Summary. Exposure to 400 r of whole-body X-radiation resulted in 100% mortality in 10 to 15 days of young male guinea pigs fed a basal diet of bran and oats plus ascorbic acid. Supplementation with cabbage or broccoli for 2 weeks before irradiation and during 30 days after irradiation significantly reduced mortalty. Lyophilized cabbage retained ability to reduce radiation mortality. Pre-feeding of guinea pigs to time of radiation exposure, signficantly delayed onset of death and enabled some animals to survive test period. Post-irradiation supplementation with cabbage also yielded a lower total mortality. Feeding of cabbage both before and after radiation exposure produced greatest amount of protection.

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Response of Ciliated Epithelia of Different Histological Origin to Virus Infections *in vitro.* (24644)

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We recently reported(1) that ciliated epithelia of the respiratory mucosa of man or monkey, exposed *in vitro* to polio- or adenoviruses, can maintain for relatively long periods their morphological and functional integrity. This phenomenon is especially evident in mixed cultures in which histologically organized epithelium is associated with dedifferentiated cells of the same species, such as HeLa or monkey kidney cells. These cells are selectively destroyed, leaving organized ciliated epithelium unharmed, in spite of high virus concentrations. The present experiments were performed to determine whether: (1) the observed phenomenon occurs also in ciliated epithelia of histological location other than respiratory tract, (2) this phenomenon can be demonstrated also with highly epitheliotropic viruses, such as herpes simplex virus, (3) the observed resistance to virus infections can be abolished by chemical or enzymatic treatment of ciliated cells.

Material and methods. Cultures of plasma imbedded rabbit or monkey trachea fragments were prepared and processed in living state or after fixation and staining, according to previously described methods(1). Cultures of rabbit Fallopian tube fragments were cut from upper convoluted oviduct, including its fimbriated extremity. Human ovarian tubes were obtained by total hysterectomy in 5 cases of uterine cancer on patients 40-56 years of age. No histological evidence of malignancy was present in the Fallopian tubes. Only fimbriated extremity of human oviducts was used for explantation. In certain experiments HeLa cells or 2nd and 3rd passage rabbit kidney cells were added to cultures of Fallopian tube fragments as previously described(1). Culture medium contained 0.5% lactalbumin hydrolysate in Earle's solution, supplemented with 0.1% yeast extract, 15% decomplemented horse serum and 3% bovine embryo extract. Poliovirus type 1 Mahoney strain, adenovirus type 1 "Ad 71" strain,† tissue culture adapted strain of Mengo encephalomyelitis virus(2), "5433" strain of herpes simplex virus(3), infectious bovine rhinotracheitis virus (I.B.R.)‡ and a strain of vaccinia virus isolated from commercial calf lymph vaccine, were used. Virus titrations were performed by serial dilutions in tube cultures of trypsinized rabbit kidney cells for herpes simplex virus and HeLa cells for other viruses.

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TABLE I. Behavior of Human Fallopian Tube and HeLa Cell Cultures Infected with Different Viruses.

Tissue culture	Age of culture*	Virus	ID_{50} introduced per culture	Max time of survival after infection	Virus titer attained† ID_{50}
Org. C. Ep.	48 hr	Polio	10^3	7 days	$10^{4.5}$
" ‡	"		"	6	10^6–10^7
Gr. Ep.‡	"		"	3	"
HeLa	"		"	48 hr	10^7
Org. C. Ep.	4 days		10^6	>7 days	
"	Fresh		10^5	9	$>10^5$
"	3 days	Mengo	"	>8	
" ‡	8		"	"	10^6
Gr. Ep.	"		"	6	"
HeLa	3		"	48 hr	$10^{7.5}$
Org. C. Ep.‡	4	I.B.R.	10^4	>10 days	10^5
"	13		"	"	
HeLa	3		"	4	"
Org. C. Ep.	3	Adeno type 1	"	12	
Gr. Ep.	"		"	5	
HeLa	"		"	3	

Org. C. Ep. = Organized ciliated epithelium present on explanted fragments.
Gr. Ep. = Dedifferentiated epithelium of outgrowth area.
* At time of virus introduction.
† Per ml of supernatant at time indicated as maximum survival.
‡ Mixed Fallopian tube and HeLa cultures.

