Replication of Mumps Virus in Human Leukocyte Cultures

HUU DUC-NGUYEN¹ AND WERNER HENLE²

Virus Laboratories, The Children's Hospital of Philadelphia, and School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

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ABSTRACT

DUC-NGUYEN, HUU (The Children's Hospital of Philadelphia, Philadelphia, Pa.), AND WERNER HENLE. Replication of mumps virus in human leukocyte cultures. *J. Bacteriol.* 92:258-265. 1966.—Human peripheral leukocyte cultures maintained in the presence of phytohemagglutinin (PHA) were found to support to some extent the replication of mumps virus. When such cultures were exposed, within 24 hr after their initiation, to a high input multiplicity of virus, successful infection, as determined by immunofluorescence and plaque assays, did not become evident before the 3rd or 4th day. On exposure of cultures 4 to 5 days old, viral replication was detectable within 2 days. In both instances, peak immunofluorescence and virus titers were reached when the cultures were 7 to 9 days old and composed mainly of blast forms. With decreasing input multiplicities of infection, cells containing viral antigen and production of infectious viral progeny became detectable with increasing delay. No significant viral replication was noted in surviving cells maintained in the absence of PHA. These results indicate that mainly, if not solely, the PHA-stimulated cells of the lymphocytic series support viral multiplication. The extent of the infectious process was limited, however, because the life span of the cultures was not significantly shortened, the yields of infectious virus per immunofluorescent cell were at all times low, and most infected cells contained only a few well-delineated small masses of antigen, suggestive of an abortive infection. Only fresh cultures were capable of synthesizing interferon on stimulation by mumps, Newcastle disease, or Sendai viruses. When the cultures were set up in the presence of PHA, this capacity was lost within 24 hr. PHA per se failed to induce detectable production of an interferon under the conditions used. The implications of these findings are discussed.

Several viruses were found to replicate to some extent in suspensions of human peripheral leukocytes which were prepared or cultured in the presence of phytohemagglutinin (PHA). This substance, originally used to separate erythrocytes from leukocytes, was shown subsequently to stimulate mytosis of lymphocytic elements and to change many into blast forms (8, 10). Berg and Rosenthal (2) were able to maintain measles virus through serial passages at 3- to 4-day intervals in fresh human leukocyte cultures. Maximal virus titers were obtained, however, only between the 4th and 7th days. Measles virus also grew in

leukocyte suspensions consisting mainly of blast forms, which were derived from leukemic patients. Berg (1) reported similar results on infection of fresh leukocyte suspensions with echovirus 9. Subsequently, Nahmias and co-workers (9) and Gresser and Chany (5) infected not only fresh but also 4-day-old leukocyte cultures with herpes simplex virus and poliovirus type 1, respectively. When fresh cultures were used, evidence of viral replication was not noted before the 4th day, and peak titers were observed by the 6th day or later. On infection of 4-day-old cultures, significant viral replication became evident and maximal titers were obtained sooner than in fresh suspensions. These results indicated that polymorphonuclear cells, which degenerate rapidly, contributed relatively little, if at all, to the maintenance of the various viruses, and that mainly the PHA-

¹ Present address: Section of Viral Oncology, National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Md.

² Career Investigator, 1-K6-AI-22,683, National Institutes of Health, U.S. Public Health Service.

stimulated lymphocytic elements were responsible for supporting viral replication. Indeed, in the absence of PHA, the cultures failed to support herpes simplex virus. The yields of virus in all instances were low in relation to the number of available cells.

The delay in viral replication and the low yields of progeny conceivably might be related to the fact that fresh leukocyte suspensions are capable of synthesizing interferon on appropriate viral stimulation (4), with both lymphocytes and polymorphonuclear cells being involved (7). Furthermore, PHA per se was reported to induce interferon synthesis in human leukocytes (14). Thus, interferon could well reduce, delay, or abort viral replication in fresh leukocyte cultures. Whether PHA-stimulated leukocytes which have been maintained in culture for some time retain the capacity of interferon synthesis has not as yet been clearly demonstrated.

