definitely in a state comparable to the "carrier state" obtained in many other animal cell-virus systems.[7, 8]

The mechanism by which the persistent release of *sp* virus arises seems completely distinct from the mechanism by which the PY virus causes the neoplastic transformation. For this reason, the mode of production of *sp* virus does not illuminate the mechanism of neoplastic transformation.

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*THE SPARING EFFECT OF RMC POLIOVIRUS ON PRIMARY AMNION CELL CULTURES**

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In previous communications,[1, 2] one mechanism whereby cell cultures persist in the presence of an ordinarily cytopathogenic virus was described. Viral inhibitory factor (VIF), probably a variety of interferon,[3] was shown to account for the diminished cytopathic effect (CPE) of a Type II MEF_1 poliovirus variant adapted to the chick embryo (RMC virus[1, 4]) in human amnion cultures receiving undiluted inocula as compared to the complete CPE in those that received diluted inocula. However, it was pointed out[2] that VIF could *not* explain all forms of resistance of amnion cultures to RMC virus, such as that of amnion cells of young *in vitro* age.[5] Further studies of certain resistant cultures have shown that not only is initial CPE overcome but their prolonged survival may be favored.

Materials and Methods.—Primary amnion cultures were prepared as previously described[2] except that instead of Enders' medium containing 5 per cent horse serum, 5 per cent beef embryo extract, 45 per cent bovine amniotic fluid, and 45 per cent Hank's balanced salt solution (BSS), Eagle's basal medium in BSS modified to contain 10 per cent horse serum and antibiotics was used. Cells were grown on the glass surface of 16 mm test tubes containing 1 ml medium. RMC virus used in these studies was from a stock chick embryo passage or from an amnion culture passage. The latter was collected from cultures of amnion cells in Roux bottles two days after stock RMC virus at input multiplicity of about 1 was inoculated. Such harvests also contained VIF.[2]

Results.—In routine titrations of RMC virus, it was customary to use amnion cultures of at least two weeks *in vitro* age and to roll them after inoculation in order to elicit viral CPE.[1, 5] Previously,[1] using amnion cultures nourished with Enders' medium under these conditions, stock RMC virus produced complete CPE.[1] However, using cultures prepared under our present conditions, complete CPE of RMC virus was not consistently observed unless chick embryo extract was added.[6] Thus, in Table 1, typical titrations of three specimens employing amnion cultures one month after they were initiated are presented. Although RMC virus from both a stock culture and from amnion passage developed typical CPE in 3–5 days, this was only partial in character. With weekly to biweekly changes of Eagle's medium, all cultures exhibiting characteristics of infection had recovered in about one month and appeared healthier and more confluent than controls. Furthermore, the cells in such cultures gradually changed in morphology, so that compared to the usual polygonal amnion cells, they were longer and more ellipsoidal and hyaline. They arranged themselves frequently in whorls, and the edges of the culture were smoother than uninfected controls. When samples of centrifuged VIF from which infective virus was removed (see Table 1) were inoculated in amnion cultures or when

TABLE 1

PROGRESSION OF CELLULAR CHANGE IN HUMAN AMNION CULTURES INFECTED WITH RMC VIRUS

Source and dilution of inoculum (0.1 ml)		Days of observation 3–5	15	30	60–120
Stock RMC Virus	10^{-0}	1+	±	sO	C
	10^{-1}	2+	±	O	C
	10^{-2}	1+	±	C	C
	10^{-3}	1+	±	sO	C
	10^{-4}	0	P	—	—
Amnion Passage RMC Virus	10^{-0}	0	O	sO	C
	10^{-1}	±	O	O	C
	10^{-2}	±	O	sO	C
	10^{-3}	1+	O	sO	C
	10^{-4}	±	O	P	C
	10^{-5}	0	P	P	PC
	10^{-6}	0	P	P	P
Centrifuged* VIF	10^{-0}	0	P	P	F
	10^{-1}	0	P	P	P
	10^{-2}	0	P	P	PC
	10^{-3}	0	P	P	—
	10^{-4}	0	P	P	F
	10^{-5}	0	P	P	F
No inoculum		0	P	P	P

* Amnion passage material was centrifuged x2 at 104,000 *g* for 90 minutes.
± = definite but minimal viral CPE; 1+ = CPE, cc 25% cells destroyed; 2+ = CPE, cc 50% cells destroyed.
O = confluent sheet of normal polygonal cells; P = cells scattered, granular, and poor; sO = small patches of normal-appearing cells; F = no viable cells visible; C = confluent sheet of "changed" cells.
PC = poor culture with some areas of "changed" cells.
— = not observed.

uninoculated cultures were kept as controls, no initial viral CPE developed and no such cellular changes were evident in most instances. As expected, the culture receiving undiluted amnion passage virus showed no initial CPE due to the presence of VIF,[1, 2] although the progress of this culture corresponded closely to those that showed early viral CPE. Whether the eventual appearance of "changed" cells in two other cultures described in the table which showed no initial CPE is due to undetected viral effect is at present undetermined. A similar cell-sparing effect consisting of repair of cell damage and appearance of cells of altered morphology

comparable to that described has been repeatedly observed after about 10^6 TCD_{50} RMC virus were inoculated in amnion cultures less than two weeks old and incubated in a roller drum or left stationary.

