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

overcrowded. After 8 months, the only tumors observed have been four lymphomas in a group of eight mice that were not immune to vaccinia virus and were given vaccinia and DMBA intraperitoneally. The probability that this incidence could have occurred by chance—in view of a group of nine immune mice that were similarly injected and had no tumors—is 2.9 percent.

Purified suspensions of poliovirus 2 ($10^{8.9}$ to $10^{9.9}$ monkey kidney $TCID_{50}$) and vaccinia ($10^{6.8}$ $TCID_{50}$) and of their respective nucleic acids were prepared (*6*), incubated at 37°C with solutions in acetone of DMBA-9-C^{14} (*7*) containing 2.7×10^{-3} to 5.4×10^{-2} millimicromole of DMBA per milliliter, and ultracentrifuged. Measurements were made with a gas-flow counter with a sensitivity of 431 to 437 counts per minute per millimicrocurie and a background of 26 to 27 counts per minute; a precision of 0.9 to 1.5 perecent was obtained with prolonged counting times. Binding of DMBA by whole poliovirus 2 significantly in excess of that by similarly purified suspensions of frozen-thawed monkey kidney cells was demonstrated. Three separate measurements of uptake, in molecules per $TCID_{50}$, were: 17,000 ± 5000 ($p < .01$); 20,000 ± 12,000 ($.05 > p > .01$); and 3100 ± 900 ($p < .01$). Significant binding of DMBA by vaccinia or by virus nucleic acids was not demonstrated.

The results obtained affirm in vivo interactions of viruses and carcinogens first described by Rous and Friedewald (*8*) and by F. Duran-Reynals (*9*) and since described further by M. L. Duran-Reynals (*10*). The results are also consistent with the report by Wisely *et al.* (*11*) of enhanced chemical carcinogenesis in mice repeatedly exposed to respiratory viruses.

Although the results suggest that common viruses may serve as carcinogen vectors, other interpretations of these interactions can be made. If such interactions occur in nature, it may prove possible to reduce neoplasia currently ascribed to chemical carcinogens by immunization against a virus (*12*).

CHRISTOPHER M. MARTIN
SIGMUNDUR MAGNUSSON*
PHILIP J. GOSCIENSKI†
GERARD F. HANSEN†

Division of Infectious Diseases, Department of Medicine, Seton Hall College of Medicine, Jersey City, New Jersey

References and Notes

1. P. Shubik and J. L. Hartwell, "Survey of Compounds Which Have Been Tested for Carcinogenic Activity," *U.S. Public Health Service Publ. No. 149, Suppl. 1* (1957).
2. This report was presented in part at the Multidisciplinary Research Forum of the American Medical Association, New York, 29 June 1961.
3. New Animal House, Taconic Farms, Germantown, N.Y. Kindly substantiated by W. P. Rowe.
4. C. Friend *et al., Proc. Am. Assoc. Cancer Research* **3**, 227 (1961); C. Friend, personal communication.
5. R. A. Fisher, *Statistical Methods for Research Workers* (Oliver and Boyd, Edinburgh, Scotland, ed. 10, 1946), p. 96.
6. J. J. Holland, *et al., J. Exptl. Med.* **110**, 65 (1959).
7. Nuclear-Chicago Corporation, specific activity 2.6 mc/mmole.
8. P. Rous and W. F. Friedewald, *J. Exptl. Med.* **79**, 511 (1944).
9. F. Duran-Reynals, *Ann. N.Y. Acad. Sci.* **54**, 977 (1952).
10. M. L. Duran-Reynals, personal communication.
11. D. V. Wisely *et al., Proc. Am. Assoc. Cancer Research* **3**, 278 (1961).
12. These studies were supported by U.S. Public Health Service research grants E-1695 (C1) and E-3257 and aided by a grant from the Tobacco Industry Research Committee. The assistance of Hugh G. Grady and Mary Lu Jacobs is gratefully acknowledged.

* Fellow of the Squibb Institute for Medical Research Foundation.

† National Institutes of Health student summer research fellow.

19 June 1961

Estradiol Stimulation of Glycine Incorporation by Human Endometrium in Tissue Culture

Abstract. The incorporation of C^{14}-labeled glycine into human endometrium grown in tissue culture is accelerated by the addition of estradiol-17β to the culture medium under certain experimental conditions. This effect is accompanied by an increased rate of disappearance of the glycine from the medium, and its demonstration is dependent upon the age of the culture and the frequency with which the medium is renewed.

Estrogens appear to influence the metabolism and chemical composition of "target organs" by increasing the rates of endergonic synthetic reactions. An enzyme system which is dependent upon minute amounts of estrogens has been isolated from several of these target organs. When activated by estrogen, it catalyzes the transfer of hydrogen from reduced triphosphopyridine nucleotide to diphosphopyridine nucleotide, which may result in increased energy to accelerate the synthetic activities of the cell (*1*).

Tissue culture techniques seemed to offer a useful system for further investigations of estrogen action. Human endometrium, which shows striking morphologic responses to estrogens, can be propagated in vitro in a variety of media (*2, 3*). Estradiol, when added to slices of endometrium in vitro, increases the rates of oxygen consumption and conversion of glucose and pyruvate to carbon dioxide. Human endometrium from different phases of the ovulatory cycle shows two peaks of oxygen consumption in vitro. These two peaks, at 6 to 10 and 22 to 24 days, correlate well with the known peak of concentration of endogenous estrogens in blood (*4*). Treatment of castrated rats with estradiol increases the subsequent rate of incorporation of radioactive glycine and formate into the protein, lipid, adenine, and guanine of surviving uteri (*5*). The estradiol-sensitive enzyme system has been identified in human endometrium (*6*). This paper is a preliminary report of the effects of estradiol on human endometrium in tissue culture.

The methods and media used were those devised by Peebles (*7*). Sterile specimens of endometrium were obtained by curettage, and sterile technique was used in all subsequent procedures. The tissue was washed once with Hanks' salt solution, then 20 ml of 0.1-percent trypsin solution was added to each specimen and the mixture was stirred rapidly for 15 minutes. The supernatant fluid was saved, and the remaining tissue fragments were treated twice more in a similar manner. The combined supernatant fluids were centrifuged at 600 rev/min for 10 minutes at 12°C. The sediment was resuspended in sufficient medium to give a final concentration of about 750,000 cells per milliliter. Each tube received 1 ml of this suspension and was incubated in a horizontal position without agitation at 35°C for the desired length of time.

The medium was composed of 5.5 parts of medium 199, 1.5 parts of beef embryo extract, 3 parts of calf serum, plus penicillin and streptomycin. Glycine-2-C^{14} and estradiol-17β were added to give final concentrations of $6.6 \times 10^{-7}M$ and $5 \times 10^{-6}M$, respectively. At the end of the growth period the medium was poured off, leaving the cells adherent to the tube wall. An equal volume of 10-percent metaphosphoric acid was added to each specimen of medium. The resulting precipitate was removed by centrifugation, and 0.1 ml of the supernatant fluid was pipetted onto stainless steel planchets, dried under an infrared light, and