through the courtesy of Dr. J. Black, Schering Corp., Bloomfield, N. J. The guanethidine supplies were provided by Dr. William Wagner, Ciba Pharmaceutical Co., Summit, N. J.

1. Rubin, A. A., Roth, F. E., Winbury, M. M., *Nature,* 1961, v192, 176.

2. Rubin, A. A., Roth, F. E., Winbury, M. M., Topliss, J. G., Sherlock, M. H., Sperber, N., Black, J., *Science,* 1960, v133, 2067.

3. Rubin, A. A., Taylor, R. M., Rosenkilde, H., *J.P.E.T.,* 1962, v136, 344.

4. Wilson, W. R., Okun, R., *Circulation,* 1963, v28, 89.

5. Finnerty, F. A., Kakaviatos, N., Tuckman, J., Magill, J., *ibid.,* 1963, v28, 203.

6. Okun, R., Russell, R. P., Wilson, W. R., *Arch. Int. Med.,* 1963, v112, 882.

7. Tabachnick, I.I.A., Gulbenkian, A., Seidman, F., *Diabetes,* 1964, in press.

8. Wolff, F. W., Parmley, W. W., *Lancet,* 1963, v2, 69.

9. Gulbenkian, A., Seidman, F., Tabachnick, I. I. A., *Fed. Proc.,* 1964, v23, 457.

10. Montgomery, R., *Arch. Biochem. and Biophys.,* 1957, v67, 378.

11. Wilson, W. R., Stone, D. B., Okun, R., Russell, R. P., *Ann. Int. Med.,* 1964, v60, 317.

12. Hertting, G., Axelrod, J., Whitby, L. G., *J.P.E.T.,* 1961, v134, 146.

13. Wolff, F. W., Langdon, R. G., Ruebner, B. H., Hollander, C., Skoglund, R. D., *Diabetes,* 1963, v12, 335.

Received July 9, 1964. P.S.E.B.M., 1964, v117.

Tumor Immunity in Hamsters Infected with Adenovirus Type 12 or Simian Virus 40. (29642)

BERNICE E. EDDY, GEORGE E. GRUBBS AND RALPH D. YOUNG
Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

It has been established that either adenovirus type 12(1) or simian virus 40 (SV_{40})(2,3,4) will induce neoplasms when injected into newborn hamsters. The minimum oncogenic dose of adenovirus type 12 for newborn hamsters as determined in cultures derived from human embryonic lung (5) is reported to be between 0.5 and 50 tissue culture infective doses ($TCID_{100}$). The minimum oncogenic dose of SV_{40} as determined by cytopathic changes in cercopithecus monkey kidney cell cultures is large. More than $10^{3.5}$ $TCID_{50}$ per 0.2 ml(3,6) was necessary to induce neoplasms in nearly every hamster, although smaller doses of virus sometimes caused tumor development in a few hamsters after a prolonged latent period.

Recently, it has been shown that the incidence of neoplasms in hamsters could be reduced or completely inhibited if additional doses of homologous virus were administered sometime after the initial infecting virus dose but before the appearance of tumors(7). The extent of the protection from tumor development varied from partial to complete. The experiments herein reported were designed to determine the effect of administering repeated large doses of virus to hamsters to determine the relationship between the degree of observed protection against tumor development with: 1) the initial infecting virus dose; 2) size of each virus dose used in treatment; and 3) total amount of virus given. The effect of the injection of repeated large doses of virus to normal hamsters beginning at 11 to 15 days of age was also tested. Except in the instance when incompletely inactivated SV_{40} was employed, all treatment was with undiluted fully virulent virus of the same lot which had been used to infect the animals when newborn, or of a similar lot.

Materials and methods. Viruses. Human adenovirus type 12, strain Huie, was obtained through the courtesy of Dr. M. J. Cook, Nat. Inst. of Allergy and Infectious Diseases. The virus was propagated in a continuous line of human carcinoma cells, HeLa(8), and the first virus lots prepared had titers of approximately $10^{2.0}$ to $10^{2.5}$ per 0.2 ml in 7 days. After several passages in HeLa cells and a

TABLE I. Comparison of Results of Treating Hamsters Uninfected, and Infected with Adenovirus Type 12 when Newborn, with 13 One ml Doses of Undiluted Homologous Virus Given Twice Weekly Starting 11 to 15 Days After the Initial Infection.

