pressed by the bile acid while the enzymic activity of both pancreatic lipase and the enzyme present in the blood after pancreatic lipase ingestion were greatly enhanced by the presence of cholic acid. Although the rate of fat absorption has been shown to be affected by lipase ingestion, the possibility remains that this increased lipolytic activity may play some role in clearing the blood of fat.

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## Host-Virus Relationships in Hamsters Inoculated with $SV_{40}$ Virus During the Neonatal Period.* (29355)

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Previous reports(1-3) from this laboratory described the isolation of $SV_{40}$ virus, presented first definitive proof of oncogenic quality of the agent and described the influence of age, virus dose, route of inoculation and other factors on tumorigenesis. The present report describes the findings in continued studies of pathogenesis of $SV_{40}$ virus in hamsters with special reference to viral latency, immunology, and age influence.

*Materials and methods. Virus and cell cultures.* Prototype strain VA 45-54 of $SV_{40}$ virus was propagated and titrated in cell cultures of grivet monkey kidney (GMK) by methods described previously(2,3). *Hamsters.* Pregnant hamsters were obtained from Lakeview Hamster Colony, Newfield, N. J., and the progeny were inoculated subcutaneously with virus inoculum at the time periods shown in the text. Animal care, clinical observations, and pathologic observations were carried out by previously described methods (2,3). *Animal tissues.* Homogenized whole animals 1 to 7 days of age were assayed for infectious virus whereas pools of various organs were prepared from older animals. Special attention was given to include tests of skin and muscle from the dorsal area of the animals which was the site of injection and the usual site for tumor appearance. The whole animals, tissue pools, and tumors were homogenized for 10 minutes at 16,000 rpm in an Omnimix cup with sufficient Hanks' balanced salt solution to give a 15% suspension. These were clarified by centrifuging at 2200 rpm for 20 minutes in the horizontal head and the supernates were stored frozen at —70°C until assayed for presence and amount of $SV_{40}$ virus. *Virus recovery.* The 15% tissue suspensions were diluted 1:2 and serial 10-fold dilutions were titrated in GMK using 4 tubes per dilution. Preparations which gave negative results were retested, whenever possible, in 20 ml amount (1:2

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dilution) in 1 liter bottle cultures. The cultures were refed after 24 hours when necessary. All cultures which were negative were observed for as long as possible and then blind passages were made in GMK. Samples were not recorded as negative until the total time on test was at least 56 days. All isolates were identified using appropriate $SV_{40}$ antiserum. Negative control tissue suspensions were always included to rule out possible induction of latent $SV_{40}$ virus from the assay system cultures. These controls were comparable suspensions of nonvirus shedding polyoma hamster transplant tumor tissue. *Serology.* Neutralization tests were carried out in GMK cultures. The hamster sera were inactivated at 56°C for 45 minutes and were tested against 10-100 $TCD_{50}$ of $SV_{40}$ virus. The virus-serum mixtures were incubated for 45 minutes at 37°C followed by overnight at 4°C and the inoculum dose was 0.2 ml.

*Results. Virus occurrence according to time period following inoculation.* Newborn hamsters were inoculated subcutaneously with 0.2 ml of $SV_{40}$ virus which titered $10^{-5.5}$ or $10^{-6.0}$ per 0.2 ml. The animals were sacrificed at the time periods shown in Tables I and II and the tissues or tumors were tested for presence of $SV_{40}$ virus. The theoretical virus titer was computed for each sample based on the weight of tissue, volume of suspending medium, assumption of no loss of infectivity of the original virus in the inoculum and localization of all the virus in the particular tissues assayed (theoretical maximum). There was gradual and progressive diminution in detectable viral content during the first 3 weeks following inoculation. In Experiment 1, no virus was detectable at 14 days but in Experiment 2 virus was still present in detectable amount at 20 days. The carcass or the whole animal was the most likely site for virus when present pretumor. No virus was found at 130-200 days in nontumor-bearing animals, at a time when tumors had begun to appear in the littermates. Virus was commonly but not always present in tumor even though the

