work coordinated by WMO carries such information once daily soon after 1600 U.T. Many geophysical stations increase their programs or carry on special experiments during disturbed periods. Prompt notification of immediately significant geophysical observations and of major solar flare events, which have important and sometimes long-lasting geophysical effects, are also undertaken through the Regional Warning Centers.

The International World Day Service was established in 1958 by the International Council of Scientific Unions (ICSU) and is administered by the International Scientific Radio Union, 7, Place Emile Danco, Brussels 18, Belgium. This calendar has been drawn up by A. H. Shapley and J. V. Lincoln in consultation with interested unions and committees of the ICSU and representatives of the WMO (*2*).

A. H. SHAPLEY
Boulder Laboratories, National Bureau of Standards, Boulder, Colorado

References and Notes

1. Copies of the calendar are available upon request to the Secretary General, International Scientific Radio Union, 7, Place Emile Danco, Brussels 18, Belgium.
2. A more complete description of the calendar has appeared in the *U.R.S.I. Information Bulletin* and other widely available scientific publications. See also, A. H. Shapley, "International geophysical calendar for 1961," *Science* **132**, 1941 (1960).

2 November 1961

Vaccinia Dermal Infection and Methylcholanthrene in Cortisone-Treated Mice

Abstract. Cortisone-treated mice were inoculated with vaccinia virus, and then five paintings of methylcholanthrene were applied over the site of inoculation. In 70 percent of the mice, tumors developed at the site of inoculation, and 35 percent of the mice, with and without skin tumors, also developed lymphomas. Identically treated control mice that were vaccinia-immune developed a significantly lower incidence (38 percent) of both skin tumors and lymphomas.

F. Duran-Reynals (*1*) showed that in cortisone-treated mice inoculated with vaccinia virus into skin previously painted with methylcholanthrene (MC), tumors developed at the site of inoculation. In these experiments the mice received the following: first, ten MC paintings; next, cortisone to enhance the skin response to the virus, because mice are naturally resistant to vaccinia infection; and last, the virus, which was inoculated into the painted flank. Skin ulcers developed at the site of inoculation and healed within 3 weeks, forming a hyperplastic scar. Several weeks later tumors, frequently malignant, developed at the site of the scar in 66 percent of the mice.

Table 1. Different types of neoplasia in mice that received cortisone, vaccinia virus, and MC and in identically treated control mice that were vaccinia immune.

Neoplasia	Mice with neoplasia (No.)	
	Experimental	Control
Papilloma	5	2
Carcinoma	10*	3
Sarcoma	9†	6
Lymphoma	6	3
Total‡	30 (34)	14 (36)

* Two of this group also developed lymphomas. † Four of this group also developed lymphomas. ‡ The total number of mice included in each group is shown in parentheses.

The procedure was reversed in the present experiments. That is, cortisone-treated mice were first inoculated with the virus, and then MC was applied over the site of inoculation. Results similar to those previously reported (*1*) were obtained with much less MC. The tumors appeared much earlier, grew faster, and were mostly malignant. In addition, a high incidence of lymphomas was observed, particularly in the absence of skin tumors. Mazurenko (*2*) has shown that a high incidence of lymphomas develops late in life in mice inoculated with vaccinia virus shortly after birth.

We used the following materials and methods: noninbred albino female mice, 16 weeks of age; the Levaditi strain of neurovaccinia grown in rabbit testes (infective titer for the rabbit skin 10^{-9}); cortisone (cortone acetate) injected subcutaneously in the groin (1 mg in 0.1 saline daily for 5 days). On the day of the last cortisone injection, the virus or an extract from normal rabbit testes was inoculated intradermally in the flank (0.1 ml of a 1:10 saline suspension). Beginning 24 hours after inoculation, 1-percent 3-methylcholanthrene in benzene or benzene alone was painted over the shaved back and flanks daily for 5 days. The mice were observed until death, when routine autopsies were performed (*3*).

Nine groups of about 40 mice each were used in the experiment. The experimental group received cortisone, virus, and MC. The results from the control groups were as follows. Cortisone and virus together or alone, in the absence of MC, induced no significant changes in the mice. Cortisone and MC or MC alone, in the absence of the virus, induced an incidence of neoplasia significantly lower than in the experimental group. Essentially the same results were obtained in vaccinia-immune control mice that received cortisone and virus and, 1 month later, received more cortisone, a second virus inoculation, and MC.