Results. General aspect of Fallopian tube cultures. In most explanted fragments of adult rabbit and human oviducts, surface sheets of ciliated epithelium maintained characteristic histological structure and coordinated, vibratory activity usually for 20-25 days. In some fragments it lasted 40 to 50 days after explantation. The outgrowth, composed chiefly of confluent sheets of epithelial cells, could be maintained in culture for several months and in some cases produced permanent cell lines.§ Isolated spots of ciliated cells were present frequently in the outgrowth, but their vibratory activity was less persistent than in fragments.

Human oviduct cultures infected with polio and other viruses. Table I shows the behavior of human oviduct cultures, and of HeLa cell cultures, infected with polio, Mengo encephalomyelitis and adenoviruses. As a general rule, organized ciliated epithelium reacted to virus infections, if at all, only after considerable delay as compared with dedifferentiated HeLa cells or cells present in outgrowth area. This difference was particularly conspicuous in mixed cultures where a perfectly active ciliated epithelium could be observed many days after complete destruction of all dedifferentiated cells.

Rabbit oviduct cultures infected with herpes simplex and other viruses. The "5433" strain of herpes simplex virus (105th-108th *in vitro* passages in rabbit kidney cell cultures) when introduced in concentrations of 10^4-10^5 ID_{50}/ml in dedifferentiated rabbit epithelial cell cultures, caused lesions characterized by generalized giant cell formation§ and specific nuclear changes resulting in complete destruction of cultures in <48 hours. Fragments of rabbit Fallopian tubes incubated 1 hour with 10^4 ID_{50}/ml virus suspension, then washed and explanted, showed for first 3 days apparently normal growth and cell migration. On 4th day specific cell degeneration started at periphery of epithelial outgrowth and became generalized on 5th day. However ciliary activity continued undisturbed until the 6th day. At this time in fixed preparations, specific herpes nuclear lesions could be seen in all cells of outgrowth area, in some cells of epithelial lining of the fragment, among them an occasional ciliated cell. In other experiments, rabbit Fallopian tube cultures grown in association with homologous dedifferentiated kidney cells, were infected with virus after 2, 5,

§ Unpublished data.

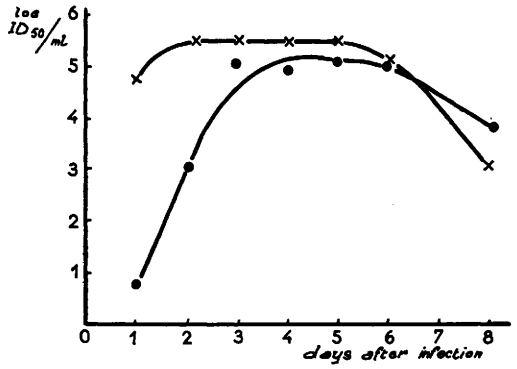

FIG. 1. Herpes virus concentration in supernatant of infected rabbit kidney dedifferentiated cell cultures (—×—) and cultures of rabbit Fallopian tube fragments (——●——). Non-adsorbed virus eliminated by washing at time 0.

7 or 10 days of preliminary culture. In these cultures outgrowth and associated cells were entirely destroyed in <3 days after infection. This was accompanied in every case by sustained virus titer in the medium of 10^5-10^6 ID_{50}/ml. In all these cultures ciliary activity could be observed for at least 48 hrs after complete destruction of the nonorganized growth. Later, the ciliated lining epithelium was progressively invaded and destroyed. Similar observations were made on rabbit trachea cultures. Comparison of curves of virus liberation in trypsin dispersed rabbit kidney cell cultures and cultures of rabbit Fallopian tube fragments (Fig. 1) shows that the virus was produced in both cases, but reached maximum levels much more rapidly in dedifferentiated kidney cells.

Rabbit oviduct cultures infected with vaccinia behaved in a similar way with herpes virus.

Virus action on chemically or enzymatically pretreated ciliated cells. It is possible that mechanical activity of cilia is by itself able to disturb fixation and penetration of virus into the cells. To check this possibility we tried, before bringing the cells in contact with the virus, to stop ciliary activity in a reversible way with appropriate anesthetics. Nembutal (Na methyl-butyl-barbiturate) acting in concentrations of 1.0 to 1.5 mg/ml during 1 hour stopped almost entirely the ciliary movement without permanent damage to either cells or cilia, which resumed their normal activity after elimination of the product.

