two ultrastructural observations are inconsistent with a thermal model of damage: the absence of significant damage to pigment epithelium and its microvilli and the predilection of lamellar disarray for the proximal rather than distal ends of rods and cones. Furthermore, the ultrastructural alterations seen here, though nonspecific in nature, do resemble very closely changes produced with ordinary light at low levels (*5–7*). These changes have been attributed to direct photic effects both on the basis of energy calculations (*7*) and on the basis of studies involving deficiency in vitamin A (*8*). Also, the changes observed do not resemble those produced directly by heat (*6*) or those produced by higher levels of coherent irradiation and, hence, indirectly by heat (*9*).

The significance of these observations is not fully established. If this morphologic phenomenon proves to be extensively reproducible, its functional effects and persistence or resolution remain undetermined. At present, we conclude that very low levels of coherent radiation are capable of producing ultrastructural alterations in sensory retina.

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Inhibition of Chemical Carcinogenesis by Viral Vaccines

Abstract. *The incidence of 3-methylcholanthrene–induced subcutaneous tumors was significantly reduced by a single injection of inactivated type C RNA viral vaccine. Rauscher leukemia virus vaccine reduced the incidence of sarcomas from 78 to 50 percent in the BALB/cCr mouse. Radiation leukemia virus vaccine and a vaccine from a wild murine leukemia virus derived from a 3-methylcholanthrene tumor reduced the incidence of sarcoma from 86 percent to 33 and 37 percents, respectively, in the C57BL/6 mouse. These reductions in tumor incidence by virus vaccines help support the concept that type C RNA viruses serve as determinants of chemically induced cancer; additional studies of vaccines made with more purified virus preparations are necessary.*

Transplantation tests with chemically induced tumors have demonstrated tumor-specific antigens (TSA) which do not protect against other chemically induced transplantable tumors (*1*). These antigenic specificities differ from those of antigens in virus-induced tumors where cross-reactivity is a common feature (*2*). Huebner and his associates demonstrated that cytoplasmic group specific antigens of the type C RNA virus were present in chemically induced tumors (*3–7*). Because of this it seemed feasible to undertake studies to determine whether type C RNA virus vaccines would inhibit induction of sarcoma by 3-methylcholanthrene (3MC) in BALB/c mice and C57BL/6 mice.

BALB/cCr mice were obtained from Microbiological Associates, Inc., Bethesda, Maryland, and C57BL/6Cum mice from Cumberland View Farms, Clinton, Tennessee. For virus preparations and viral titrations, newborn mice 24 to 72 hours old were used. Female mice, weanling (4 weeks old), were used for vaccination studies. Mice were housed, fed, and observed as described (*5, 6*).

Rauscher leukemia virus (RLV) (*8*) was partially purified and concentrated by the Moloney procedure (*9*) as modified by Huebner *et al.* (*10*) from infected BALB/c spleen tissue and resuspended in phosphate-buffered saline. The concentrated virus was titrated by the spleen antigen test in which BALB/c newborn mice were inoculated intraperitoneally with 0.05 ml per $\log_{10}$ dilution of virus; two litters were used for each dilution, and the spleens were harvested individually for half the mice at 21 days and for the remaining mice at 42 days; induction of infection was determined by testing the spleens in the complement-fixation test for the group specific (gs) antigen of type C RNA virus (*11*). Tests for both 21- and 42-day titrations indicated that the infectious dose (ID_{50}) was $10^{5.5}$ per milliliter. The virus was kept for 2 weeks at 4°C in formalin (final concentration of 0.1 percent), and inactivation was established by inoculating (intraperitoneally) undiluted viral

Table 1. Effect of inactivated type C RNA virus vaccine on 3-methylcholanthrene tumor induction in BALB/c and C57BL/6 mice. CI, Carcinogenic index; PBS, phosphate-buffered saline.

Treatment		Tumor			
		Incidence			
Vaccination	3MC* (μg)	Tu/T†	Percent	Average latency (weeks)	CI
		BALB/c mice			
None	None	0/24	0		
RLV + adjuvant	None	0/24	0		
None	150	21/27	78	14.8	75
PBS + adjuvant	150	22/29	76	14.0	77
RLV + adjuvant	150	12/24	50	15.6	46
		C57BL/6 mice			
None	None	0/25	0		
RadLV + adjuvant	None	0/27	0		
PBS + adjuvant	150	19/22	86	17.3	71
RadLV + adjuvant	150	9/27	33	17.8	27
Wild MuLV + adjuvant	150	10/27	37	16.3	33

* All mice received trioctanoin, the vehicle, whether or not they were given 3MC. † Tu/T, Number of tumors/total numbers of mice at risk for 5 months.

material into two litters of BALB/c mice; the spleens of all of these were devoid of gs antigen at 42 days. The COMUL (complement fixation of murine leukemia virus) tests on the inactivated vaccine material in BALB/c mouse embryo tissue culture also failed to demonstrate infectious virus (*14*). The inactivated virus was homogenized with an equal volume of Freund's complete adjuvant (Difco).