Exp No.	Treatment started on day:	Observation period (days)	Hamsters		
			Uninfected when newborn and treated	Infected when newborn	
				Untreated	Treated with adenovirus type 12
1	15	90	0/19*	8/9	1/10
2	12	83	0/9	6/8	4/9
3	11	76	0/12	13/13	2/13
4	15	69	0/11	5/7	1/18
Total			0/51	32/37	8/50

* Numerator indicates number of hamsters which developed neoplasms and denominator the number of hamsters injected.

change in the serum used, the titers increased to approximately $10^{2.5}$ to $10^{3.0}$ per 0.2 ml. Different lots of virus were used for each experiment.

SV_{40} was isolated from rhesus monkey kidney cell cultures(3) and propagated in cercopithecus monkey kidney cell cultures. Different lots of virus were employed, and the titers of the preparations ranged from $10^{7.5}$ to $10^{8.5}$ per 0.2 ml.

Cells and media. Eagle's medium(9) containing 100 units of penicillin G and 100 mg of streptomycin per ml and sera was used for all the cell cultures. The HeLa cells were trypsinized and grown in media containing 10% calf serum. Prior to inoculation with virus, the fluid was replaced with medium containing 3% calf serum. Recently, medium has been used in which 2.5% "Newborn Agamma Calf Serum" (Hyland Laboratories, Los Angeles) replaced the calf serum.

The cercopithecus monkey kidney cell cultures were prepared in the Tissue Culture Section of the Division of Biologics Standards. A medium containing 2% calf serum was used in this preparation; but for the propagation of SV_{40}, the medium was changed to one containing one percent calf serum.

Heated virus. In one experiment, both unheated and heated virus were employed in treatment. One lot of virus was divided. Magnesium chloride(10) was added to one portion to make a molar solution, and the virus was dispensed in 10 ml volumes in 15 ml vials. These containers were submerged in water heated to 50°C and kept there for one hour. The virus was then pooled and dialyzed to remove the high salt content. This treatment did not inactivate all the SV_{40}. Cytopathic changes which were characteristic of SV_{40}(11,12) developed in cercopithecus monkey kidney cell cultures incubated with the heated virus.

Injection of virus. When <1 to 4 days of age, hamsters (*Cricetus auratus*) in 2 to 5 litters were each injected subcutaneously with 0.2 ml of adenovirus type 12 or SV_{40}, undiluted or diluted in 4-fold steps. Later, but before additional virus was given, the hamsters were redistributed so that each group was made up of an approximately equal number of animals from each litter. In a few experiments, the newborn hamsters were redistributed before being injected with virus. In these instances some of the hamsters were not infected when newborn. These hamsters, along with a similar group which had been infected with virus when newborn, were set aside and given repeated large doses of virus when older. Except in animals which developed tumors before the conclusion of the experiment, the usual course of treatment was 13 doses of virus. In certain experiments the number of doses of virus used in treatment was varied. As mentioned above, one group of hamsters was treated with heated virus.

Results. In Table I are shown the results of treating normal hamsters with repeated large doses of undiluted adenovirus type 12 beginning at approximately 11 to 15 days of age. Within the periods of observation, these

TABLE II. Influence of Variations in the Initial Infecting Dose of Virus Given to Newborn Hamsters on the Protective Effect of 13 Additional Doses of Homologous Virus.

Virus	Treatment started on day:	Observation period (days)	Dilutions of virus used to infect newborn hamsters							
			Undiluted		1:4		1:16		1:64	
			Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Adenovirus, type 12	14	145	10/11*	4/10	9/11	0/11				
SV_{40}	25	153	8/8	1/7	3/4	0/5				
SV_{40}	25	139	7/7	3/7	6/6	2/6	2/2	0/2	3/7	0/7
									5/7	0/6

* Numerator indicates number of hamsters which developed neoplasms and denominator indicates number of hamsters injected.

hamsters did not develop neoplasms. Of a group of 37 hamsters which received 0.2 ml of adenovirus type 12 when newborn, 32 hamsters (86%) developed neoplasms. In a similar group which, in addition to receiving 0.2 ml of virus when newborn, was treated with repeated one ml doses of undiluted homologous virus, only 8 of 50 (16%) developed neoplasms.

