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.

1 December 1964

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.

My purpose has been to apply a more precise and objective cytotoxic technique, developed by Wigzell (*3*) for the H-2 system of mice, to the detection and measurement of antigen specific for Moloney tumor (tumor induced by Moloney virus) in cells and subcellular fractions. In this technique the target cells are labeled with $Na_2{}^{51}CrO_4$; after treatment of the cells with antibody and complement, cell death is assessed by measuring the isotope which is released into the supernatant.

Under appropriate conditions the technique is applicable to the Moloney tumor–specific system (Fig. 1). Moloney tumors are not uniformly sensitive to the cytotoxic action of antibody; the tumor routinely used in this study is an ascites form of YAC, a lymphoma originally induced in an A-strain mouse by neonatal injection of Moloney virus. The cells, suspended at a concentration of 5×10^7 per milliliter in saline containing 5 percent by volume of normal calf serum, are labeled for 30 minutes with $Na_2{}^{51}CrO_4$ (20 μc/ml) at 37°C, washed, and suspended in 5 percent calf serum at a concentration of 3×10^7 per milliliter. Labeled cells (20 to 40 μl) are incubated with equal volumes of diluted antiserum for 15 minutes at 37°C, and unfixed antibody is removed by centrifugation and replaced with an equal volume of 50 percent guinea pig serum as a source of complement. After further incubation for 45 minutes, a portion of the centrifuged supernatant is removed for estimation of liberated ^{51}Cr by means of a scintillation counter.

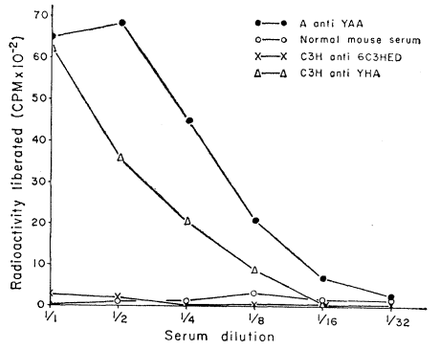

Fig. 1. Liberation of ^{51}Cr from labeled ascites tumor cells by antiserum specific for Moloney tumor in the presence of guinea pig complement. The target cells used were YAC, an ascites lymphoma induced by Moloney virus in A-strain mice. YAA and YHA are Moloney lymphomas of A and C3H strains, respectively; 6C3HED is an ascites leukemia of C3H mice unrelated to Moloney virus. CPM, count/min.

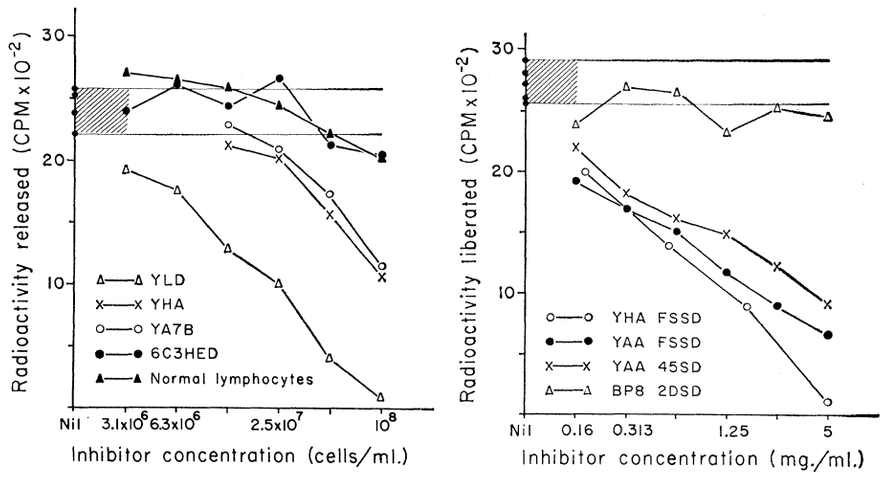

Fig. 2. Specific inhibition of cytotoxicity, as measured by ^{51}Cr release. Portions of antiserum to the Moloney tumor antigen were incubated with antigen and tested for residual cytotoxic antibody against YAC target cells labeled with ^{51}Cr. (Left) Whole cells used for inhibition; YHA, YLD, and YA7B are Moloney lymphomas of C3H, C57L, and AXC57B1 mice, respectively. 6C3HED is a leukemia of C3H not related to Moloney virus. (Right) Subcellular fractions used for inhibition. YHA FSSD was a freshly prepared material; the others were reconstituted after lyophilization (*4*). BP8 2DSD, known to be a highly active preparation of H-2 antigen, was prepared from an ascites sarcoma of C3H mice (*4*). CPM, count/min.

For measurement of tumor-specific antigen, the technique has been used as an inhibition test. Portions of antiserum, at a dilution which liberates about 25 percent of the total radioactivity from the target cells, are incubated for 15 minutes at 37°C with decreasing dilutions of antigen. Labeled target cells are then added, and the test is continued as already described. Antigen can be measured by this method either in whole cells or in separated subcellular fractions (Fig. 2).

The fractions used were prepared by the method described earlier for the separation of cell fractions rich in H-2 antigen (*4*); fractions rich in H-2 antigen are also usually rich in tumor-specific antigen.

This technique for measuring antibody and antigen specific for Moloney tumors offers (i) complete objectivity; (ii) a high degree of precision; (iii) lack of interference with the cytotoxic effect of residual antibody on labeled target cells by intact cells used as an inhibitor (such intact cells need not be removed before the test is continued); (iv) convenient assay of subcellular fractions which may be difficult to sediment; (v) greater accuracy and economy by avoidance of the absorption test, in which a relatively large amount of antibody is absorbed with antigen and residual activity is titrated after removal of the antigen-antibody complex.

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5. I thank Prof. Georg Klein for facilities and materials, Barbro Jansson for technical assistance, and the International Union against Cancer for awarding an Eleanor Roosevelt Fellowship. Work supported by grants from the Damon Runyon Memorial Fund (DRG-598), the Jane Coffin Childs Memorial Fund for Medical Research, the Swedish Cancer Society, and Lotten Bohmans Fund.

14 December 1964

Charge-Transfer Self-Complex Formed by 8-Azaguanine

Abstract. *The perpendicular distance between the planes of successive molecules of 8-azaguanine, in crystals of 8-azaguanine monohydrate, is 3.25 Å. This distance indicates intermolecular interaction of the charge-transfer type. 8-Azaguanine may act as a cell poison by forming a charge-transfer complex within the bacterial RNA.*

Brockman *et al.* (*1*) have shown that 8-azaguanine is a cell poison. Tracer studies with 8-azaguanine-2-^{14}C in vivo with *Streptococcus faecalis* have shown