Mature male C. D. mice (Charles River Breeding Laboratories, Inc.), 12-16 weeks of age, weighing approximately 40 g, were used. Preliminary experimentation showed that the degree of vascular injury in the testis caused by cadmium varied directly with the dosage. Cadmium was given as $CdCl_2$ in a single subcutaneous injection in the interscapular area. Administration of 0.03 mmole/kg of $CdCl_2$ (0.2 ml of 0.006 M solution) to mature C. D. mice consistently produced marked edema, capillary hemorrhage and thrombosis with resulting interstitial tissue and tubular necrosis. This degree of response was graded as a maximal reaction and is illustrated in Fig. 2; compare with the uninjected control, Fig. 1. With a lower dose of $CdCl_2$, 0.01 mmole/kg (0.2 ml of 0.002 M solution), there was edema, but no capillary hemorrhage and no interstitial or tubular necrosis; this was graded as a minimal response (Fig. 3). In the hormone treatment experiments to be presented, the degree of vascular injury in the testis from 0.03 mmole/kg of $CdCl_2$ was graded as a maximal, minimal or negative response, based on the classifications described above. A total of 87 male C. D. mice was used. Mice were subjected to saline, sesame oil, testosterone propionate, estradiol benzoate or stilbestrol treatment of various duration as described below. At the end of the treatment period, one testis was removed surgically under ether anesthesia, fixed in Bouin's fluid and processed for microscopic study by routine histological techniques. Following surgery, mice were given a single subcutaneous injection of 0.03 mmole/kg of

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