TABLE I. *Experiment 1.* Titers of Infectious Virus in Tissues of Hamsters Inoculated with $SV_{40}$ Virus when Newborn.

| Day after inoculation | No. of animals assayed | Tissue assayed | Neg. $\log_{10}$ infectivity titer (.2 ml) Theoretical* | Measured |
|---|---|---|---|---|
| — | — | Virus inoculum | — | 5.5 |
| 0 | 9 | Whole animal suspension | 3.4 | 3.7 |
| 1 | 8 | *Idem* | 3.2 | 1.7 |
| 7 | 10 | " | 2.9 | 1.0 |
| 14 | 9 | Various organs† | 2.6 | <0 |
| Pretumor§ | | | | |
| 130-200 | 8 | " " ‡ | 1.5 | <0 |
| Tumor | | | | |
| 173 | 1 | Tumor (A 1) | 3.2 | 2.5 or > |
| 173 | 1 | " (D 3A) | 2.8 | 1.5 |
| 173 | | " (D 3B) | 3.7 | <0 |
| 210 | 1 | " (A 2) | 2.0 | 1.5 |
| 210 | 1 | " (A 3) | 2.2 | <0 |
| 215 | 1 | " (A 4) | 2.3 | 1.5 |
| 215 | 1 | " (D 2) | 3.0 | <0 |

* Calculation based on weight/volume and assuming no loss of initial infectivity (theoretical maximum).

† Heart and lungs; liver and spleen; kidneys, other viscera; carcass (eviscerated and decapitated remains).

‡ Skin and muscle; viscera; remains.

§ After littermates developed tumor.

TABLE II. *Experiment 2.* Titers of Infectious Virus in Tissues of Hamsters Inoculated with $SV_{40}$ Virus when Newborn.

| Day after inoculation | No. of animals assayed | Tissue assayed | Neg. $\log_{10}$ infectivity titer (.2 ml) Theoretical* | Measured |
|---|---|---|---|---|
| — | — | Virus inoculum | — | 6.0 |
| 1 | 5 | Whole animal suspension | 3.8 | 2.7 |
| 7 | 5 | *Idem* | 3.4 | .7 |
| 12 | 5 | Head | 3.8 | 0 ‡ |
| | | Viscera | 3.7 | 0 |
| | | Carcass† | 3.6 | .2 |
| 15 | 5 | Head | 3.6 | <0 |
| | | Viscera | 3.4 | 0 |
| | | Carcass | 3.2 | 0 |
| 20 | 5 | Head | 3.4 | <0 |
| | | Viscera | 3.2 | <0 |
| | | Carcass | 3.0 | 0 |
| Pretumor | | | | |
| 126 | 3 | Skin, muscle, other | 2.0 | <0 |
| Tumor | | | | |
| 120 | 1 | Tumor (F 1) | 3.6 | 2.0 |
| 197 | 1 | " (B 1) | 3.2 | 2.0 |

* Calculation based on wt/vol (theoretical maximum) and assuming no loss of initial infectivity.

† Eviscerated and decapitated remains.

‡ 0 = Undiluted.

TABLE III. Effect of Age at Time of Inoculation of Hamsters with $SV_{40}$ Virus on Retention of Infectious Virus and on Development of Neutralizing Antibody.

| Age at time of inoculation (days) | Neg. $\log_{10}$ infectivity titer: Day after inoc. | No. animals assayed | Tissues assayed (pools) | Neg. $\log_{10}$ $SV_{40}$ titer (.2 ml) | $SV_{40}$ neut. antibody*: No. pos./total tested (28 days post-inoc.) |
|---|---|---|---|---|---|
| — | — | — | Original inoculum | 7.0 | — |
| 0 | 7 | 5 | Whole animal | .5 | 0/19 (0%) |
| | 14 | 5 | Viscera | <0 | |
| | | | Carcass | <0 | |
| 7 | 7 | 5 | Viscera | <0 | 8/20 (40%) |
| | | | Carcass | 1.0 | |
| | 14 | 5 | Viscera | <0 | |
| | | | Carcass | <0 | |
| 14 | 7 | 5 | Viscera | <0 | 10/12 (83%) |
| | | | Carcass | .7 | |
| | 14 | 5 | Head | <0 | |
| | | | Viscera | <0 | |
| | | | Carcass (individual) | All <0 | |
| 21 | 7 | 5 | Head | <0 | 14/16 (88%) |
| | | | Viscera | <0 | |
| | | | Carcass (individual) | All 0† | |
| | 14 | 5 | Head | <0 | |
| | | | Viscera | <0 | |
| | | | Carcass (individual) | All <0 | |