To avoid repetition, only the results from the experimental and vaccinia-immune control mice will be summarized in detail. Skin ulcers developed at the site of virus inoculation in all the experimental mice. Beginning 3 weeks after treatment, in 50 percent of the mice the virus-induced lesions showed a series of changes, such as extensive, confluent hyperplasia followed by chronic ulceration, which led to the development of malignant skin tumors. In the remaining mice the virus lesions healed rapidly forming a hyperplastic scar. Skin tumors also developed at the site of the scar in 20 percent of the mice. Thus, 3 months after treatment skin tumors had developed in 70 percent of the experimental mice and in only 16 percent of the control mice. When the experiment was terminated, neoplasia—skin tumors and lymphomas—had developed in 88 percent of the experimental mice and in 38 percent of the control mice.

The number of mice with different types of neoplasia is shown in Table 1. The number of mice with carcinomas and lymphomas appears to be significantly lower in the control group. Lymphomas developed in 25 percent of the 24 experimental mice with skin tumors and in none of the 11 control mice with skin tumors. The small size of the control group may account for this difference. In the case of mice without skin tumors, however, the size of the experimental and control groups was approximately reversed, and lymphomas developed in 60 percent of the smaller group of ten experimental mice and in only 12 percent of 25 control mice. These results have been shown to be consistently reproducible.

The results suggest that the chance of malignancy may be significantly increased in a host exposed to MC during vaccinia infection. The role that nonspecific factors, such as the skin injury and tissue repair, may play in these results is being investigated (*4*).

M. L. DURAN-REYNALS*
BARBARA STANLEY
Department of Microbiology, Yale School of Medicine, New Haven, Conn.

References and Notes

1. F. Duran-Reynals, *Ann. N.Y. Acad. Sci.* **68**, 430 (1957).
2. N. P. Mazurenko, *Problems of Oncology* **6**, No. 6, 873 (1960).
3. The histological diagnoses were confirmed by Dr. Richard Siegler, assistant pathologist, Children's Hospital, Philadelphia, Pa., and visiting investigator, Institute of Microbiology, Rutgers University.
4. This work was supported by grants from the National Institutes of Health and from the American Cancer Society.

* Present address: Institute of Microbiology, Rutgers University, New Brunswick, N.J.

10 July 1961

Common Human Viruses as Carcinogen Vectors

Abstract. Single doses of pairs of viruses and organic carcinogens (in amounts too small in themselves to induce tumors) were administered to male Swiss mice free of polyoma virus. Malignant tumors developed in groups of mice injected with five of the carcinogen-virus pairs. Prior immunization against the virus of a pair prevented tumor formation by that pair. Carcinogen binding by poliovirus 2 was demonstrated in vitro.

It has been a continuing paradox in the field of experimental neoplasia that carcinogens strongly implicated in human tumorigenesis, though present in the human environment in only trace amounts, will ordinarily induce neoplasia in animals only when administered in relatively large amounts, or when given together with various physical or chemical "cocarcinogens" (*1*). Speculation on possible natural cocarcinogens led us to consider the role of common, nontumor viruses. Viruses are ubiquitous, often occur in family or household patterns, are most easily spread in urban environments, and with relative ease penetrate susceptible, nonimmune cells, and commonly, cell nuclei. This report (*2*) presents evidence of in vitro and in vivo interactions between common human viruses and chemical carcinogens; the results suggest a hypothesis that viruses may serve as natural vectors for the transport of otherwise innocuous amounts of environmental carcinogens (mutagens) to susceptible intranuclear chromosomal loci.

Studies were performed in vivo on male Swiss white mice (Webster strain), which were obtained from a colony proved free of polyoma virus (*3*) and which were reported to have a low incidence of *de novo* tumors (thymoma and lymphoblastic leukemia) (*4*). The viruses used (vaccinia, ECHO 9, Coxsackie B_4, and poliovirus 2) were harvested fluids of fully infected tissue cultures of monkey kidney; by the routes given, they evoked negligible mortality or morbidity. The carcinogens injected and their respective doses, judged to be too small to induce tumors (*1*), were: 9,10-dimethylbenzanthracene-1,2 (DMBA), 100 μg; 2-aminofluorene (AF), 100 μg; and 1,2,5,6-dibenzanthracene (DBA), 75 μg.

