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Susceptibility of Human Lymphoblasts (RPMI 7466) to Viral Infections *in Vitro* (33638)

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Since the reports of Pulvertaft (1) and Epstein and Barr (2) on the successful serial culture of lymphoblasts from Burkitt's human lymphoma, several other lymphoblast cell strains derived from Burkitt's lymphoma (3–5) and from normal (6, 7) or leukemic (8–11) peripheral blood have been described. These cell strains were of particular interest because of their infection with a herpes-type virus (12) and because of the possible association of this virus with the human malignancies from which the cells were derived.

Little is known of the general viral susceptibility of human cells of this type. Attempts have been made in this laboratory to isolate viruses from sera of persons with infectious hepatitis, using a strain of lymphoblasts (RPMI 7466) derived from normal human peripheral blood (6). In these studies, this strain of lymphoblasts was found resistant to the cytopathic effect (CPE) of some viruses which normally replicate in human cells in tissue culture. These cells were capable however, of supporting persistent viral infections in most instances, and an investigation of cell–virus relationships in lymphoblasts infected with poliovirus revealed some unusual features. Results of these studies are presented here.

Materials and Methods. Tissue cultures. Human lymphoblasts (strain RPMI 7466), derived from peripheral blood of a person without malignant disease (6), were obtained from Dr. George Moore, Roswell Park Memorial Institute. These cells, which consist predominantly of immature lymphoid cells, and a few large multinucleate cells and mature lymphocytes, multiply freely in the fluid medium of agitated or stationary suspension cultures.

Cells were propagated in stationary Blake culture bottles[1] in Moore's 1640 medium (6), supplemented with 1% by volume of bovine fetal serum and the following per liter:

[1] Corning Glass Cat. No. 1285.

Bactopeptone, 1.0 g; human serum albumin, 2.5 g; folic acid, 3.5 mg, and chlorotetracycline 25 mg. Cultures used in experiments were prepared in 5- or 10-ml vials and contained 1.5–3.0 ml each of 30×10^4 viable lymphoblasts/ml. Cell viability was determined by direct microscopic count, in a hemacytometer, of the number of cells unstained by eosin (13).

Human diploid lung fibroblasts strain Led-130 were propagated as previously described (14). The 48-hr monolayer cultures in 16×150-mm tubes were replenished with 1.8 ml of the above medium immediately before use in viral assays. Cell cultures were stoppered and maintained at 37°.

Viruses. Polioviruses were the prototype strains, type 1 (Mahoney), type 2 (YSK) and type 3 (Leon). These were propagated and titered for infectivity in rhesus monkey kidney (RMK) cell cultures; types 1 and 2 titered $10^{7.0}$ and type 3 titered $10^{6.0}$ tissue culture infectious doses ($TCID_{50}$/0.1 ml. *Vaccinia* was the commercial calf lymph vaccine propagated on the chorioallantoic membrane of chick embryos and titered $10^{6.5}$ $TCID_{50}$/0.1 ml in Led-130 cell cultures. *Vesicular stomatitis* (VSV) was the Indiana strain propagated and titered in chick embryo cell culture, and contained $10^{5.0}$ $TCID_{50}$/0.1 ml. *Herpes simplex* (HSV) was a 10% mouse brain homogenate which contained 10^4 lethal mouse doses/0.1 ml. Later titrations were performed in Led-130 cell cultures. *ECHO-11* (Gregory strain) was propagated and titered in Cercopithecus monkey kidney (CMK) cell cultures and contained $10^{6.5}$ $TCID_{50}$/0.1 ml. *Human adenovirus type 5,* obtained from the American Type Culture Collection, was propagated and titered in Led-130 cell cultures and contained $10^{5.0}$ $TCID_{50}$/0.1 ml. *Reovirus type 1* (Lang strain) was propagated and titered in Led-130 cell cultures and contained $10^{5.0}$ $TCID_{50}$/0.1 ml. Rhinovirus type 2 (HGP Salisbury strain) was propagated and titered in Led-130 cell cultures and contained $10^{4.0}$ $TCID_{50}$/0.1 ml. *Rubella* virus was the M33 strain of Parkman *et al.* (15) further attenuated in these laboratories (16). It titered $10^{3.3}$ $TCID_{50}$/0.1 ml in CMK cell culture by the ECHO-11 virus interference test. *Mumps virus* (Jacobson strain) was isolated and adapted to chick embryo cell culture in these laboratories (17). It titered $10^{5.2}$ $TCID_{50}$/0.1 ml by hemadsorption test in chick embryo cell culture. *Newcastle disease* virus (NDV) was the MD strain which had been propagated alternately in cell cultures of Yoshida rat tumor cells and chick embryo; the titer in chick embryo cell culture was $10^{6.0}$ $TCID_{50}$/0.1 ml. *Parainfluenza virus* type 3 (Strain 64-2389) was isolated by Dr. J. Conchola of the Children's Hospital of the District of Columbia, and was adapted to passage in the allantoic cavity of the chick embryo by Dr. P. H. Frickey of these laboratories. The infected allantoic fluid used contained 4000 hemagglutinating units/0.5 ml.

