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

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* Fellow of the Squibb Institute for Medical Research Foundation.

† National Institutes of Health student summer research fellow.

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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

Table 1. Effect of estradiol on incorporation of glycine into endometrium in tissue culture. The number of determinations made is shown in parentheses. Each sample was counted long enough to obtain a probable error of counting of less than 1 percent.

Growth	Cellular radioactivity per tube (count/min)	
	Control	Estradiol
1+	1.20 (10)	1.10 (4)
2+	1.00 (5)	
3+	1.80 (3)	2.61 (8)
4+	2.95 (2)	3.86 (5)

counted in a continuous gas flow windowless counter. The cells adhering to the tube walls were washed once with 0.9-percent sodium chloride and were then mixed with 10-percent metaphosphoric acid. The precipitate was centrifuged and transferred quantitatively, with the aid of a little water, to planchets and then dried and counted.

Attempts were made to culture nine specimens of endometrium. Four samples showed no evidence of viability after 5 days culture in the basic medium. One of these was from a postmenopausal woman, and the other three were obtained from premenopausal women shortly before their predicted menstrual periods. Of the five samples that did grow, all showed evidence of growth within 2 days. One of these was obtained 2 days after cessation of menstruation and 1 day after injection with a large dose of synthetic estrogen. Another specimen was from a patient with hyperplastic endometrium, and the other three were from women in the first 2 weeks of their ovulatory cycle. That tissue from the proliferative phase grows better than specimens from the secretory phase has been noted by other investigators (*3*).

The cultures, examined microscopically in the living state, exhibited great differences in the number of viable cells adherent to the walls of the tubes. Two basic cell types were often noted in the same tube (*3*). The greater number of cells had a long, spindle-shape, with processes which appeared to connect with other similar cells. Less often, scattered clumps of round or polygonal-shaped cells were seen.

In one incubation experiment, 43 tubes were initially planted. In addition to the basic medium, all tubes contained labeled glycine during the initial growth period; estradiol was added to 21 tubes. At the end of 3 days, there were all degrees of growth in both groups. The average amount of radioactivity per tube in the cellular material and in the medium was not significantly different in the two groups.

In another experiment three conditions were compared. The control set contained no added estradiol and the other two sets contained estradiol at concentrations of 10^{-7} and $10^{-6}M$. Each group of tubes was grown for 1 week in its own medium; then the old medium was replaced by fresh medium of the same type, and the tubes were left to grow for another week. At the end of this time, 8 of the 13 tubes containing the higher estradiol concentration showed complete cellular necrosis. All of the other tubes contained viable cultures. This prolonged exposure to estradiol appeared to bring about an early death of the cell cultures, but did not increase incorporation or utilization of glycine from the medium.

In a third experiment 40 tubes, grown in the basic medium for 3 days until growth was established, received fresh medium containing labeled glycine. Estradiol was added to half of the tubes at random, and the cultures were incubated for four more days. The degree of growth in each tube was judged by inspection and graded 1+ to 4+ (Table 1). The amount of glycine incorporated is correlated with the estimate of cellular growth, and in the two groups with greatest growth the estradiol significantly increased the incorporation. The mean for all tubes containing estradiol was 2.5 counts per minute while that for the control was 1.4 counts per minute. This difference is small, but it is statistically significant at the 1-percent confidence level (t test). The average radioactivity remaining in the medium in all of the tubes containing estradiol was 9.8 counts per minute, while the control value was 15.9 counts per minute. This difference is statistically significant at the 0.1-percent confidence level.

This experiment indicates that estradiol can increase the uptake of glycine from the medium and its incorporation into the cellular material of human endometrium in tissue culture. This effect is dependent upon the duration of contact between the tissue and medium, as shown by the second experiment. The results suggest that the method of tissue culture will be of value for future investigations into the mechanism of action of estrogens (*8*).

GARY L. ROBERTSON
DWAIN D. HAGERMAN
GEORGE S. RICHARDSON
CLAUDE A. VILLEE
Departments of Biological Chemistry and Surgery, Harvard Medical School, Boston, Massachusetts

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Action of Vasopressin on the Permeability of Mesentery

Abstract. Contemporaneous movements of Rb^{86} cations and of P^{32} orthophosphate across isolated rabbit mesentery display kinetic patterns that are generally associated with passive diffusion. Vasopressin at a concentration of 100 milliunit/ml produced a significant increase in the permeability constant for P^{32} and at the same time a significant decrease in the permeability to Rb^{86}. At lower hormone concentrations (0.1 milliunit/ml) the P^{32} response was less marked but still significant ($P < .01$), while the Rb^{86} effect was not ($P > .05$). Hyaluronidase did not mimic these actions of vasopressin.

The scientific literature is replete with studies on the actions of vasopressin (ADH) on transport systems. Ussing and Zerahn (*1*) demonstrated its stimulatory effect on the active sodium transport in frog skin and presented evidence for a similar action on the passive sodium flux. With the isolated urinary bladder of the toad (*Bufo marinus*) Leaf and Dempsey (*2*) showed that vasopressin increased active sodium transport, but they were unable to establish an effect on the passive flux. Sawyer (*3*) and others have explored the ability of this hormone to promote