* Serum diluted 1:4 and tested with 10 $TCD_{50}$ of $SV_{40}$ virus. † 0 = Undiluted.

agent was not detectable in the preceding period.

*Influence of age at time of inoculation on viral disappearance.* Hamsters were inoculated as above at 0, 7, 14, or 21 days of age and groups of animals were tested for presence of virus, 7 and 14 days later and for $SV_{40}$ neutralizing antibody in bloods drawn 28 days after virus. As shown in Table III, virus was detected in the whole animal or carcass pools at 7 but not at 14 days following inoculation in all groups. No virus was present in the viscera at either time period. Neutralizing antibody was absent 4 weeks after inoculation in animals receiving $SV_{40}$ when newborn but appeared in an increasing proportion of animals which were given virus at 7, 14 or 21 days of age, respectively. It seemed that infectious virus had disappeared from the newborn group of animals by the time they had reached the age for development of immunologic competence.

*Relationship between tumor weight and virus presence.* It was noted previously that virus was more likely to be present in large than in small tumors(2). Fig. 1 shows the results of isolation attempts with 11 tumors in a single experiment and indicates good correlation between tumor weight and virus titer. This finding was substantiated further in the experiments with 35 tumors summarized in Table IV in which more than

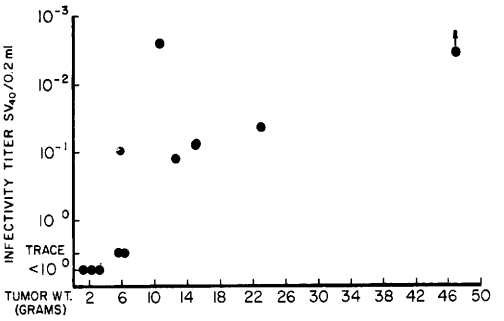


FIG. 1. Relationship between tumor weight and titer of $SV_{40}$ virus present.

TABLE IV. Relationship Between Tumor Weight and Presence or Absence of Infectious $SV_{40}$ Virus.

| Wt of tumor (g) | Virus isolation results (No. of tumors): Positive | Negative | % positive |
|---|---|---|---|
| <10 | 5 | 12 | 29% |
| >10 | 13 | 5 | 72% |

TABLE V. Infectivity Titer of $SV_{40}$ Virus in Large Compared with Small Primary Subcutaneous Tumors Which Occurred in the Same Animal.

| Hamster | Tumor wt (g) | Neg. $\log_{10}$ infectivity titer (.2 ml) |
|---|---|---|
| A 2 | 11.0 | 0 |
| | 1.0 | <0 |
| B 2 | 15.5 | 1.5 |
| | 2.1 | .5 |
| D 3 | 9.0 | 1.5 |
| | 1.2 | <0 |
| D 04 | 42.5 | 1.5 |
| | .6 | 0 * |

* 0 = Undiluted.

twice the proportion of tumors weighing > 10 g showed virus than did those weighing < 10 g. These findings suggest that the virus, which is below the level of detection or absent during the latent period, replicates to form infectious virus some time after the tumor has made its appearance.

It was considered possible that biologic variation from host to host might influence virus isolation results and accordingly, assays were performed in 4 instances in which a large and a small tumor occurred in the same animal. The findings are given in Table V. The greater amount of virus in large tumor was confirmed giving further validity to the relationship between tumor size and virus content.