Experiments with human Fallopian tube cultures pretreated with Nembutal, were then infected by incubation with a suspension containing 10^6 ID_{50}/ml of poliovirus. During 8-day observation no difference in response to virus infection could be found between normal and Nembutal-pretreated cultures. In other experiments tissue fragments were pretreated with enzymes to eliminate from their surface hypothetic substances which could interfere with the intimate cell-virus contact. Thus monkey trachea and human Fallopian tube fragments were submitted 30 min. at 37°C to 20 t.u./ml of bovine hyaluronidase‖ or treated similarly with 0.25% buffered (pH 7.5) solution of trypsin. The tissue fragments were cultured *in vitro,* then infected with poliovirus and observed. Hyaluronidase pretreated cultures behaved exactly as did untreated controls.

The trypsin solution acting on rabbit or human Fallopian tube fragments caused detachment of the lining sheets of ciliated epithelium, and a suspension of ciliated cells, single or in small groups, was obtained. These cells remained alive and active for at least 4 days in microdrops of medium placed on bottom of Petri dish under a 3-4 mm sheet of liquid paraffin.¶ When infected by incubation with 10^5 ID_{50}/ml of poliovirus suspension, and then washed with specific antipoliovirus serum, most of these trypsin pretreated cells maintained their ciliary activity as long as non-infected controls (4 days). Virus production in the microdrops containing ciliated cells could not be detected until 4th day. HeLa cells prepared and infected concurrently under same conditions, were all destroyed in <48 hrs. This was accompanied by virus production evidenced in droplets at end of this period.

Discussion. Resistance to the cytopathic action of viruses *in vitro,* observed previously on tracheal and bronchial ciliated epithelium (1), could be regarded possibly as specific defense adaptation of the respiratory tract mu-

‖ Preparation supplied by Pasteur Inst. (Paris).

¶ These were prepared according to technic used by Dr. John Paul, Glasgow.

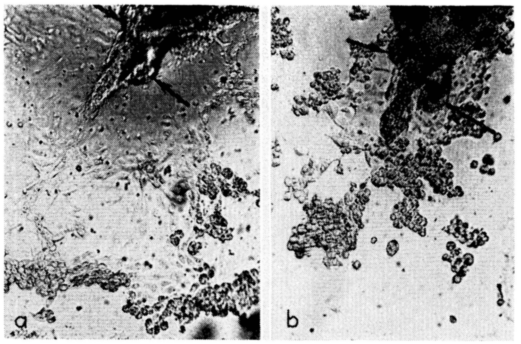

FIG. 2. 4-day-old rabbit Fallopian tube culture (a) 24 hr and (b) 48 hr after infection with herpes virus. Centripetal progression of lesions in outgrowth area and a persistent zone of ciliary activity (arrows).

cosa. However in view of above observations, this phenomenon appears as a more general one, since it can be observed as well in Fallopian tube ciliated epithelium infected with different viruses.

It must be emphasized that we are actually observing only a *relative* resistance, since even differentiated ciliated epithelium in most cases finally succumbs to virus infection. For each tissue virus combination a well defined gap exists between time of disorganized tissue destruction and attack on organized ciliated epithelium by a given virus. For I.B.R. virus and human Fallopian tube cultures f.ex., we never observed a definite action of the virus on ciliated cells, in spite of its rather strong cytopathic effect on human dedifferentiated epithelium *in vitro*. For human oviduct cultures infected with poliovirus, there were 5 to 6 days between onset of degeneration of dedifferentiated cells and cessation of ciliary activity. While in herpes infected rabbit Fallopian tube cultures the ciliated epithelium survived only 2 to 3 days longer than the non-organized cells.

In studying response of organized tissue cultures to virus infections, Bang and Niven recently reported(4) negative results with the common cold and influenza viruses in human embryo respiratory tract mucosa cultivated *in vitro*. In a similar system adult ferret nasal mucosa and embryonic chick skin were destroyed when infected with influenza, vaccinia or herpes viruses. However these authors did not specifically compare the cytopathic effect and virus production in organized and dedifferentiated tissues.

