irradiated sample. A check was made for Mn^{54} γ-activity, and it and samples of Mn_3O_4 and Fe_2O_3 were sealed in quartz ampules and irradiated in the isotope-tray position of the Argonne CP-5 Research Reactor for 1 week. The thermal neutron flux was 1×10^{12} n cm^{-2} sec^{-1}. After the irradiation, carriers were added, and manganese and iron separated from each sample. These elements were counted with a γ-counter and, in most cases, recycled and counted again. The Mn^{54} activity in the manganese from Odessa Mn sample was also determined by x-ray counting.

The Mn^{54} activity produced by the irradiation was referred to the 2.58-hour Mn^{56} activity produced in the reaction Mn^{55} $(n,\gamma)Mn^{56}$. The two activities are given by the conventional irradiation equations

$$A_{56} = \sigma_{55}N_{55}f(1 - e^{-\lambda_{56}t_1})e^{-\lambda_{56}t_2} \quad (1)$$
$$A_{54} = \sigma_{53}N_{53}f(1 - e^{-\lambda_{54}t_1})e^{-\lambda_{54}t_2} \quad (2)$$

where A = activity, σ = cross section, N = number of atoms, f = thermal neutron flux, λ = decay constant, t_1 = irradiation time in the reactor, and t_2 = decay time after removal from the reactor. Dividing Eq. 2 by Eq. 1, substituting $N_{53} = A_{53}T_{53}/0.6931$ (where T_{53} = half-life of Mn^{53} and A_{53} = activity of Mn^{53} measured prior to irradiation), and solving for $\sigma_{53}T_{53}$ yields the relation

$$\sigma_{53}T_{53} = \sigma_{55}N_{55}\frac{0.6931}{A_{53}} \times \frac{A_{54}(1 - e^{-\lambda_{56}t_1})e^{-\lambda_{56}t_2}}{A_{56}(1 - e^{-\lambda_{54}t_1})e^{-\lambda_{54}t_2}} \quad (3)$$

If t_1 = 1 week, t_2 = 0, and σ_{55} = 13.2 barns, then

$$\sigma_{53}T_{53} = (566 \text{ barns})\frac{N_{55}A_{54}}{A_{53}A_{56}} \quad (4)$$

The activity in the 0.835-Mev gamma peak was measured shortly after the removal of the irradiated material from the reactor to obtain the counting rate for Mn^{56} and again after the Mn^{56} had decayed to obtain the counting rate for Mn^{54}. The counting rate for the 0.84-Mev Mn^{56} peak was corrected for contributions from the Compton regions of the 1.81- and 2.13-Mev gammas and the counting rate for Mn^{54} was corrected for Mn^{54} from Mn^{55} or Fe^{54}.

The results are presented in Table 1 along with the specific activity or composition calculated from these data. In the case of the Odessa Mn sample the contributions to the Mn^{54} activity made by the reactions Fe^{54} $(n,p)Mn^{54}$ and Mn^{55} $(n,2n)Mn^{54}$ are respectively $\leq$0.013 disintegrations per minute (dpm) and $\leq$42 dpm. These activities are much smaller than the 1.19×10^4 dpm for Mn^{54} activity found and may therefore be neglected. The value for $\sigma_{53}T_{53}$ calculated from these data by Eq. 4 is $(350 \pm 100) \times 10^6$ barn-years. The error on this number was estimated from the uncertainty in the specific activity of Mn^{53} in the Odessa Mn sample and is much larger than the error based on the counting statistics after activation. If a half-life of 2×10^6 years is assumed, then σ_{53} is 170 barns. An increase in specific activity by a factor of 320 is achieved under these irradiation conditions. This large amplification will greatly facilitate the detection and measurement of Mn^{53} in the future.

Table 1. Counting results for Mn^{56} (half-life = 2.58 hours), Mn^{54} (half-life = 300 days), and Fe^{59} (half-life = 45.0 days). The errors shown are standard deviations based on counting statistics only.

