nificance can be attached to this effect of the hypnotic drugs. That the drugs have a site of action preceding D-glucuronic acid is more probable in the light of the present and previous data(3), and the elegant work of Hollmann and Touster(18).

Summary. The temporal sequence of changes in activity of L-gulono-γ-lactone hydrolase, in concentration of ascorbic acid in serum and in urine during replacement therapy of hypophysectomized rats with somatotropin was examined. Results agree with the view that variations in activity of the hydrolase are responsible for diminished synthesis of ascorbic acid after hypophysectomy and increased synthetic activity when somatotropin is administered. The prompt decrease in activity of the hydrolase found after removal of the hypophysis did not occur in severe alloxan diabetes, indicating that permissive action of insulin may not be required for effects of somatotropin on hepatic protein anabolism. However, DL-ethionine rapidly depressed the activity of the hydrolase and of L-gulonate NADP oxidoreductase in a way analogous to long-term consequences of hypophysectomy. It appears that an alternative to the step involving lactonization of L-gulonic acid cannot be quantitatively important for synthesis of ascorbic acid. Inhibition of the hydrolase by Chloretone and chloral hydrate *in vitro,* in apparent contradiction since these drugs stimulate biosynthesis of ascorbic acid, is probably of no relevance *in vivo.*

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Received November 18, 1963. P.S.E.B.M., 1964, v115.

Transplant Immunity to Polyoma Virus Induced Tumors. I. Correlations with Biological Properties of Virus Strains.* (29043)

J. DONALD HARE† (Introduced by Herbert R. Morgan)
Louis A. Wehle Virus Research Laboratory, Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The neoplastic conversion of hamster cells by polyoma virus (PV), *in vivo* and *in vitro,* is accompanied by the appearance of an "antigen" specific for tumor cells induced by PV(1,2) but distinct from those induced by

* Supported by grants from Nat. Cancer Inst., U.S.P.H.S. and United Funds of Clayton and Alexandria Bay, N. Y.

† Supported by a Career Research Development Award from the U.S.P.H.S.

SV_{40} virus(3,4,5). The source of this antigenic specificity is of basic importance to an understanding not only of viral neoplasia but of neoplasia in general, since it has now been repeatedly demonstrated that chemically induced neoplasms may also show new antigenic characteristics(6,7,8). Thus, two major hypotheses to explain viral oncogenesis are currently under investigation: 1) viral genetic material is directly responsible for the coding of this new antigenic material and must persist throughout the life of the cell and be passed on to its progeny; or 2) abortive, noncytolytic interaction between virus and prospective tumor cell results in a specific alteration of cellular genetic material, chromosomally or extrachromosomally located, which is responsible for the synthesis and perpetuation of the antigenic character in the absence of the viral genetic material.

A study was begun to investigate the relationship between tumor cell antigen and a series of specific biological markers available in several PV strains. It was hoped that by studying the biological expression of viral genetic information and correlating this with oncogenesis and transplant immunity, it would be possible to learn how the virus might induce the neoplastic conversion of normal cells. A variant of PV, SE210-H^-, described recently(9), showing not only antigenic but other biological characters sufficient to distinguish it from a second strain, SE3049-H^+, has been studied. Data will be presented demonstrating the similarity between the "antigens" induced in hamster tumor cells by these 2 virus strains which are also equally capable of stimulating resistance to transplanted tumor cells *in vivo* in spite of widely different oncogenic capacity. A third strain of PV, Dulbecco's "large plaque" variant, was found to induce little transplant immunity against tumor cells induced by the first 2 strains, pointing to possible heterogeneity within the PV-tumor cell antigen system.

Materials and methods. Polyoma viruses were the high hemagglutinin producing strain (H^+), SE3049-H^+, the low hemagglutinin producing strain (H^-), SE210-H^-, previously described(9), and the "large plaque" strain of Dr. Renato Dulbecco (PV^{1p}). These were propagated in P388 D_1 cells using Eagle's basal medium and 5% bovine fetal serum. Two hamster tumor cell lines, described in Table I, were induced by 2 strains of PV by injection of newborn, random-bred, golden hamsters and carried in tissue culture in Eagle's basal medium with 10% calf serum. Suspensions of single tumor cells from tissue culture and solid tumors were prepared by dispersion with 0.2% trypsin in citrate buffer(10). Cell viability always exceeded 85-90%. For tests of transplant immunity, appropriate tumor cell suspensions were counted, diluted in fresh calf serum medium, and injected subcutaneously in 0.1 ml volumes over the thin abdominal wall of hamsters. Two doses of tumor cells were usually inoculated into each animal, using the right and left lower abdominal quadrants. Immunizing doses of virus were given intraperitoneally 2 to 3 weeks prior to tumor cell challenges. Tumor growth was followed at weekly intervals for 4 to 6 weeks, at which time the animals were sacrificed and bled, and the tumors were harvested and weighed. Hemagglutination-inhibition tests were carried out on the sera of immunized hamsters by a technique described previously(11). Plaque assays for infections PV were performed on mouse embryo monolayers in plastic Petri dishes with an initial overlay of 6 ml of Eagle's basal medium (double strength), 0.5% lactalbumin hydrolysate and 5% bovine fetal serum followed by 4 ml of the same medium without serum on day 7. Neutral red, at a final concentration of 1:30,000, was added on the 13th day and plaques counted the following day. All overlay medium contained 0.9% agar (Noble).