An apparent contradiction exists between relative resistance of ciliated epithelium to virus infections *in vitro,* which we demonstrated, and observations on presence of degenerated ciliated cells in bronchial secretions of animals(5) or man(6) infected with respiratory viruses. Our observations support the evidence that at least in many instances, dedifferentiated normal or cancer cells in tissue culture, are more sensitive to virus infections than organized tissue cells even when these represent typical pathological location of a given virus infection *in vivo*. Studying relative sensitivity of brain and dedifferentiated visceral tissues of hamsters to encephalomyelitis viruses, Kissling(7) came to a similar conclusion.

One could suppose that the belated response of ciliated epithelium to action of cytopathic viruses *in vitro* is due to a period of "maturation" or metabolic transformation after explantation *in vitro* necessary for appearance of virus receptivity. It is however remarkable that the delay between introduction of virus and arrest of activity in ciliated, organized sheets of epithelium, did not seem to depend either on age of culture or on chemical or enzymatic treatment of cell surface. Another possibility is normal penetration of virus in differentiated ciliated cells followed by slowed down virus development in these cells. This might not interfere immediately with normal activity of cell organelles such as cilia. As a matter of fact poliovirus fixation and development could be demonstrated by us in pure suspension of fresh trypsinized human oviduct ciliated cells followed by virus production markedly delayed, as compared with that in similar conditions with dedifferentiated cells.

The particular behavior of ciliated epithelial cells with regard to virus infection *in vitro* can be compared with observations on peritoneal exudate cells and poliovirus(8), on human embryo heterografted skin infected with the same virus(9), on different chick cells infected with avian myeloblastosis virus(10) and many others. In general, one should not expect to find uniform pattern and timing of viral infectious processes in animal cells, even

for a specific species - virus combination. With the variety of highly differentiated and specialized cells of the animal organism, one must anticipate rather a multiplicity of cell - virus reactions, much wider than that established for monocellular organisms and their viruses.

Summary. 1) Adult human and rabbit Fallopian tube fragments, which maintained in *in vitro* cultures their specific histological structure and coordinated vibratory activity, were submitted to infection with polio, adeno, bovine rhinotracheitis, herpes and other viruses. 2) The organized ciliated epithelium when compared with dedifferentiated epithelium in the same cultures showed in all cases a relative resistance to the cytopathic action of viruses, manifested by a marked delay in cell destruction, more pronounced in the case of bovine rhinotracheitis or polio and less marked in the case of herpes or vaccinia viruses. 3) This resistance could not be abolished either by immobilization of cilia with anesthetics or by treatment of cell surface with hyaluronidase or trypsin. 4) The meaning and implication of these data are discussed.

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Effect of Parathyroidectomy on Distribution of Radiocalcium. (24645)

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Tracer experiments with injected Ca^{45} have demonstrated rapid exchange between blood and bone calcium which can vary with age and with the metabolic state of bone(1,2). It has been suggested that parathyroid hormone might directly affect the amount of "exchangeable bone calcium"(3), but recent evidence indicates that the hormone can act on that portion of bone unavailable for rapid exchange(4,5). While it appears established that parathyroid hormone directly increases effective solubility of bone mineral(6), it is not certain how this effect might influence the amount of bone calcium available for rapid exchange. In the present study the effect of parathyroidectomy on the early exchange of a tracer dose of Ca^{45} in rats was examined to shed some light on this question.

Methods. Distribution of a tracer dose of Ca^{45} was studied in groups of young (70 to 82 days) and old (289 to 302 days) male Holtzman rats. In 3 groups of young rats, Ca^{45} was given at intervals of 1 day, 6 to 8 days, and 28 to 30 days after parathyroidectomy or after sham operation. In one group of old rats, Ca^{45} was given 7 days after parathyroidectomy or sham operation. Parathyroidectomy was performed by electro-cautery under nembutal anesthesia. In sham operations the parathyroids were identified but not cauterized. In each animal distribution of radioactivity was measured 6 hours after intraperitoneal injection of a tracer dose of 3 to 6 μc of Ca^{45} with less than .005 mg of carrier calcium dissolved in 0.5 ml of normal saline. Plasma radioactivity was measured in samples of tail vein blood obtained 2 and 4 hours

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