The present studies, concerned with infection of leukocyte cultures by mumps virus, were undertaken to clarify further the cultural conditions required for viral replication, the extent of cellular susceptibility, and the roles played by PHA and interferon in the infectious process.

MATERIALS AND METHODS

Leukocyte cultures. The technique described by Moorhead et al. (8) was used with some modifications. Blood treated with heparin was allowed to settle at 37 C within a syringe kept in an upright position. When about one-third of the volume had been cleared of red cells, the leukocyte-rich plasma was expelled upward through a bent needle into a centrifuge tube, and the cells were sedimented at $600 \times g$ for 10 min. The cells were washed three times in medium 199 supplemented with 10% inactivated fetal calf serum (30 min at 56 C), and transferred to plastic or glass flasks. After being gassed with a mixture of air and 5% CO₂, the flasks were incubated in a prone position at 37 C for 1 hr to permit attachment of polymorphonuclear cells and monocytes to the surface of the vessels. The suspension of unattached cells was decanted and counted. Differential counts revealed that the granulocytes were reduced by this procedure by, at most, 85%. The desired numbers of cells were sedimented and resuspended in medium 199 containing 20% fetal calf serum, 1% phytohemagglutinin-P, and penicillin (100 units per ml) and streptomycin (100 µg/ml). The final suspensions, containing between 10^6 and 2×10^6 cells per milliliter, were dispensed into Erlenmeyer flasks, prescription bottles, or screw-capped vials, leaving an air space of about 80% of the total volume. Half of the medium was replaced on the second day, care being taken not to disturb the settled cells. Further feedings did not seem to prolong the survival of the cultures and, therefore, were usually omitted. Cell counts were made at intervals, by use of a 0.1% solution of trypan blue as

diluent to differentiate between viable and dead (staining) cells.

Viruses. The Ricki strain of mumps virus, isolated in chick embryos from a case of parotitis, was used in its 7th to 9th amniotic passage. The amniotic fluid seeds, stored at -70 C, contained approximately 10^8 plaque-forming units (PFU) and $10^{2.6}$ hemagglutinating (HA) units per ml. The virus was added to leukocyte cultures in a concentration sufficient to attain desired input multiplicities. Nonadsorbed virus was usually, but not always, removed after adsorption periods of 1 to 3 hr at 37 C by washing of the cells twice with medium.

For production of interferon, Sendai or Newcastle disease (Victoria strain) virus was used in the form of infected chick embryo allantoic fluids. For assay of interferon activity, the Indiana strain of vesicular stomatitis virus (VSV) was used as challenge. Stock virus was prepared from infected chick embryo fibroblast cultures.

Hemadsorption plaque assay of mumps virus. Monolayers of chick embryo fibroblasts were prepared in plastic flasks (Falcon Plastic Co.) according to routine procedures (6). Enough cells were seeded to obtain confluent sheets within 18 hr. After washing with Hanks solution, 0.4-ml amounts of the test materials were introduced, and the virus was permitted to adsorb for 1 hr at 37 C. The cell sheets were then overlaid with the following mixture: methyl cellulose (Methocel; viscosity, 1,500 centipoises; Dow Chemical Co., Midland, Mich.), 1.5%; medium 199 (10X), 3%; Scherer's maintenance medium (10X), 5%; inactivated horse serum, 20%; NaHCO₃ solution (7.5%), 5%; and antibiotics. After incubation at 37 C for 10 days, the flasks were placed at 4 C for 1 hr, the overlay was poured off, and the cell sheets were washed with cold phosphate-buffered saline (PBS) solution until all cellulose was removed. After addition of 3 ml of a 0.6% suspension of chicken red cells in PBS, the flasks were kept at 4 C for 30 min, drained, washed, and examined for foci of hemadsorption. These were readily identifiable (Fig. 1) and countable under illumination by a 60-w light source.