Viral susceptibility tests, viral assays. Either 0.1 ml of undiluted or a 10-fold dilution of virus was inoculated into cultures of 7466 lymphoblasts or Led-130 cells; 3 cultures of each cell strain were inoculated with each virus dilution. All cultures were maintained at 37° for 2 weeks, and where persistent infections were established in 7466 lymphoblasts, for 1–7 months. The Led-130 monolayer cultures received a medium renewal each 5 days; 7466 lymphoblasts received a medium renewal each 3–4 days and were subcultured once each week. At this time, lymphoblast suspensions were centrifuged at 1000 rpm for 10 min, resuspended in twice the original volume of medium and subdivided among fresh culture vials.

Cultures were examined each 2–3 days for CPE; Led-130 cultures were read by direct microscopical examination of the culture tubes, 7466 cultures by determination of cell viability in the presence of eosin. Culture fluids were collected each 3–4 days and assayed for virus infectivity (see "Viruses"). Virus titers were calculated by the method of Reed and Muench (18).

Plaque assays of cell suspensions from polio-infected cultures were performed on RMK monolayers in 60-mm plastic dishes.[2] A 0.2-ml volume of each dilution to be tested

[2] Falcon Plastics Division of Becton, Dickinson Co., Los Angeles, California.

was inoculated onto washed cell monolayers and incubated at 37° for 30 min, followed by 1 hr at room temperature. One-half ml of warm (43°) "Lapagt" (19) medium, containing agar at 0.9% and neutral red at 0.005% was added to each dish. When this shallow layer had hardened, 4.5 ml of the same agar medium was added and plates were incubated at 37° in an atmosphere of 5% CO_2 in air. They were examined for necrotic areas (plaques) 6 days later.

Disruption of polio-infected lymphoblasts was accomplished by subjection of cell suspensions to three successive 30-sec periods of ultrasonication at 0 to 4° by means of a 60-cycle Blackstone ultrasonic probe, model BP-2.

Chromosome analysis, mitotic synchrony. The 7466 lymphoblasts were prepared for chromosome analysis essentially as described by Moorhead *et al.* (20). Preparations were stained with aceto-orcein and examined under a 40× objective; well-spread metaphases were projected for analysis by means of a camera lucida.

Mitotic synchronization of cultures was induced by incubating cell suspensions at 37° with colcemide at 0.06 μg/ml as described by Stubblefield and Murphree (21).

Assays for interferon. Tissue culture fluids from control and virus-infected human lymphoblast cultures were acidified at pH 2.0 with HCl, stored 4 days at 4°, adjusted to pH 7.0 with NaOH, subjected to low speed centrifugation and then centrifuged twice for 2 hr at 100,000 *g*. These supernatants were assayed on monolayers of Led-130 cells in 16 × 150-mm tubes. One and one-half ml of undiluted fluids or fluids diluted 1:2 or 1:4 with medium without serum were left in contact with the cells for 18 hr. The cultures were then washed with Hanks' BSS (22), renewed with fresh medium, and inoculated with 0.1 ml of VSV containing 10 $TCID_{50}$/0.1 ml. Cultures were read at 3 and 6 days for inhibition of CPE.