The concentration of the infecting virus dose was known to be an important determinant(6,13) of the incidence of tumor development and the length of the latent period; it also appeared to influence the degree of protection afforded by subsequent treatment with large doses of homologous adenovirus type 12 (Table II). When the hamsters infected with adenovirus type 12 diluted 1:4 were treated with repeated doses of undiluted virus, complete protection was obtained; but the same treatment of hamsters infected neonatally with undiluted virus was effective in protecting only 6 of 10 hamsters (60%). The influence of the initial infecting dose of SV_{40} was also important. Suggestive evidence was also obtained that the protective effect of large doses of SV_{40} to hamsters infected when newborn was less when the initial infecting dose of virus was great.

The influence of the virus dose used in treatment of the neonatally infected hamsters is shown in Table III. The larger dose of virus was more effective. Only 2 of 14 hamsters (14%) treated with one ml doses of homologous adenovirus type 12 developed neoplasms as compared with 6 of 14 (43%) treated with 0.25 ml doses of virus. The variation in the virus dose used for treatment of SV_{40} infected hamsters was only 2-fold, and there was a difference of only one hamster in the groups treated with 0.5 and 1.0 ml doses respectively. The one hamster which developed a neoplasm was in the group treated with only 0.5 ml doses of virus.

The total amount of virus used in treatment seemed to be more important in some experiments than in others. Some protection was afforded hamsters neonatally infected with adenovirus type 12 by 1, 3 and 8 doses of homologous virus, but more was obtained

TABLE III. Effect of Variations in Dose of Virus Administered to Hamsters to Achieve Inhibition of Tumor Development in Hamsters Infected when Newborn.

Virus	Treatment started on day:	Observation period (days)	Hamsters infected when newborn			
			Untreated	Treated with 13 doses of homologous undiluted virus. Each dose: .25 ml	.5 ml	1 ml
Adenovirus, type 12	14	159	12/14*	6/14		2/14
SV_{40}	26	168	13/13		1/12	0/8

* Numerator indicates number of hamsters which developed neoplasms and denominator indicates number of hamsters injected.

TABLE IV. Effect of Variations in Number of Homologous Virus Doses Used in Treatment of Hamsters Infected when Newborn, and also Effect of Treatment with Virus Heated in Presence of a Molar Solution of Magnesium Chloride.

Virus	Treatment started on day:	Observation period (days)	Hamsters infected when newborn					
			Untreated	Treated with 1 ml of homologous virus: With unheated virus: 1×	3×	8×	13×	With heated virus: 13×
Adenovirus, type 12	10	105	10/10*	6/10	6/10	6/10	1/8	
SV_{40}	29	265	5/7	0/7	1/7	1/8	0/8	
SV_{40}	30-37†	259	12/12	10/11	6/11	4/11	6/11	
SV_{40}	26	196	7/8				0/8	5/8

* Numerator indicates number of hamsters which developed neoplasms and denominator indicates number of hamsters injected.

† These hamsters were infected when newborn, then 30 days later treatment was started on approximately one-half of each group and 37 days later treatment was started on the remaining half.

when 13 doses were given (Table IV). In one experiment with SV_{40}, only 5 of 7 of the neonatally infected untreated hamsters developed neoplasms; one hamster in each of the groups treated with 3 doses and with 8 doses of homologous virus developed tumors while none of the animals in the group treated with 1 or 13 doses developed neoplasms. In the second experiment with SV_{40}, all hamsters which were infected when newborn and which received no additional virus developed neoplasms between day 70 and day 133, and were all dead by day 205. Among the treated animals, only one of 11 hamsters which had been given one additional large dose of virus remained free of neoplasms; protection was not complete whether 3, 8 or 13 additional doses of virus were given. The protection was as good when 3 doses as when 13 doses of virus were administered. When heated virus was used in treatment, 5 of 8 hamsters developed tumors as compared to 7 of 8 untreated hamsters. No tumors developed in the hamsters treated with the same lot of fully viable virus.