Immunofluorescence assays. Cells were sedimented from culture samples and, after decanting of the supernatant fluids, were resuspended in the remaining medium. Drops of this suspension were spread evenly over cover slips (6 by 30 mm), and allowed to dry at 37 C. The preparations were fixed in fresh acetone at room temperature for 10 min, and then exposed for 1 hr at 37 C to fluorescein isothiocyanate-conjugated human γ -globulin obtained from donors who had been hyperimmunized against mumps. The cover slips were thoroughly washed in two changes of PBS, dipped in distilled water, dried, fixed on slides by means of a semipermanent mounting medium (12), and examined under ultraviolet illumination (Osram HBO 200-w lamp) with a Zeiss microscope. The percentage of cells containing viral antigen was determined by counting from 200 to 1,000 cells, depending upon the extent of the infection.

Interferon production and assay. Suspensions containing up to 10^7 viable cells per milliliter of medium (2% fetal calf serum) were exposed to mumps, Sendai,

FIG. 1. Hemadsorption plaques induced by mumps virus on monolayers of chick embryo fibroblasts. The flasks had been inoculated with a 10^{-2} , 10^{-3} , and $10^{-3.5}$ dilution of virus.

or Newcastle disease virus (NDV) at input multiplicities in excess of 1, and incubated at 37 C for 24 to 36 hr. The clarified medium was concentrated twofold with Carbowax polyethylene glycol compound 20-M (Union Carbide Chemical Co.). This material was usually dialyzed at 4 C successively against 100 volumes of KCl buffer (pH 2), phosphate buffer (pH 7.2), and Hanks solution, each for 24 hr. For some tests, the medium was used merely after clarification at $100,000 \times g$ for 1 hr. For assay of interferon activity, serial twofold dilutions were added to human amnion or human embryonic kidney cultures (purchased from Flow Laboratories, Rockville, Md.). These were challenged 24 hr later with about 10^4 TCID₅₀ of VSV. Virus controls showed marked cytopathic effects in 24 hr and complete destruction of the cell sheets in 48 hr. The results were read at this time, and the highest dilution of an interferon preparation protecting at least 75% of the cells was taken as the end point. In one test, HeLa cells were used according to the method developed by Paucker (11), based on changes in pH of the cultures.

RESULTS

Development of virus-specific immunofluorescence. In initial experiments, leukocyte cultures were set up with or without PHA and exposed immediately to mumps virus at input multiplicities ranging from 0.1 to >5 . After incubation for varying periods of time, cover-slip preparations of cells were made and tested for virus-specific immunofluorescence. In the PHA-stimulated cultures, only rare cells, if any, were found to contain viral antigen within the first 48 hr. Stainable cells appeared thereafter in gradually increasing numbers ranging from 30 to 80% of the total population. The appearance of the stained cells is illus-

trated in Fig. 2. The infected cells generally were larger than the lymphocytes present at start of the cultures, and contained viral antigen in well-delineated round or oval masses of varying sizes. Cultures set up without PHA and infected with mumps virus failed to reveal detectable numbers of antigen-containing cells at any time.

The gradual spread of the infection in PHA-stimulated cultures is shown in Fig. 3. In this experiment, leukocytes of one donor were exposed to mumps virus at an input multiplicity of 1.1, and replicate cultures were set up for assay of viable cells and virus-specific immunofluorescence at the indicated intervals. The cell counts, after the usual initial decline, revealed evidence of cellular replication, and the cultures, consisting at that time largely of blast forms, survived for 3 weeks. The percentage of infected cells reached its peak by the 13th day and declined thereafter, well in advance of the death of the culture.