*Virus in visceral tumors.* $SV_{40}$ virus was recovered only infrequently from visceral tumors. Only 3 of 15 such tumors yielded virus even though all the animals had primary subcutaneous tumors as well (Table VI). The visceral tumors were of small size and negative results might have been expected on this basis alone. Alternatively, the visceral tumors might have derived principally from nonvirus shedding metastatic cells or the visceral tissue might not have supported complete viral replication following its transformation. It is worthy of note, as previously shown(3), that only a small proportion of hamsters developed visceral tumors following peritoneal inoculation of a large amount of $SV_{40}$ virus and most of these occurred in the subcutaneous tissue at site of injection.

TABLE VI. Infectivity Titer of $SV_{40}$ Virus in Visceral Tumors of Hamsters.

| Hamster | Tissue | Neg. $\log_{10}$ infectivity titer (.2 ml) |
|---|---|---|
| A 02 | Kidney | .5 |
| | Lung | 0 |
| G 3 | " | 0 |
| 10 hamsters | " , kidney | All <0 |

*Virus in transplant tumors.* Attempts to recover virus from primary tumors and from transplant tumors deriving from them did not give consistent findings. Thus, in certain instances, virus was isolated from primary but not transplant tumors and in other instances the transplants yielded virus while the primary tumor did not. One transplant line (C1) was carried through 18 generations and readily yielded infectious $SV_{40}$ virus at every passage except one (passage 15). Another line (F 5-1) yielded only a small amount of virus in the original tumor and was negative for 29 passages thereafter, even when tested extensively.

*Relationship between antibody development and tumor.* As noted previously(3) and in Table III above, there was an increase in the proportion of animals which showed neutralizing antibody response and a decrease in the proportion of animals which developed tumor with increase in age at time of inoculation with $SV_{40}$ virus. However, presence or absence of detectable antibody 28 days following inoculation of 7 day old animals did not appear to affect the subsequent development of tumors since ultimate incidence of tumor was roughly the same in those with antibody as in those without (Table VII). A few of the 0 day animals which failed to develop antibody initially, did so within 28 days after appearance of their tumors.

*Discussion.* The well studied oncogenic viruses of animals and human origin generally fall into 3 groups, viz., simple cubic nuclear DNA (papovavirus, adenovirus); complex helical cytoplasmic RNA (Myxovirus-like); or complex cytoplasmic DNA (poxvirus)(4,5). Tumors caused by the first named group of agents are characterized by the lack of need for continued proliferation of or for continued presence of infectious

virus in order to maintain their malignant quality.

The present findings concerning the pathogenesis of $SV_{40}$ virus, a papovavirus, in tumor induction in hamsters are generally consistent with those presented earlier(2,3) and with data reported by others as reviewed by Black and Rowe(6). Thus, there is limited proliferation or no proliferation of infectious $SV_{40}$ virus following inoculation into baby hamsters and there is complete or nearly complete absence of detectable virus within 3 weeks after the agent is given. Once absent, no infectious virus can be detected at the inoculation site or in distal tissues and organs until it appears in the tumors. The amount of virus demonstrable in the tumors is generally related to tumor size, even when the tumors are derived from the same animal. Thus, virus amount increases with enlargement of tumor but in very large tumors, as noted by Black and Rowe(6), tests for virus may give negative results. This may be due to neutralization by the large amount of antibody which may be present in animals bearing large tumors. Efficiency of tumorigenesis by $SV_{40}$ virus is age-related and is greatest in newborn animals with progressive decrease in tumor incidence as the age at time of inoculation is increased. Newborn animals generally fail to develop antibody against $SV_{40}$ virus within a month following inoculation. These animals, however, are fully capable of forming antibody when inoculated with the same virus some weeks later(2,3). A portion of animals inoculated when 7 days of age or older do develop neutralizing antibody and such antibody or lack of antibody appears to be unrelated to whether the animal ultimately develops tumor.