Discussion. The failure of normal hamsters to develop tumors when treated with large doses of viable virulent virus beginning at 11 to 15 days of age demonstrated that the protective mechanism is not dependent on stimulation resulting from an infection when newborn. Hamsters up to 14 days of age have been reported to be susceptible to the oncogenic effect of a single dose of adenovirus type 12(5). As might be expected, an important factor which appeared to be correlated inversely with protection from tumor induction was the concentration of the infecting virus dose. As to the amount of virus needed for protection, in general larger doses of virus were more effective than smaller doses, but protection was obtained over a range of virus doses. This may have been correlated with the initial virus dose, although this was not shown in the present investigations.

Thus far there is no indication that a booster dose of virus is needed following the series of virus doses administered. Some neonatally infected hamsters treated with large doses of SV_{40} have remained alive and well for over 400 days, and adenovirus-infected and treated hamsters have remained free of tumors for 275 days.

The SV_{40} virus heated in the presence of magnesium chloride and used in treatment of SV_{40}-infected hamsters did not afford protection from tumor development. This may or may not indicate that protection can be achieved only by treatment with viable virus. It is possible that protection could be achieved by another means of inactivation. Immunogenic potency of other infective viruses are reported to be retained when heated with high concentrations of divalent cations(14).

Summary. The oncogenicity of adenovirus type 12 and SV_{40} administered to hamsters when newborn could be inhibited or prevented by administration of large doses of the homologous virus at later dates. The protection achieved appeared to vary inversely with the concentration of virus in the initial infecting dose. The administration of greater initial infecting doses of virus resulted in fewer tumor-free animals in the treated groups. The size of the dose of virus used in treatment was a factor, and a large dose (1 ml) gave a little more protection than a 0.25 or 0.5 ml dose. The usual course of treatment was a total of 13 doses of virus administered at twice weekly intervals. Smaller numbers of doses used in treatment were sometimes effective. Treatment with virus heated to 50°C for an hour in the presence of magnesium chloride was not effective in preventing the induction of tumors.

1. Trentin, J. J., Yabe, Y., Taylor, G., *Science,* 1962, v137, 835.

2. Eddy, B. E., Borman, G. S., Berkeley, W. H., Young, R. D., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 191.

3. Eddy, D. E., Borman, G. S., Grubbs, G. E., Young, R. D., *Virology,* 1962, v17, 65.

4. Girardi, A. J., Sweet, B. H., Slotnick, V. B., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 649.

5. Yabe, Y., Trentin, J. J. Taylor, G., *ibid.,* 1962, v111, 343.

6. Eddy, B. E., Grubbs, G. E., Young, R. D., *ibid.,* 1962, v111, 718.

7. Eddy, B. E., Young, R. D., Grubbs, G. E., *Perspect. Virology* (Gustav Stern Symposium), v4, in press.

8. Scherer, W. F., Syverton, J. T., Gay, G. O., *J. Exp. Med.,* 1955, v97, 695.

9. Eagle, H., *Science,* 1955, v122, 501.

10. Wallis, C., Melnick, J. L., *Texas Rep. Biol. Med.,* 1961, v19, 701.

11. Sweet, B. H., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 420.

12. Eddy, B. E., *Progr. Exp. Tumor Res.,* (Karger, New York), 1964, v4, 1.

13. Girardi, A. J., Sweet, B. H., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 662.

14. Yang, C-A., Tai, F.-H., Wallis, C., Melnick, J. L., *J. Immunol.,* 1963, v91, 283.

Received July 9, 1964. P.S.E.B.M., 1964, v117.

Prolactin Secretion *in vitro*: Effects of Gonadal and Adrenal Cortical Steroids.* (29643)

CHARLES S. NICOLL† AND JOSEPH MEITES‡
Department of Physiology, Michigan State University, East Lansing

Several experimental observations indicate that gonadal and adrenal cortical steroids can influence prolactin secretion *in vivo*. Administration of estrogens (E) cause pronounced elevation of pituitary prolactin content(1,2), induces pseudopregnancy in rats (3,4) and initiates lactation in several spe-

* Published with approval of Michigan Agri. Exp Station as Journal Article No. 3424.

† Present address: Nat. Cancer Institute, Bethesda, Md.

‡ This work supported in part by grant from N.I.H.