These results indicated that mainly the PHA-stimulated lymphocytic elements of the culture supported the synthesis of viral antigen(s). This is further evident from the fact that, upon inoculation of mumps virus into 3- to 5-day-old cultures, many cells became infected within a few days, their number depending, of course, upon the multiplicity of infection. In the experiment presented in Table 1, PHA-stimulated leukocytes were infected on the 3rd day at varying multiplicities. With the larger doses of virus, more than 50% of the cells contained antigen 48 hr later, but as the dose was decreased antigen became detectable successively later. In other experiments extending over longer periods, immunofluorescence

FIG. 2. Virus-specific immunofluorescence in cultured human leukocytes. (a) Controls, 10-day-old culture; (b) infected on day 0 and stained on day 10; (c, d) infected on day 5 and stained on day 10. $\times 1,500$.

appeared as late as 6 days after infection at a multiplicity of 0.0001. In confirmation of the data shown in Fig. 1, the percentage of infected cells, after reaching a peak, declined again, whereas the cultures still contained many viable cells.

Production of infectious viral progeny. The above experiments have shown that cultured leukocytes in the presence, but not in the absence, of PHA are capable of supporting synthesis of mumps antigen(s). To test for production of infectious viral progeny, cultures, with and without PHA, were exposed to mumps virus on different days after their initiation, and assayed for infectious virus yields by use of the hemadsorption-plaque technique. In the first experiment (Fig. 4), freshly obtained PHA-stimulated and PHA-free suspensions of leukocytes, as well as cell-free medium, were inoculated with mumps virus at an input multiplicity of 1.2. At the indicated intervals, 0.2 ml of medium was withdrawn,

diluted 1:10 in medium, and stored in sealed ampoules at -70°C until assayed. The virus in cell-free medium was completely inactivated within 4 days of incubation at 37°C . In the PHA-free cultures no evidence of viral replication became evident, but inactivation proceeded at a slightly slower rate until no virus was detectable by the 7th day. In contrast, the virus titers in the PHA-stimulated cultures, after an initial decline, showed a significant rise on the 3rd day and possibly a second peak on the 8th day. This culture as well as uninfected controls failed to survive for more than 12 days.

A similar experiment, including cell counts, is shown in Table 2. The PHA-free cultures revealed a continuous decline in virus titer, even though some viable cells were left by the 6th day. In the PHA-stimulated cultures, the virus titers reached a plateau between the 8th and 12th days, and declined thereafter when the cell counts were still

FIG. 3. Viable-cell counts and percentage of immunofluorescent cells in leukocyte cultures infected on the day of preparation.

TABLE 1. Relation between multiplicity of infection and development of virus-specific immunofluorescence

Day after infection ^a	Input multiplicity				
	8	4	0.4	0.04	0.004
1	0 ^b	0	0	0	0
2	3	2	0	0	0
3	1	3	3	0	0
4	1	1	2	1	0

^a The leukocyte cultures were inoculated 3 days after their initiation in the presence of PHA.

^b Symbols: 0, no fluorescence; 1, 1 to 20%; 2, 21 to 50%; 3, 51 to 80% of the cells showed positive fluorescence.

relatively high. A comparison of the cell counts and plaque-forming units revealed that the yields of infectious virus per cell were very low.

The two suggestive peaks in virus titers seen in Fig. 4 were evident also when other cultures were infected at multiplicities of 0.5 to 5 on the day of preparation or 1 day later (Fig. 5A). Depending on the general viability of leukocyte cultures from individual donors, the final decline in virus titers occurred between the 8th and 15th days. The loss of the cultures could not be ascribed to viral action, because control, uninfected cultures, as a rule, failed to survive longer. When cultures were infected at similar multiplicities on the 5th day after their initiation, peak virus titers were reached within 2 to 3 days thereafter, and only small, secondary peaks were noted after another 4 to 6 days (Fig. 5B).

When low multiplicities of infection were employed, evidence of viral replication was consider-

ably delayed as was observed also in the immunofluorescence tests. In the experiment shown in Fig. 6, leukocyte cultures were exposed 24 hr after their initiation to virus at input multiplicities of 4.5 and 0.0045. The results obtained with the larger dose conformed to those just described. With the smaller dose, virus became temporarily detectable 6 days after infection of the culture, and was in significant titers only after the 8th day. The cell counts for the two cultures failed to show striking differences during the observation period in spite of the fact that the yields of virus differed on the average by a factor of 10. This showed again that the mumps infection does not markedly reduce the survival time of the leukocyte cultures.