TABLE VII. Comparison, Neutralizing Antibody Levels at Various Time Intervals Follownig $SV_{40}$ Virus Injection at Various Ages in Tumor-Bearing and Nontumor-Bearing Hamsters.

| Age at inoculation (day) | Tumor status | Reciprocal of $SV_{40}$ neutralizing antibody titers of hamsters at time periods following virus inoc. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 28 days | | | | | Tumor appearance | | | | 28 days after tumor appearance | | |
| | | 0 | 2–4 | 8–16 | 32–64 | No. pos. /total | 0 | 4 | 16 | No. pos. /total | 0 | 4 | 8 | No. pos. /total |
| 0 | Tumor | 35* | — | — | — | 0/35 ( 0%) | 33 | — | 1 | 1/34 | 24 | 4 | 0 | 4/28 |
| 7 | Tumor | 14 | 2 | 3 | 3 | 8/22 (36%) | 12 | 3 | 0 | 3/15 | 9 | 1 | 1 | 2/11 |
| | No tumor | 5 | 2 | 0 | 1 | 3/8 (38%) | — | — | — | — | 3 | 2 | 1 | 3/6 |
| 21 | No tumor | 31 | 10 | 17 | 8 | 35/66 (53%) | — | — | — | — | — | — | — | — |

* No. of animals.

Various evidences have been presented (6-11) to suggest that $SV_{40}$ viral nucleic acid may be incorporated into the cell genome, probably into most of the cells, with continued production of a new antigen under the viral genetic code. A majority of tumors in the present study contained infectious virus but this was not always the case. The transplant $SV_{40}$ tumor line designated F 5-1, *e.g.*, has been carried in hamsters in our laboratory for a total of 29 passages and has failed to yield detectable virus at any point beyond the original tumor when tested in grivet kidney cell cultures incubated and observed for as long as 60 days. Thus, presence of infectious virus in tumor occurs only sometimes and is not essential to continued malignant property.

Sabin and Koch(9,10) reported increase in synthesis of infectious virus by propagat-

ing tumor cells *in vitro*, or by application of X-ray, and Gerber(11) demonstrated infectious $SV_{40}$ virus in sensitive indicator cells cultivated *in vitro* in contact with $SV_{40}$-induced hamster ependymoma cells which were free of detectable virus. The state of the virus in the cell which is essential to or associated with continued malignancy and the state of the virus in the cell which can be caused to produce infectious virus are unknown and they may be quite different. Thus, capacity to yield infectious virus might be only an incidental property of certain of the cells which are carrying the virus in a "latent" state contrasted to a more "integrated" form of viral nucleic acid essential to expression of cancer. Such difference in virus state might explain why some tumor lines yield virus and others do not.

*Summary.* Findings relative to the pathogenesis of $SV_{40}$ virus in inducing tumor in hamsters are discussed. Infectious virus disappeared rapidly from the animal and was not demonstrable until its appearance in tumor. The quantity of virus was related to tumor size. Infectious virus was commonly demonstrable in tumor but was not always present and appeared not to be essential to retention of oncogenic quality. Initial neutralizing antibody response in a portion of animals to inoculated $SV_{40}$ virus appeared unrelated to subsequent development or failure to develop tumor.

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## Immunofluorescent Study of Experimental *Trypanosoma cruzi* Infection.* (29356)

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The fluorescent antibody technique has been utilized in the identification of organisms ranging in size from viruses to protozoa (1). Two techniques may be employed, the direct and indirect. The direct technique involves application of a conjugate containing the antibody to the smear or section; the indirect technique involves application of the specific antibody which when bound to the antigen is identified by staining with a conjugate containing an antibody against the specific antibody. The indirect technique has the advantages of being more sensitive and offering a mechanism for serologic testing.

The presence of antibodies in a given serum is demonstrated by applying the test serum to a known antigen. The excess serum is washed away and a fluorescein-conjugated antibody to the test serum is applied. If an antibody to the known antigen is present in the test serum, the antigen will be stained, but otherwise it will remain unstained when examined in fluorescent light. This method of testing has been used to identify antibodies to various organisms including viruses and bacteria(1-2).

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