Separated element	Radioactive nuclide	Activity in total sample (dpm)	Computed specific activity or composition
		Before irradiation of Odessa Mn	
Total sample	Mn^{54}	$\leq 4.2 \pm 3.4$	
		After irradiation of Odessa Mn (sample wt. = 3.5 mg; Mn = 1.63 mg; Mn^{53} = 37 ± 11 dpm)	
Mn	Mn^{56}	$(1.79 \pm .09) \times 10^{10}$	$(1.10 \pm .06) \times 10^{10}$ Mn^{56} dpm/mg Mn
Mn	Mn^{54}	$(1.29 \pm .02) \times 10^4$	
Mn recycle	Mn^{54}	$(1.19 \pm .01) \times 10^4$	$(0.73 \pm .01) \times 10^4$ Mn^{54} dpm/mg Mn
Mn recycle	Mn^{54} (x-ray)	$(1.47 \pm .03) \times 10^4$	
Fe	Fe^{59}	$\sim 6.5 \times 10^3$	
Fe recycle	Fe^{59}	$\sim 6.0 \times 10^3$	3.2 μg Fe/mg Odessa Mn sample
		After irradiation of Mn_3O_4 (sample wt. = 10.0 mg; Mn = 6.60 mg)	
Mn	Mn^{56}	$(7.29 \pm .41) \times 10^{10}$	$(1.10 \pm .06) \times 10^{10}$ Mn^{56} dpm/mg Mn
Mn	Mn^{54}	$\leq 168 \pm 7$	$\leq 26 \pm 1$ Mn^{54} dpm/mg Mn
Fe	Fe^{59}	$\leq 420 \pm 25$	
Fe recycle	Fe^{59}	$\leq 450 \pm 25$	$\leq .08$ μg Fe/mg Mn_3O_4 sample
		After irradiation of Fe_2O_3 (sample wt. = 6.2 mg; Fe = 4.3 mg)	
Mn	Mn^{54}	$\leq 19.2 \pm 5.3$	$\leq 4.5 \pm 1.2$ Mn^{54} dpm/mg Fe
Fe	Fe^{59}	$(2.56 \pm .02) \times 10^6$	
Fe recycle	Fe^{59}	$(2.33 \pm .01) \times 10^6$	$(5.42 \pm .01) \times 10^5$ Fe^{59} dpm/mg Fe

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Brain Tumors (Gliomas) Induced in Hamsters by Bryan's Strain of Rous Sarcoma Virus

Abstract. *Gliomas and choroid plexus papillomas were induced by intercerebral inoculation of Rous sarcoma virus, Bryan's strain, in newborn hamsters. Of five pools of virus tested, four were effective when used at high dose.*

After the original isolation of a filterable agent responsible for the chicken myxosarcoma (*1*), a number of strains of Rous sarcoma virus (RSV) with different biological properties have been described. These variants differ with respect to antigenicity (*2*) and host range (*3, 4*). They also show varying pathological effects in chicken fibroblasts grown in vitro (*5*). Differences occur in the morphology of the pock-like lesions produced on the chorioallantoic membrane of embryonated hen's eggs and in the cell comprising the infective foci in chicken embryo tissue culture (*6*).

Whether these variants occur naturally or whether the original strain has been undergoing antigenic or mutational changes as a result of varying techniques for the preparation of virus stocks in different laboratories is not clear at present. The RSV is not species specific (*3, 7*). However, the demonstration that some strains of RSV are capable of inducing neoplastic lesions in rats (*4, 8*) showed that the virus is not class specific.

This stimulated extensive research on its oncogenic activity in mammals (*9*). Bryan's high-titer strain of RSV, which permitted, for the first time, quantitative assay of a tumor-inducing virus both in vivo (*10*) and in vitro (*11*), has never been shown to induce tumors in mammals. However, vacuolization of monkey and human kidney cells in tissue culture (*12*) and some degree of mitotic inhibition of human leukocytes in vitro (*13*) have been observed after infection with RSV, Bryan's strain.