Fluorescent antibody tests. Cell suspensions to be examined by the indirect test were smeared onto clean slides, air dried, and fixed in acetone at 4° for 30 min. Poliovirus-infected smears were incubated with horse antipoliovirus or normal horse serum for 30 min at 37°, then washed and incubated for another 30 min with fluorescein-tagged rabbit antihorse gamma globulin. Mumps virus-infected cell smears were incubated with human antiserum to mumps virus, followed by washing and incubation with fluorescein-tagged rabbit antihuman gamma globulin. Slides were mounted in 90% glycerin and examined with a Zeiss fluorescent micoscope illuminated by an Osoram HBO-200 mercury vapor lamp.

Results. The comparative susceptibility of human lung fibroblasts Led-130 and lymphoblasts 7466 to some viruses which infect human cells *in vitro* is summarized in Table I. As shown, lymphoblasts 7466 are resistant to the CPE of most viruses tested, and unlike Led-130 cells, are capable of supporting persistent infections with most of these viruses. Only HSV, Coxsackie A-15, VSV, Vaccinia, and ECHO-11 viruses were cytopathic for lymphoblasts 7466, and only with HSV, Coxsackie A-15, and VSV, was viral replication always accompanied by total destruction of the culture. Both ECHO-11 and vaccinia virus, which also produced CPE when high concentrations of virus were used, were able to establish persistent infections when 7466 cultures were inoculated with lower virus doses. The Led-130 cells and lymphoblasts 7466 were equally susceptible to infection and lysis by HSV and also to Coxsackie A-15 virus, after the latter had undergone at least three culture passages in lymphoblasts 7466. Five culture passages of ECHO-11 and vaccinia virus in lymphoblasts 7466 did not increase the cytopathogenicity of these two viruses for these cells.

Human adenovirus type 5, all 3 types of poliovirus, rhinovirus type 2, and reovirus type 1, though highly cytopathic for Led-130 cells, replicated to high titer in 7466 lymphoblasts without evidence of CPE or effect on growth rate of these cultures.

Cultures of lymphoblasts 7466 were negative for virus during the first 3 weeks following inoculation with mumps virus; virus was first detected at titers of $10^{2.2}$ $TCID_{50}$/ml in

TABLE I. Comparative Viral Susceptibility of Human Diploid Lung Fibroblasts (Led-130) and Human Lymphoblasts (RPMI 7466) *in Vitro*.

Virus	Highest virus dilution causing CPE[a]		Replication in RPMI 7466		
	Led-130	RPMI 7466	With CPE[a]	As persistent infection without apparent CPE	Virus titer in RPMI 7466 cultures
Herpes simplex	$10^{-5.0a}$	$10^{-5.0a}$	Yes	No	6.0[b]
Coxsackie type A-15	$10^{-5.5}$	$10^{-5.0c}$	Yes	No	6.0
VSV	$10^{-5.0}$	10^{-0} (delayed)	Yes	No	5.5
Vaccinia	$>10^{-6.0}$	$10^{-3.0}$ (delayed)	Yes	Yes	6.0
ECHO-11	$10^{-6.0}$	$10^{-1.0}$ (delayed)	Yes	Yes	7.5
Human adeno type 5	$10^{-5.0}$	None	No	Yes	7.0
Polio type 1	$10^{-6.0}$	None	No	Yes	6.0
Polio type 2	$10^{-6.0}$	None	No	Yes	6.5
Polio type 3	$10^{-5.0}$	None	No	Yes	5.5
Rhino type 2 (HGP)	$10^{-5.0}$	None	No	Yes	5.5
Reo type 1	$10^{-6.0}$	None	No	Yes	7.5
Mumps	None	None	No	Yes	2.2
Rubella	None	None	No	No	0
Parainfluenza type 3	None	None	No	No	0
NDV	$10^{-3.5}$	None	No	No	0