Some preliminary experiments have been undertaken to elucidate the role of RMC virus in producing this sparing effect.

1. To compare cell multiplication in infected and in control amnion cultures, 20 tube cultures were initiated with a cell suspension containing about 400,000 cells per ml. To each of ten cultures a 0.1 ml inoculum of about 10^6 TCD_{50} RMC stock virus was added, and ten control cultures received 0.1 ml of uninfected chick embryo material. At intervals ranging from two days to two weeks, culture fluids were collected for virus titrations, and the number of cells adhering to the glass surface of each culture was determined by counting in a hemocytometer after they were removed with trypsin. The cell count in both the infected and control cultures was about 45,000 two days after initiation of the cultures and it reached a maximum of about 120,000 in 12 days. After $1^1/_2$ months, however, the inoculated cultures were confluent and healthy in appearance, whereas the cells in the controls were in nonconfluent patches. The cell counts were 70,000 and 50,000 respectively. It appears from a number of such experiments that the presence of virus probably has no accelerating effect on cellular multiplication, but it somehow renders the cells relatively resistant to spontaneous degeneration.

2. To determine the extent and presence of virus in inoculated amnion cultures, representative samples of fluids from cultures used in the experiments described in the above paragraph and in Table 1 were titrated by inoculating 0.1 ml of serial 10X dilutions in amnion cultures, which in parallel experiments were shown to be satisfactory in titrating a stock RMC virus specimen. Similar titrations were carried out in 2–4 day suckling mice, which received 0.02 ml of a dilution intracerebrally. Intracellular virus was released by freezing and thawing a cell suspension 3X. RMC virus was only sporadically detected in these persistent cultures. For example, no virus was detected by using amnion cultures as a test system in the fluid of cultures 1 and 4 months after they had received 10^{-1} stock RMC virus. Parallel tests of fluids from a culture that received 10^{-1} amnion passage virus were similarly negative. However, four months after a culture had received 10^{-2} amnion passage virus, death was produced at 10^{-1} dilution in suckling mice when both fluid and cells were tested together for viral activity. It appears that the altered state of amnion cultures inoculated with RMC virus is accompanied by little if any multiplication of infectious virus. However, the importance of small amounts of virus, which may not always be detectable under the described conditions, is not precluded.

3. To ascertain the role of virus in the fluid phase of these persisting cultures, monkey antiserum against Type II poliovirus previously shown to be sufficient to neutralize infective virus (1:50) was added to two persistent cultures $3^1/_2$ months after they had received RMC virus. Observation for three weeks failed to reveal any difference between these cultures and other persistent cultures without antiserum, showing that the presence of infective virus in the fluid phase is probably not essential for the persistence of the cultures.

Comment.—It appears that when the ordinarily cytocidal RMC virus is added to primary amnion cultures under various conditions, one may observe not only

a spectrum of cell destruction ranging from complete to absent CPE but also a paradoxical sparing effect on the cultures. Further studies are required to ascertain the genetic and nutritional conditions underlying this phenomenon. This sparing effect has some superficial resemblance to that of certain tumor viruses on cell cultures, but there is as yet no evidence that the cells spared have undergone genetic transformation.

Summary.—Primary human amnion cells cultured under defined conditions were inoculated with a variant of Type II poliovirus and observed for over four months. The initial cytopathic effect was overcome, and gradually confluent sheets of healthy-appearing cells of altered appearance developed. In contrast, uninoculated control cultures underwent gradual spontaneous degeneration.

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*REGULATION BY COLIPHAGE LAMBDA OF THE EXPRESSION OF THE CAPACITY TO SYNTHESIZE A SEQUENCE OF HOST ENZYMES**

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A small group of closely linked genes specifying the enzymes of galactose metabolism is capable of being transduced by the coliphage lambda.[1] A region of the bacterial chromosome or a replica thereof encompassing the transduced genes is inserted into the genome of the transducing phage, replacing a portion of the viral genetic material.[2–4] This relatively stable situation makes possible investigation of the expression of bacterial genes situated in a foreign environment, i.e., within the genome of a prophage. Upon inducing the replication of transducing phage, cells may be obtained prior to lysis, each possessing several copies of a short region of bacterial genetic material. The present work was originally undertaken to investigate the position and gene dosage effects afforded by a study of *Escherichia coli* harboring transducing lambda. Its course has been altered somewhat by an unexpected result obtained in a control experiment using *E. coli* lysogenic for normal lambda alone. Following induction of phage replication, the activity of an enzyme of galactose metabolism was found to increase whether or not the capacity for synthesis of this enzyme, galactose-1-phosphate uridyl transferase, was carried by the phage. Increases in enzyme activity, apparently due to increased dosage of transduced genes, must be interpreted in the light of the increases in