We now describe the effect of this strain of RSV on newborn hamsters inoculated intracerebrally. Cell-free pools of RSV, Bryan's strain, from different sources (Table 1) were prepared by differential centrifugation (*14*). The virus was concentrated and resuspended in phosphate-buffered saline *p*H 7.2 (PBS). The virus titers of the inocula for the different pools are shown in Table 1.

Newborn Syrian hamsters (*Cricetus auratus*) less than 24 hours old were inoculated with 0.02 ml of the different virus suspensions into the midportion of the right cerebral hemisphere. The results are presented in Table 1. Many animals showed cranial enlargement at an early age. The neurological symptoms consisted of lethargy, lateral posture of the head, and paralysis; the paralysis resulted in death. At autopsy the brain was enlarged and swollen owing to hydrocephalus. Gross examination indicated focal areas of malacia. Microscopically these consisted of multiple neoplasms within the cerebral hemispheres (Fig. 1*a*). Some of these extended into the ventricles with proliferation of ependymal cells. There were two cell types in these tumors. One type, consisting of large or medium-sized round elements arranged in sheets, showed delicate fibrillar strands but no collagen or reticulum. These tumors showed the characteristics of gliomas (Fig. 1*b*). The other form had cords of polygonal eosinophilic cells largely arranged in papillary strands. These tumors showed the characteristics of a choroid plexus papilloma (Fig. 1*c*). The latter tumors were always associated with the solid gliomas. Both types had numerous mitotic figures.

Control hamsters inoculated intracerebrally with the suspending medium (PBS), alone or with extracts of normal chicken brain, failed to give evidence of intracranial tumors or of pathologic changes after 3 months of observation.

The results (Table 1) suggest that high doses of the agent are needed for the oncogenic effect. This is consistent with the fact that the virus does not replicate in this host and is in agreement with the high titers required for the induction of papillary ependymomas in the same species with SV40 virus (*15*).

The induction of gliomas in hamsters with the Schmidt-Ruppin strain of RSV has been reported (*16*). The possibility of this variant's contaminating Bryan's virus strain is small because several pools, from different sources, proved effective at high doses. On the other hand, below 3.6×10^4 plaque-forming units (PFU) all the pools proved ineffective. Pool A has been kept frozen in sealed vials since 1960. At that time the Schmidt-Ruppin strain of RSV had not yet been introduced into the United States. Pool B proved ineffective when