Each animal received a single dose, at the same time and in the same site, of two substances: (i) virus suspension, or frozen-thawed monkey kidney tissue culture cells, or tissue culture nutrient medium; and (ii) carcinogen or carcinogen solvent (acetone or propylene glycol). Randomized groups of 6 to 12 animals, 20 to 23 days old, were injected subcutaneously, intraperitoneally, or intranasally and dispersed in multiple cages; animals that survived for 12 months were killed. Cannibalism prior to 3 months of age was heavy, presumably because of overcrowded cages.

Lymphomas, myeloid leukemias, a reticulum cell sarcoma, and a subcutaneous fibrosarcoma—malignant tumors other than those reported to arise *de vovo* in this strain (*4*)—occurred in five groups of mice that received carcinogen-virus pairs, and in no other groups (Tables 1 and 2). The calculated probabilities (*5*) that the tumor incidences in these groups could have occurred by chance are: DMBA and vaccinia, 2.0 percent; DMBA and poliovirus 2, 0.7 percent; DMBA and Coxsackie B_4, 62 percent; AF and Coxsackie B_4, 16.5 percent; and AF and ECHO 9, 20 percent. When the chi-square test with Yates's correction is applied to all the data (Table 1), the probability that tumor incidence associated with the following conditions is due to chance alone is: virus, with and without carcinogen, $.05 > p > .01$; carcinogen, with and without virus, $.05 > p > .01$; virus plus carcinogen, $p < .01$. Four localized thymomas were found in the 161 mice alive after 3 months. Multiple pulmonary adenomas occurred in five mice that received DMBA intranasally, with or without virus.

Half of a group of 108 mice were immunized against vaccinia virus and half against frozen-thawed monkey kidney cells. Each was given a single simultaneous intraperitoneal or subcutaneous injection, as described above, of either vaccinia virus or frozen-thawed monkey kidney cells plus either DMBA or propylene glycol. The cages were not

Table 1. Results obtained by injecting mice with carcinogen-virus pairs. Data from two experiments are included.

Mice injected with carcinogen									Mice injected with carcinogen solvent		
DMBA*			AF			DBA					
No. with malignant tumors	No. alive at 3 mo.	No. injected	No. with malignant tumors	No. alive at 3 mo.	No. injected	No. with malignant tumors	No. alive at 3 mo.	No. injected	No. with malignant tumors	No. alive at 3 mo.	No. injected
					With vaccinia virus						
5	9	16	0	11	16				0	8	12
					With ECHO 9 virus						
0	11	22	2	7	12	0†	6	10	0	12	23
					With Coxsackie B_4 virus						
1†	13	22	2	6	12	0	4	10	0	14	23
					With poliovirus 2						
5	7	12				0†	6	12	0†	6	11
					With tissue culture cells						
0	8	13	0	8	12				0	7	12
					With tissue culture media						
0	6	10				0	6	10	0	6	10

* Pulmonary adenomas in DMBA groups: ECHO 9, 1; polio 2, 2; tissue culture cells, 1; tissue culture media, 1.
† One thymoma.

Table 2. Routes, tumors, and latent periods after injection of various carcinogen-virus pairs in mice. Abbreviations: i.p., intraperitoneal; s.c., subcutaneous; i.n. intranasal.

Carcinogen-virus pair	Route	Tumor	No. of mice with tumors	Latent period (days)
DMBA and vaccinia	i.p.	Lymphoma	3	152; 239; 344
DMBA and vaccinia	i.p.	Myeloid leukemia	1	202
DMBA and vaccinia	s.c.	Fibrosarcoma	1	168
DMBA and polio 2	i.n.	Lymphoma	4	249; 307; 307; 307
DMBA and polio 2	i.n.	Myeloid leukemia	1	241
DMBA and Coxsackie B_4	i.p.	Reticulum cell sarcoma	1	307
AF and Coxsackie B_4	i.p.	Lymphoma	2	151; 202
AF and ECHO 9	i.p.	Lymphoma	2	298; 298