[a] Cytopathic effect.
[b] Log_{10} of the $TCID_{50}$/ml.
[c] Dilution causing CPE following 3 passages in RPMI 7466 cultures.

tissue culture fluids on day 25 after inoculation. Mumps virus replicated and persisted at these titers in cultures observed up to 2 months after inoculation. At this time, fluorescent antibody tests revealed 25–45% of the cells in infected cultures to contain mumps virus antigen.

Rubella virus replicates without CPE in Led-130 cells as evidenced by the ECHO-11 virus interference test, and NDV replicates in these cells producing CPE at virus dilutions of $10^{-3.0}$. Lymphoblasts 7466 were not susceptible to infection with either of these viruses.

Persistent poliovirus type 1 infection in cultures of lymphoblasts 7466. When lymphoblasts 7466 were exposed to poliovirus type 1 and maintained in continuous cultures, no cell destruction or decrease in cell multiplication could be measured. Virus titers of tissue culture fluids ranged from $10^{5.0}$ to $10^{6.0}$ $TCID_{50}$/ml, and usually represented a low (less than 1.0) virus to cell multiplicity. Most of the virus appeared to be extracellular, as disrupted cells with culture fluid did not yield larger amounts of virus than the cell-free culture fluids. Simultaneous virus release and cell multiplication continued in these persistently infected cultures, designated 7466-p, which revealed no evidence of cytopathology during 7 months of observation. Table II shows cell multiplication and virus release in control and polio-infected cultures maintained 99 days, representing 15 cell culture passages following poliovirus infection.

The number of virus-producing cells in 7466-p cultures, virus release. To determine what proportion of the cells in 7466-p cultures were capable of transmitting infection, cells were collected from cultures which had undergone 14–18 *in vitro* passages after poliovirus inoculation. The 20×10^6 viable cells were washed with Hanks' BSS, incubated with 1.5 ml of hyperimmune horse antipoliovirus type 1 serum[3] for 1 hr at 37°, again washed 3 times each with 30 ml of Hanks' BSS, then

[3] The horse anti-poliovirus serum used was capable of neutralizing 7 logs of poliovirus type 1 at dilutions greater than 1:16.

TABLE II. Effect of Poliovirus Type I on Cell Multiplication in Cultures of Human Lymphoblasts RPMI 7466.[a]

Lymphoblast culture	No. of cells/ml ($\times 10^4$)												
Days:	0	7	9	15	18	22	24	35	45	59	62	69	99
Control													
Viable	25	119	95	75	66	119	139	160	200	93	212	206	230
Nonviable	9	95	25	38	48	63	52	70	75	32	154	82	120
Polio-infected													
Viable	25	152	89	98	105	105	158	190	220	185	243	280	210
Nonviable	9	74	32	47	70	86	71	69	62	41	47	91	110
Poliovirus infectivity titer	5.5[b]		6.0		5.0	5.0	6.0	5.5	6.0	5.5	6.0	6.0	5.0
Virus:cell ratio	1.26		1.05		0.15	0.08	0.71	0.19	0.5	0.03	0.47	0.48	0.04

[a] One and one-half ml of lymphoblasts at 25×10^4 cells/ml were initially inoculated with 0.15 ml of poliovirus having a titer of $10^{5.5}$ $TCID_{50}$/ml. Cultures were subdivided once each week.

[b] Log_{10} of the $TCID_{50}$/ml.

serially diluted with culture medium. Decreasing numbers of cells were tested for infectivity by direct inoculation into culture tubes of Led-130 or RMK cells and onto culture plates of RMK cells. Approximately 100 $TCID_{50}$/0.1 ml of residual poliovirus type 1 was detected in the last wash fluid of the immune serum-treated cells. All cell suspensions tested however, were used at dilutions exceeding the dilutions at which residual virus could be detected. Results in Table III show that at least 125 7466-p cells are required to infect 50% of Led-130 cultures, and about 12 cells to infect the more sensitive

TABLE III. Number of 7466-p Cells Required to Infect Led-130 or Monkey Kidney Cell Cultures.