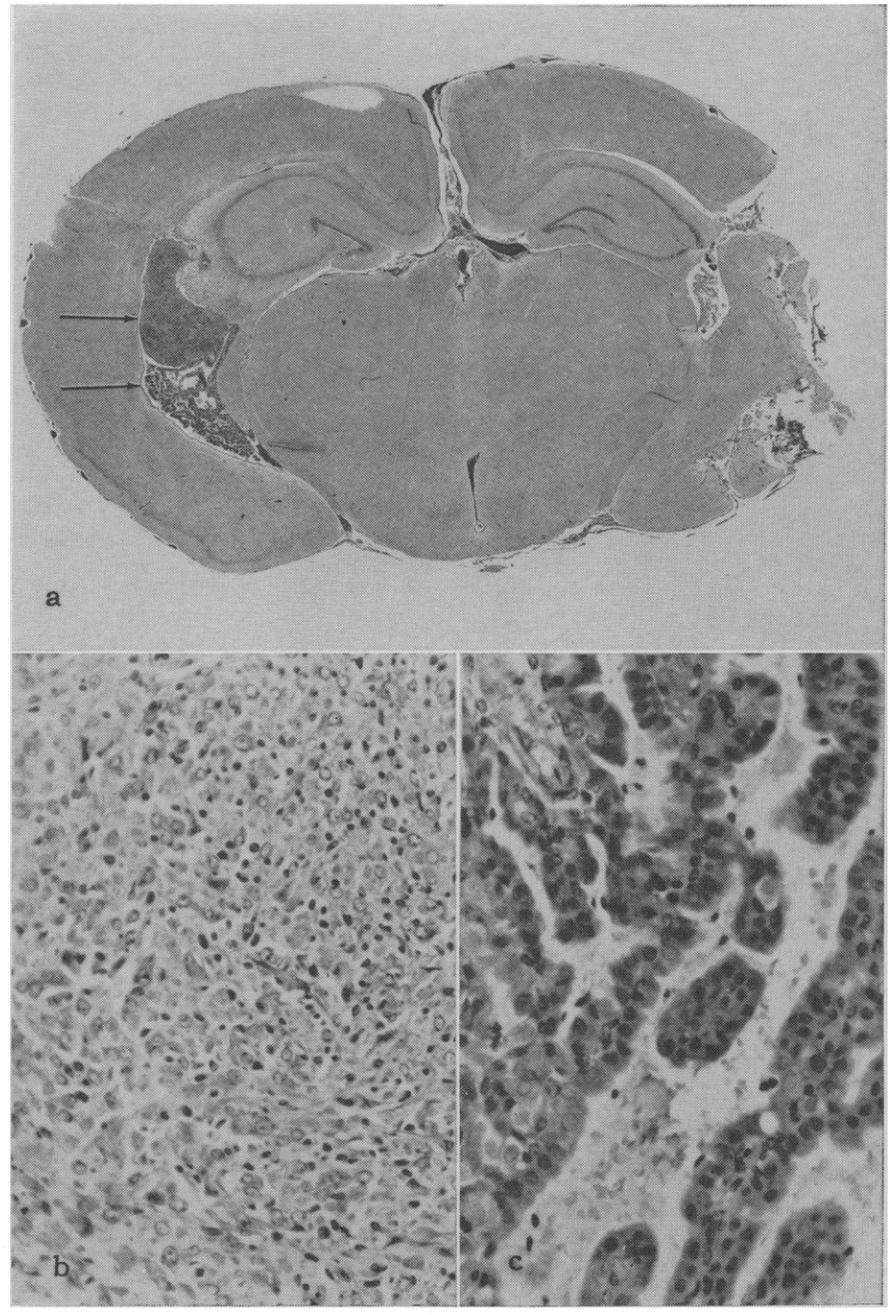

Fig. 1. Tumor induced in hamster brain by pool A of Rous sarcoma virus, Bryan's strain. *a*, Two types of tumors are visible one above the other (about × 25). *b*, Upper tumor of Fig. 1*a* is an undifferentiated glioma (about × 350). *c*, Lower tumor of Fig. 1*a* is a choroid plexus papilloma (about × 350).

Table 1. Newborn hamsters injected intracerebrally with RSV, Bryan's strain. The virus inoculum was titrated in hen's chorioallantoic membrane and is expressed as plaque-forming units (PFU).

Inoculum titer (PFU × 10^5/.02 ml)	Weaned hamsters (No.)	Appearance of neurological symptoms (av. days)	Hydrocephalus (%)	Gliomas (%)
*Pool A, 66th chicken brain passage of RSV infected brain**				
2.2	7	26	57	43
Pool B, chicken wing web tumor†				
0.15	17			
2.5	9	33	22	55
5.5	43	28	23	90
8.2	9	29	33	66
Pool C, 57th chicken brain passage of pool A				
0.08	16			
0.16	25			
0.22	10			
Pool D, chicken wing web tumor ct. 958‡				
0.30	6			
0.35	19	36	5	5
0.65	19	27	42	16
0.72	12	32	41	33
Pool E, chicken wing web tumors induced with Pool C§				
N.T. ‖	7	40	57	43
Controls				
PBS ¶	26			
N.C.B. #	41			