TABLE I. Hamster Tumor Cell Lines Induced by Polyoma Virus Strains.

HTC-3049-3	Induced by SE3049-H^+ 85 transfers *in vitro* Chromosome number: 42-46
HTC-210-27	Induced by SE210-H^- 40 transfers *in vitro* Chromosome number: 42-46

Results. Biological characteristics of virus strains. Table II summarizes the various dis-

TABLE II. Biological Characteristics of Polyoma Virus Strains Studied.

Character*	Virus strain SE3049-H+ (Eddy)	PV1p (Dulbecco)	SE210-H− (Eddy-Hare)
Plaque size	~70% large	~75% large	>98% small
Cytopathic effect in P388 D_1 cells	Slow Incomplete	Slow Incomplete	Rapid Complete
Hemagglutinin production	High	High	Low
Antigenic structure	A	A	B
Oncogenicity in newborn hamsters	>90%†	~87%	30% or less
Immunogenicity vs 3049 tumor cell	4+	±	4+

* See text for description of the character indicated.
† Percent of animals with solid tumors or hepatic hemorrhagic cysts.

tinguishing characteristics identifiable in the 3 strains of PV studied. Plaque diameter was 0.5 to 1.5 mm for the small plaque particles and 3 to 5 mm for the large plaque particles. The low hemagglutinin strain (H^-) produced titers usually less than 2 to 6 $\log_2$ hemagglutinin units (HAU) per ml at the time of complete cytopathic effect in P388 D_1 cells, while the high hemagglutinin strains (H^+) produced 10 to 14 $\log_2$ HAU/ml, often detectable prior to the onset of significant cytopathic effect. The rapidly cytopathic strain always destroyed P388 D_1 cultures completely in 4 to 6 days when infected with a multiplicity of 1, while the slow cytopathic strain frequently entered into a carrier state with the P388 D_1 cell without ever destroying the culture. An antigenic difference has been demonstrated between several polyoma strains(9) and will be described more fully elsewhere.

The oncogenicity of the various strains has been tested over a 2-year period. The SE3049-H^+ strain has been of high oncogenic capacity, a dose of 10^6 to 10^7 TCD_{50} producing sarcomas in more than 90% of hamsters inoculated when less than 7 days of age. When injected at a similar dose, the "large plaque" strain produced sarcomas in 87% of suckling hamsters while the SE210-H^- strain induced sarcomas in approximately 30% of newborn hamsters. Both the SE3049-H^+ and "large plaque" strains produced hemorrhagic liver lesions frequently, while this was rare in animals inoculated with the SE210-H^- strain.

Graded response of hamsters to polyoma virus. To compare the immunogenic potency of the various strains, several experiments were conducted to test the immune response of adult hamsters to decreasing doses of virus. The serum hemagglutination-inhibiting antibody level was measured at each virus dose as well as transplant immunity to the HTC 3049-3 cell line. Table III shows the results of these experiments. Several points should be noted. In the first place, there was a direct relationship between HI antibody response and infectious dose of the 3 virus strains injected. This type of response suggested that PV behaves as an antigenic material which either fails to replicate or replicates to a limited extent in adult hamsters. This finding differs from that seen in the mouse(12) where the virus multiplies to high titer when only a few infectious particles are inoculated, thereby stimulating full antibody production at limiting virus dilutions.

The second point to note is that transplant immunity induced with either of the first 2 strains, SE3049-H^+ or SE210-H^-, showed a similar direct relationship to dose, except in the case of the highest dose of SE3049-H^+ where an unusual number of challenge tumors developed after inoculation of 10^5 cells. These two findings indicate that the virus strains tested must be limited in their ability to replicate in hamsters and that the ultimate immunologic response will be dependent primarily on the initial dose administered.

Finally, the SE3049-H^+ and SE210-H^-

TABLE III. Immunogenic Potency of Polyoma Virus Strains Related to Dose of Virus Administered.