No. of 7466-p cells seeded	Led-130 cultures (tubes with CPE[a] /no. inoculated)	Monkey kidney cell cultures		
		Tubes with CPE/no. inoculated	No. of plaques/plate[b]	No. of cells/plaque
1000	6/6			
500	6/6			
250	3/6			
200	—	3/3	8	
125	3/6	—	8	
100	—	3/3	—	
62	0/6	—	4	16
50	—	3/3	3	16
31	0/6	—	3	10
25	—	2/3	2	12
15	0/6	—	—	
12	—	2/3	1	12
8	0/6	—	—	
6	—	0/3	0	
No. of cells/$TCID_{50}$	125	12		

[a] Cytopathic effect.

[b] Average of 3 or 4 plates.

RMK cultures. From 12 to 16 cells were required to produce one virus plaque on RMK monolayers, indicating that 1 out of 12 to 16 cells, or less than 10% of the 7466-p population were capable of transmitting infection at the time of assay.

Efforts were also made to determine the number of infectious units present in the infected cells. This was done by disrupting the washed cell suspensions with ultrasonication prior to dilution and assay. Results were identical to that obtained in tests using intact cells, indicating that the limited number of cells in 7466-p cultures which are producing virus probably contain not more than one infectious unit at any given time. This might also suggest that infectious virus units are produced one at a time and released from the cell as soon as completed. This interpretation appears to be supported by the repeated failure to demonstrate poliovirus antigen in the 7466-p population, using fluorescent antibody techniques.

The effect of the horse poliovirus type 1 antiserum on 7466-p cultures was tested in cultures prepared from the washed immune serum-treated cells described above. Cultures were treated with normal or polio-immune horse serum at 10% concentration for 12 days of continuous culture, at which time the added horse serum was discontinued. Results, illustrated in Fig. 1, show that treatment of 7466-p cultures with antipoliovirus serum can effectively neutralize the output of infectious virus. When treatment with antiserum was discontinued after 12 days, 7466-p cultures were completely cured of poliovirus infection, in that no virus could be detected in culture fluids when tested at any time following treatment. These results suggested an entirely extracellular spread of virus.

In order to determine whether cured cell populations were resistant to reinfection, poliovirus type 1 was reinoculated into cultures of cured 7466-p cells. A persistent poliovirus infection was again immediately established indicating that cured 7466-p populations were as susceptible to reinfection with poliovirus type 1 as uninfected lymphoblasts 7466.

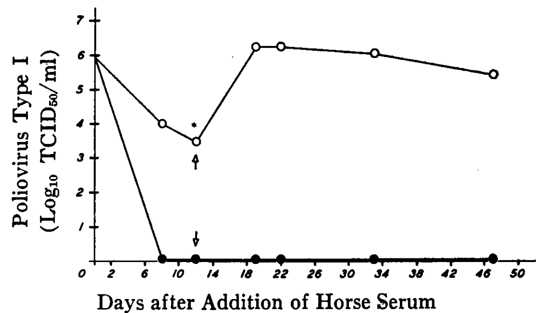

FIG. 1. Effect of immune horse antipoliovirus serum on poliovirus type 1 replication in 7466-p cultures: (○), cultured in presence of normal horse serum at 10% concentration; (●), cultured in presence of immune horse anti-poliovirus serum at 10% concentration; (*), horse serum omitted from culture medium.

Tests for viral interference, interferon production. To test for viral interference in 7466-p cultures, replicate cultures of 7466-p and of control 7466 lymphoblasts were inoculated with decreasing dilutions of HSV or vaccinia virus ranging from 10^6 to 10^2 $TCID_{50}$/inoculum. Cultures were incubated at 37° and were examined for pH change and for the percentage of nonviable cells each 3–4 days. Results revealed 7466-p populations to be equally susceptible as control uninfected lymphoblasts to the cytolytic action of HSV and vaccinia virus. No viral interference to CPE of these 2 viruses could therefore be demonstrated in 7466-p cultures.