Interferon synthesis. Leukocyte cultures were tested for their capacity to produce interferon after exposure to mumps, Sendai, or Newcastle disease (NDV) virus. Fresh cultures produced

FIG. 4. Viral titers in leukocyte cultures with or without phytohemagglutinin and infected on the day of preparation.

TABLE 2. Viable-cell counts and mumps virus titers in PHA-stimulated and PHA-free leukocyte cultures

Day after infection ^a	No PHA		PHA-stimulated	
	Viable cells per ml	Virus titer (PFU/ml)	Viable cells per ml	Virus titer (PFU/ml)
1	3.04×10^5	5.82×10^2	1.62×10^5	5.59×10^2
2	2.03×10^5	1.25×10^2	1.12×10^5	1.33×10^2
6	8.00×10^3	2.50×10^1	5.40×10^4	1.20×10^3
8	0	0	8.80×10^4	1.70×10^4
9	0	0	9.60×10^4	1.20×10^4
12	0	0	3.40×10^4	9.75×10^3
14	0	0	1.80×10^4	4.60×10^2

^a The leukocyte cultures were infected on the day of their preparation.

FIG. 5. Viral titers in leukocyte cultures of different ages. (A) Infection of fresh or 1-day-old cultures. (B) Infection of 5-day-old cultures. The input multiplicities were 5 (▲), 2 (○), and 0.5 (●).

appreciable amounts of interferon whether or not PHA was present (Table 3). However, in the presence of PHA, the ability to synthesize interferon was apparently blocked within 24 hr, whereas in its absence surviving cells still yielded interferon when stimulated 1 or 2 days after initiation of the cultures. Media from control cultures with or without PHA per se or inoculated with normal amniotic fluid were free from inhibitory activity. Since stimulation of leukocytes merely by PHA was recently reported to yield an interferon-like inhibitor which is not stable at pH 2 (14) additional experiments were carried out in which the culture media were clarified at $100,000 \times g$ for 60 min and tested before and after dialysis against acid buffer. The controls failed to protect the test cultures also under these conditions.

It was shown previously (4) that Sendai virus induces interferon synthesis in human leukocytes but that the virus could not be maintained in such cultures. Live NDV infected many of the cells under the same conditions used for mumps virus, as evident from specific immunofluorescence, and

FIG. 6. Viable-cell counts and viral titers in leukocyte cultures exposed 2 days after preparation to different input multiplicities of virus.

the cultures failed to survive. In the experiment in which 5-day-old cultures were exposed to ultra-violet-inactivated NDV, no staining was obtained, and the cells failed to produce interferon (Table 3) but acquired resistance to challenge 24 hr later by VSV. Only rare cells developed VSV-specific immunofluorescence, whereas, in the controls, nearly all cells were stainable 48 hr after infection, more than 10 PFU of VSV were produced per cell, and the cultures were destroyed.

DISCUSSION

The data presented have shown that mumps, as well as NDV and VSV, can be added to the growing list of viral agents which are capable of replicating at least to some extent in human peripheral leukocyte cultures. This type of host system is far from ideal, however, for the maintenance of viruses. Without stimulation by PHA, all types of cells survive for only a short time. On addition of PHA, mitosis is stimulated in cells of the lymphocytic series and many of these are changed into blast forms (10), but the granulocytes disappear also under this condition. Attempts to remove the granulocytes by adsorption onto glass prior to setting up of the cultures were only partially successful. Thus, early PHA-stimulated cultures are composed of degenerating granulocytes and, as yet, small but mostly live lymphocytic elements. As time progresses, mainly enlarged lymphocytes