Transplants of lymphoma induced by radiation leukemia virus (RadLV) were removed 20 days after passage in C57BL/6Cum newborn mice, and the virus was purified and concentrated by the Moloney procedure (*12*). The concentrated virus contained $10^{3.3}$ ID_{50} per milliliter when titrated by the spleen antigen test in C57BL/6Cum newborn mice. Inactivation by formalin (as described above) was complete, as demonstrated by the spleen antigen and COMUL tests. The inactivated virus was homogenized with an equal volume of Freund's complete adjuvant.

A wild murine leukemia virus (MuLV) was isolated from a 3MC tumor induced in a C57BL/6J mouse. This virus did not produce tumors or leukemia; however, when injected intraperitoneally in newborn C57BL/6Cum mice, gs antigen was detected in the spleen and thymus tissues at 21 and 42 days. The spleens from 20 litters of mice infected as newborns were pooled at 42 days; the virus was purified and concentrated by the modified Moloney technique and gave an ID_{50} of $10^{3.3}$ per milliliter by the spleen antigen test in C57BL/6 newborn mice. The virus concentrate was inactivated with formalin and contained no infectious virus, as judged by results of inoculating C57BL/6 newborn mice and by the COMUL test. The inactivated virus was homogenized with an equal volume of Freund's complete adjuvant.

Mice were immunized by a single injection of 0.5 ml of vaccine given intraperitoneally at 4 weeks of age. Control mice received 0.5 ml of a placebo inoculation consisting of phosphate-buffered saline, Freund's adjuvant, and formalin (0.05 percent final concentration). The chemical carcinogen used in these studies was 3-methylcholanthrene (Eastman). Mice were injected subcutaneously in the intrascapular region with 150 μg of 3MC dissolved in 0.05 ml of trioctanoin (Eastman). For ascertaining that the vehicle had no activity, mice were given 0.05 ml of trioctanoin alone (*5*). The 3MC and trioctanoin were administered 4 weeks after vaccination when the mice were 8 weeks old. The mice were observed weekly for subcutaneous tumors at the site of inoculation for a period of 5 months. The tumor incidence, average tumor latency period, and carcinogenic index were computed in the manner described (*5, 7*) and are summarized in Table 1.

There was no significant difference in tumor incidence (> 0.45) between BALB/c mice receiving only 3MC and those given phosphate-buffered saline and Freund's adjuvant 4 weeks prior to 3MC treatment (Table 1). In BALB/c mice vaccinated with RLV in Freund's adjuvant the tumor incidence was significantly reduced from 76 to 78 percent to 50 percent ($P < .05$). There was no difference in the latency period; therefore the difference in the carcinogenic index is related to the reduction in tumor incidence. The data for the C57BL/6 mice vaccinated with RadLV or with a wild MuLV are summarized in Table 1. The differences in tumor incidence with C57BL/6 mice treated with 150 μg of 3MC in phosphate-buffered saline and Freund's complete adjuvant and vaccinated with RadLV or wild MuLV are highly significant ($P < .001$ and $P < .01$, respectively). The carcinogenic indexes for the vaccinated C57BL/6 mice, both for RadLV and the wild MuLV, are approximately half those for mice given the placebo. These differences are related to the reduction in tumor incidence, since the latency period was not affected.

Transmissible murine type C RNA leukemia viruses have been demonstrated in spontaneous and radiation-induced mouse lymphomas (*12, 13*) and in certain lymphomas induced by carcinogenic chemicals (*4, 14*); however, most sarcomas and other types of nonlymphomatous "solid" tumors occurring spontaneously or induced by chemicals have generally failed to yield transmissible RNA tumor-inducing viruses (*4*). However, the demonstrated high prevalences of the gs antigen expression of type C RNA viral genome in chemically induced tumors supports the concept that endogenous RNA virus derepression or activation provides a significant determinant of chemically induced cancer (*3–7*). The significant reduction in 3MC-induced sarcomas by type C virus vaccines provides additional corroborative evidence implicating the type C RNA viruses in the etiology of the chemically induced tumors. Each of three different murine leukemia viruses were shown to be effective vaccines: RLV has been used extensively in the study of experimental mouse leukemia, RadLV was isolated from radiation induced leukemia in the C57-BL/6 mouse (*12*), and the wild MuLV was isolated in our laboratory from a 3MC-induced tumor in a C57BL/6 mouse. The greater response to vaccine in C57BL/6 mice may be related to the well-known higher level immune responses (*15*).

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