Human lymphoblast cell lines derived from Burkitt's lymphoma, acute leukemia, or infectious mononucleosis were reported to produce interferon (23). This finding suggested that interferon production in 7466-p cultures may play a role in the establishment and maintenance of a persistent infection. To test this, culture fluids from 7466-p cultures representing 16 cell culture passages since poliovirus infection, and control fluids from uninfected lymphoblasts 7466 cultures were collected 2 days after the last subculture and assayed for interferon (see "Materials and Methods"). Results showed that neither control fluids nor fluids from 7466-p cultures were effective in protecting Led-130

TABLE IV. Chromosome Numbers of Cells from 7466-p and Control Lymphoblast 7466 Cultures.

Culture	No. of chromosomes/cell											Total no. of cells
	<42	43	44	45	46	47	48	49	50	51	>54	
Lymphoblasts 7466[a] (controls)	3	4	5	10	15	9	7	0	0	1	3	57
7466-p[a]	4	5	4	8	18	10	3	1	0	0	5	58

[a] Both groups of cultures had been maintained through 18 tissue culture passages since infection of 7466-p cultures with poliovirus.

cells against subsequent challenge with VSV. These tests indicate that interferon production in 7466-p cultures occurs not at all or in amounts too small to be measured by the method employed.

Human lymphoblast cell lines are known to transmit interference when propagated in direct contact with indicator cells and this cell-mediated interference has been shown due to the production by the lymphoblasts of a viral inhibitor with properties of interferon (23). That a cell-mediated type of interference is present in 7466-p cultures could therefore not be excluded. If this were a limiting factor in poliovirus infection of the greater proportion of the cells at any given time, an inhibitor of interferon production might enhance poliovirus replication in these cultures. Actinomycin D is known to inhibit interferon production (24). The 7466-p cultures were therefore treated for 4 hr at 37° with actinomycin D at concentrations ranging from 5.0 to 0.0015 μg/ml. Cells were then centrifuged, washed with Hanks' BSS, resuspended in culture medium and incubated for 4 days. Sample cultures were removed daily for determination of cell viability and virus infectivity. Results showed that actinomycin D was toxic when in contact with 7466-p cells for 4 hr at concentrations of 0.0125 μg/ml or greater. Poliovirus output was not enhanced in 7466-p cultures treated with any concentration of actinomycin D tested.

Chromosome analysis, effect of mitotic synchrony. The reinfection of cured 7466-p cultures with poliovirus type 1 and establishment of a persistent infection having characteristics of initially infected 7466 lymphoblasts indicate that selection of cell populations in 7466-p is not occurring. To obtain more information on the genetic constitution of these populations, cells from 7466-p cultures in tissue culture passage 18 after infection and cells from uninfected lymphoblast 7466 cultures, maintained as controls, were analyzed for chromosome number and gross abnormality. Results shown in Table IV reveal most cells of both control and 7466-p cultures to have around 46 chromosomes. This conforms to that previously reported for this cell strain (6). No increase in the number of polyploids nor in cells with obvious chromosome abnormalities were noted in 7466-p preparations. Thus, no apparent selection of cells with different karyotypes was found to occur after long-term culture in the presence of a persistent poliovirus type 1 infection.