* Obtained from F. Rauscher. Labeled B 1711 A, 65th CB, RSV and was kept at −60°C since 27 October 1960. † Univ. Lab. Pool TV. 19. ‡ Obtained from J. Kvedar. § Pool C at low doses showed inactivity. ‖ Not titrated. ¶ Phosphate buffer saline *p*H 7.2 as diluent. # Cell-free extract of normal chicken brain, prepared by the method used for infected chicken brain or chicken tumor.

given at the dose of 10^4 PFU. At this dose the Schmidt-Ruppin strain of RSV is highly oncogenic (*16*). These data make any possibility of contamination of pool B very unlikely. Pool C proved ineffective at the low doses available, but when its titer was increased by passage through the chicken wing web and the virus was harvested it became oncogenic (pool E). Pool D was prepared in Bryan's laboratory.

The intracranial tumors induced in hamsters by Bryan's strain of RSV are different from those induced by the same virus in chicken brain. These tumors in chicken brain are myxomatous lesions arising in the pial-arachnoid spaces (*17*).

Some points can be raised by the demonstration of the oncogenic effect of Bryan's strain of RSV in hamsters. One is the inoculation of high concentrations of the agent into a very susceptible organ such as the brain. The oncogenic dose of RSV for hamster brain is about 10^5 PFU for the Bryan's strain and 10^3 PFU for the Schmidt-Ruppin strain. One may then assume the presence of a population of at least two variants in the Bryan's RSV pools used: one affecting birds only (heterogenic), and the other affecting both birds and mammals (xenogenic). Both mutants are present in different relative amounts in each of the pools. High doses of Bryan's strain of virus would then bring the low concentration of the xenogenic variant of this pool to the critical level for carcinogenesis. On the other hand, the possibility exists that the immunological competence of newborn hamsters is higher for Bryan's virus than for the Schmidt-Ruppin virus. The resistance may then be overcome by large doses of virus. An alternative to the latter hypothesis may be that the hamster is genetically somewhat resistant to infection by the avian sarcoma viruses, and it may be more resistant to Bryan's strain than to the Schmidt-Ruppin strain (*18*).

Since the discovery of Rous sarcoma virus it has been thought that the oncogenic effect of this agent was confined to tissues of mesenchymal origin. The induction of gliomas in hamsters shows the oncogenic effect of this virus on a tissue of ectodermic derivation such as neuroglia.

Furthermore, the determination of the oncogenic dose of this virus for the hamster brain is possible since the agent does not replicate in this host. On the contrary, in the chicken, due to the growth of the virus in this host, one can determine the infective dose but not the oncogenic dose.

To the best of our knowledge this is the first time that the Bryan's strain of RSV is shown to be oncogenic in a mammalian species.

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18. Since this report was submitted a new pool of RSV, Bryan's strain, designated as ct. 955 batch #3, and the Harris strain of RSV have been shown active in inducing the same type of brain tumors in hamsters.
19. We thank Drs. W. R. Bryan, W. Heston, R. Manaker, J. Moloney, F. Rauscher, H. Stewart, and G. Suskind for helpful discussions during the preparation of the manuscript.

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Moloney Virus–Induced Leukemias of Mice: Measurement in vitro of Specific Antigen

Abstract. *A modified test for cytotoxic antibody may be used to measure antibody directed against the tumor-specific antigen of leukemias of mice induced by Moloney virus; cell death is detected by liberation of ^{51}Cr. An inhibition test based on this technique permits accurate measurement of tumor-specific antigen in cells and subcellular fractions.*

Leukemias induced in mice by infection with Moloney virus (*1*) contain a common antigen, against which specific transplantation immunity is present in syngenic hosts after their rejection of grafts of Moloney leukemias from allogenic mice or of subthreshold isografts, or after prior treatment of the mice with homogenates of cells containing Moloney virus (*2*).

Immune mice develop a serum antibody which reacts specifically with tumors induced by Moloney virus. This antibody has been detected by the indirect fluorescent antibody technique and by its ability, in the presence of complement, to kill leukemia cells induced by Moloney virus (see *2*); the criterion of cell death has been the failure of the cells to exclude trypan blue.