Virus	TCD_{50}	HI antibody/ml	Tumor cell challenge—HTC-3049 1×10^5	Tumor cell challenge—HTC-3049 1×10^4
SE3049-H+	10^7	12,800*	4/5†	1/5
	10^5	6,400	1/5	1/5
	10^3	<400	5/5	5/5
	10^1	<400	5/5	5/5
SE210-H-	10^7	6,400	2/5	1/5
	10^5	1,600	4/5	1/5
	10^3	800	4/5	4/5
	10^1	<400	5/5	5/5
Control	—	<400	11/11	10/11
PV^{1p}	10^7	3,940	—‡	3/3
	10^6	1,968	—	3/3
	10^5	1,212	—	3/3
	10^4	800	—	3/3
	10^3	800	—	3/3
Control	—	<400	—	3/3

* Expressed as reciprocal of last dilution producing 50% inhibition of 8 units of the homologous hemagglutinin in a 2-fold dilution series.
† No. with tumors/No. injected.
‡ Not tested.

strains produced an equivalent degree of immunity against a strain of tumor cells—HTC-3049-3 originally induced by SE3049-H+ strain. On the other hand, the PV^{1p} strain of Dulbecco has failed in several experiments to stimulate significant immunity to this tumor cell line (Tables III and V).

Ability of polyoma virus to replicate in hamsters. To test the hypothesis that polyoma virus replicates poorly in hamsters, 4 adult animals were inoculated intraperitoneally with 10^7 plaque-forming units of the SE3049-H+ strain, and 2 were then sacrificed at 7 days and 2 at 14 days post-infection. The liver, spleen and kidneys were harvested, frozen and thawed once, and homogenized in 2 ml of growth medium. The quantity of virus in each organ was measured by plaque titration. Table IV presents the results of this experiment. The liver and kidney failed to retain virus after 7 days except in 1 of 2 animals. The spleen proved to have infectious virus at both 7 and 14 days, but at a relatively low titer. Although not extensive, these data support the antibody studies which suggest limited replication of this virus in adult hamsters.

TABLE IV. Polyoma Virus in Hamster Organs After Intraperitoneal Inoculation.

Animal*	Day sacrificed	Infectious virus/ml of organ homogenate† Spleen	Liver	Kidney
A	7	2×10^2‡	$<10^1$	$<10^1$
B	7	2.6×10^4	4×10^2	$<10^1$
C	14	4×10^1	$<10^1$	$<10^1$
D	14	2×10^2	$<10^1$	$<10^1$

* 1×10^7 plaque-forming units of SE3049-H+ inoculated intraperitoneally on day 0.
† The spleen, 1 kidney and 1 cm cube of liver homogenized in 2 ml of buffer.
‡ Expressed as plaque-forming units/ml.

TABLE V. Comparative Immunogenicity of 3 Polyoma Virus Strains.

Virus	HI antibody/ml	Tumor-cell challenge—1×10^4 cells HTC-3049-3	HTC-210-27
Control	< 100*	13/14†	14/14
SE3049-H+	>3200	3/11	0/11
SE210-H-	6400	5/11	1/11
PV^{1p}	6400	12/12	8/12
Control	< 400	5/5	5/5
SE210-H-	1600	1/7	1/7
PV^{1p}	3200	6/6	5/6

* See Table III for appropriate explanation.
† " " " " " " "

Immunogenic capacity of polyoma strains. Transplantable hamster tumor cell lines were induced in hamsters inoculated with PV strains SE3049-H+ and SE210-H− (Table I). These tumor cells were then used to challenge animals immunized with different PV strains. Table V shows 2 experiments in which cross-immunity studies were carried out simultaneously. It is clear that the 2

TABLE VI. Influence of Simultaneous Infection with 2 Polyoma Virus Strains on Transplant Immunity.

Treatment	HI antibody/ml	HTC-3049-3 tumor cell challenge on day 28			
		9×10^4 cells		9×10^3 cells	
		Tumor takes	Tumor size	Tumor takes	Tumor size
			(mg)		(mg)
Control	400*	6/6†	216	4/6	38
PV^{1p} – day 0	6,400	5/5	180	5/5	32
SE3049-H^+ – day 14	6,400	4/6	92	1/6	10
PV^{1p} – day 0 SE3049-H^+ – day 14	12,800	6/6‡	118	6/6	118

* Expressed as reciprocal of dilution at the endpoint of a 2-fold dilution series.
† No. with tumors/No. injected.
‡ Three of 6 animals had hemorrhagic liver lesions and small peritoneal tumors at autopsy.

strains, SE3049-H^+ and SE210-H^-, were capable of producing transplant immunity to tumor cells induced by either virus, although the immunity against the HTC-3049-3 line was less active than the HTC-210-27 line. Of even more interest is the fact that a third strain of PV, the "large plaque" virus of Dulbecco, failed to produce immunity of significant degree to either of these cell lines in spite of the fact that the SE3049-H^+ and the PV^{1p} strains are quite similar in their biological characters as well as their ability to stimulate an HI antibody response (Table III).