It was considered that the small numbers of cells producing virus in 7466-p populations represented cells susceptible to infection and able to synthesize virus only at a certain stage of the mitotic cycle. To test this, efforts were made to synchronize mitotic division in 7466-p cultures (see "Materials and Methods"). Following a 3-hr treatment with colcemide, 81 cells in metaphase/1000 cells counted were found, as compared with 12 cells in metaphase/1000 cells in untreated 7466-p cultures. Further incubation of the treated cells in the absence of colcemide resulted in their continued multiplication without evidence of cell death from colcemide treatment. Viral assays of culture fluids over a 5-day period following treatment revealed poliovirus titers ranging from $10^{4.5}$ to $10^{6.0}$ $TCID_{50}$/ml and indistinguishable from that of nonsynchronized 7466-p cultures. Results

of plaque assays of control and colcemide-treated cells immediately after removal of the colcemide showed that 11–18 7466-p cells were required to produce one viral plaque with no differences between colcemide-treated and untreated cells. Although a significant increase in mitotic synchrony had been achieved in 7466-p cultures, this did not increase the number of cells capable of transmitting poliovirus infection.

Discussion. Except for HSV and Coxsackie virus type A-15, human lymphoblasts 7466 were resistant to the CPE of the viruses tested, but were able to multiply in "carrier" cultures with these viruses for long periods. In contrast to the susceptibility of lymphoblasts 7466 to the CPE of HSV, lymphoblasts derived from Burkitt's lymphoma have been reported to multiply in carrier cultures with HSV for long periods (25).

Conditions generated in cultures which permit simultaneous cultivation of both viruses and cells for indefinite periods has been elucidated for L (MCN) strain cells infected with NDV, and presented in an excellent review of this subject by Henle (26). Cellular resistance in these cultures was dependent on production of incomplete, noninfectious virus and stimulation of interferon production by this incomplete virus in other cells. Results of our experiments do not exclude the possibility that similar mechanisms are operating in 7466-p cultures, although the latter have some characteristics unlike those of previously described persistent infections.

During the 7 months of continuous culture of 7466-p cells, concomitant virus replication and cell multiplication occurred without recognizable cell destruction by virus, reduction in growth rate of the cells, or measurable interference to infection and CPE with 2 other viruses. Results of tests suggest that less than 10% of the cells are infected at any given time. The complete cure of the cultures with serum antibody to poliovirus suggests an extracellular spread of virus and that cells producing virus are eventually destroyed. No selective destruction of genetically susceptible cells and replacement by resistant cells appeared to be taking place. Nevertheless, it must be assumed that only a few cells in the total population are in an appropriate physiological state at the time of exposure to virus to permit infection and virus reproduction. The factors responsible for the refractive state of the majority of the population at any given time has not been ascertained by our experiments.

Although interferon could not be detected in 7466-p cultures, it may be present at levels too low to be detected in tissue culture fluids. Moreover, the levels of actinomycin D which were nontoxic for these lymphoblasts may have been ineffective in inhibiting interferon production. Cellular resistance in 7466-p populations induced by interferon and transmitted to other cells through cell-to-cell contact cannot be ruled out.

The production of immunoglobulins by human lymphoblast lines (27) and by RPMI 7466 cells (6) was reported. Enhancement of IgM production in human Burkitt lymphoma cell lines upon infection with HSV (25) and the production of specific neutralizing inhibitors for the infecting agent upon infection of these cell lines with HSV or T_2 phage has also been described (25,28). It is conceivable that similar inhibitors produced and effective at the cellular level may be a factor in controlling and maintaining persistent viral infections in these human lymphoblast cell lines. Further efforts to elucidate these mechanisms are important because of their possible bearing on latent viral infections in the living host.

Summary. Comparative viral susceptibility tests using human diploid lung fibroblasts (Led-130) and human lymphoblasts (RPMI 7466) have been made. Lymphoblasts 7466 were found resistant to the cytopathic effect of most viruses tested, but were capable of supporting persistent viral infections with many of these. Exceptions were herpes simplex and Coxsackie type A-15 viruses which were highly cytopathic for lymphoblasts 7466; viral replication in these instances was accompanied by total destruction of the culture. Concomitant virus replication and cell multiplication occurred in lymphoblasts 7466 persistently infected with poliovirus type 1

during 7 months of continuous culture. No recognizable cell destruction, reduction in growth rate, or interference to infection with two other viruses could be measured in these cultures. The mechanisms which operate to control and maintain persistent virus infections in cultures of human lymphoblasts has been studied and discussed.

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