Modification of oncogenic and immunogenic activity by simultaneous infection with 2 similar strains. The failure of the Dulbecco "large plaque" strain to immunize against a tumor cell line produced by a virus strain virtually identical with it (SE3049-H^+), raised the question as to whether this was because the 2 strains induce different antigenic determinants in the tumor cells and thus would not cross-immunize, or whether there was a more subtle immunologic or physiologic explanation such as interference with the tumor rejection apparatus. Work is in progress to obtain tumor cell lines induced by this virus to test the cellular antigenic specificity. Table VI shows the results of a second type of experiment in which the "large plaque" virus was injected on day 0, SE3049-H^+ on day 14 and the animals challenged with 9×10^4 and 9×10^3 HTC-3049-3 cells on day 28. It should be noted that the SE3049-H^+ strain alone produced immunity, while the PV^{1p} strain alone failed to induce resistance and the size attained by tumors produced by the tumor cells was equal to controls. However, in the animals given both viruses in the above sequence, tumors developed in all animals at both tumor cell doses. Of further interest is the fact that at the lower tumor cell dose, the tumor size in the doubly-infected animals was approximately 3 times as large as those in the controls or the PV^{1p}-injected animals. A second finding of interest was that 3 of the 6 animals in the doubly-infected group also developed hepatic hemangioendotheliomas and an occasional solid peritoneal tumor, whereas none of the other animals showed evidence of tumor formation. Therefore, not only was growth of the inoculated tumor cells enhanced, but the oncogenicity of the combination of strains for adult animals was likewise increased. This experiment raises more questions than it answers, but it does indicate that there are rather complex interrelationships between PV strains that are not fully explainable on the basis of cellular induction of neoplasia or on strictly immunologic grounds.

Discussion. The studies presented here were undertaken to determine whether any or all polyoma virus strains, irrespective of their oncogenic efficiency, plaque morphology, antigenic structure, hemagglutinin production, and so forth, could function as an immunizing stimulus against tumor cells induced by different virus strains. Most studies thus far have examined the specificity of

tumor cell antigens induced by single polyoma strains and found them to be indistinguishable(2,13).

It was felt that by examining the antigenic structure of tumor cells induced by two quite distinct polyoma virus strains, we might find evidence of a heterogeneity that could be used as a tool to pinpoint the mechanism of induction of the tumor antigen. Furthermore, by comparing the immunogenic efficiency of a virus strain that is poorly oncogenic (SE210-H$^-$) with a more neoplastic agent (SE3049-H$^+$), we might find variations in immunizing activity that could provide insight into the synthesis of the antigen and its relationship to the neoplastic process. The results show that the two virus strains which differ most widely in their biological activities are equally potent inducers of transplant immunity in adult hamsters and that each induces similar antigens in tumor cells. A striking finding is the aberrant action of the "large plaque" strain of Dulbecco which failed to immunize against tumor cells induced by either preceding strain and in fact seemed to enhance tumor growth, both primary and challenge, when inoculated before the SE3049-H$^+$ virus into the same animals. It is of interest, therefore, that Habel found (13) that his polyoma virus strain would not protect hamsters against Dulbecco's tumor cell line, Py 177, induced by a virus strain which we find fails to produce transplant immunity.

Finally, the distinct, direct relationship between the dose of virus injected and the resulting HI antibody level, as well as transplant immunity, further indicates the minimal capacity of polyoma virus to replicate in hamsters, as pointed out previously(14,15).

These findings raise doubts as to the completely homogeneous behavior of polyoma strains and perhaps suggest that neoplastic and antigenic conversion are not simultaneous events. Further studies are in progress.

Summary. Evidence is presented to show that 2 polyoma strains, 3049-H$^+$ and 210-H$^-$, which differ in a number of biological characteristics, induce similar degrees of transplant immunity in adult hamsters, and induce similar "antigens" in tumors produced in neonatal animals. A third strain, Dulbecco's "large plaque" virus, is nearly identical with the 3049-H$^+$ strain, but fails to stimulate transplant immunity against tumor cells induced by the 3049-H$^+$ virus; instead, it appears to enhance tumor growth. Evidence is also presented to show that polyoma virus replicates poorly in the hamster (as measured by virus content of liver, spleen, and kidney) and may localize more specifically in an immunologically active organ, the spleen.

The author wishes to acknowledge the technical assistance of Mrs. Judith Vande.

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Received November 18, 1963. P.S.E.B.M., 1964, v115.