TABLE 3. *Interferon synthesis in leukocyte cultures*

Expt no.	Leukocyte culture			Virus	Interferon (units/ml)		
	Viable cells per ml ($\times 10^6$)	Age of culture	PHA		Cells used for assay ^a	pH 2 dialysis	No dialysis
		<i>days</i>					
1	10	0	—	None	HA	<4	
	10	0	—	Mumps	HA	200	
2	7	0	—	Sendai	HA	240	
	6	4	+	Sendai	HA	<4	
3	9	2	—	Mumps	HA	220	
	6	5	+	Mumps	HA	<4	
4	3	0	+	None	HeLa	0	
	3	0	+	NDV	HeLa	24	
	1.8	2	+	NDV	HeLa	0	
	1.5	4	+	NDV	HeLa	0	
	1.6	6	+	NDV	HeLa	0	
5	3.3	5	+	None	HEK	0	
	3.3	5	+	NDV ^b	HEK	0	
6	2.4	0	—	None	HEK	<4	<4
	2.4	0	+	None	HEK	<4	<4
	2.4	0	—	Mumps	HEK	24	32
	2.4	0	+	Mumps	HEK	48	64
	2.4	1	—	Mumps	HEK	6	8
	2.4	1	+	Mumps	HEK	<4	<4
	2.4	2	—	Mumps	HEK	4	4
	2.4	2	+	Mumps	HEK	<4	<4

^a HA = primary human amnion cultures; HEK = primary human embryonic kidney cultures.

^b Ultraviolet-irradiated.

and blast forms are present, but these, too, gradually die and cultures fail to survive for more than 3 weeks. Thus, the cultures were at no time composed of a single type of cell; they always contained a variable proportion of degenerating or dead cells, which complicates quantitative evaluation such as accurate determination of the proportion of infected cells by use of virus-specific immunofluorescence.

From the sequence of events following infection of fresh leukocyte cultures by mumps virus, it seems evident that the PHA-stimulated cells of the lymphocytic series are the ones supporting viral replication, as was noted also with herpes simplex virus (9) and poliovirus (5). This conclusion is supported by several facts. (i) In the absence of PHA, neither virus-specific immunofluorescence nor increases in infectious virus were noted. (ii) In fresh PHA-stimulated cultures, virus-specific immunofluorescence could not, as a rule, be elicited prior to the 3rd day after inoculation, and at this time an increase in infectivity titers also became noticeable. (iii) The highest incidence of cells containing viral antigen was noted only on the 6th day, or later, after initiation of the cultures at which time second peaks of infectious virus were measured; i.e., when most of the cells present were blast forms. (iv) When PHA-stimulated cul-

tures were inoculated on the 3rd day or later, virus-specific immunofluorescence could be elicited already after 1 or 2 days in many cells, and peak virus titers were attained correspondingly early.

The mumps infection of leukocyte cultures was of a relatively mild nature as is evident from the following facts. (i) The life span of the cultures was not significantly shortened by the infection when compared with that of uninfected control populations. (ii) The yields of infectious virus were very small, in that the ratios between the numbers of plaque-forming units and infected (immunofluorescent) cells at given times were generally well below, and never exceeded, 1. (iii) The viral antigen in mumps-infected cells was often restricted to a few, small, well-delineated masses, indicative of an "abortive" infection; whereas, in a "virulent" infection, antigen is usually well dispersed throughout the cytoplasm and over the cell surface (13).

The possible role of interferon in the limitation of the infectious process has not been clearly defined in these experiments. Only fresh leukocytes responded on appropriate exposure to virus with interferon synthesis. If PHA was used for initiation of the cultures, this capacity was lost within 24 hr. In contrast to a previous report (14) no

interferon-like inhibitor was found in the media of PHA-stimulated control cultures, even when dialysis against pH 2 buffer was omitted. The conditions used for induction or assay of this inhibitor may have been less than optimal. It is conceivable that subdetectable amounts of interferon were produced by a proportion of the cells in response to PHA, and that these cells became refractory within 24 hr to restimulation of interferon synthesis by the various viruses. This suggestion is based upon the observation (3) that, after completion of virus-induced interferon production, cells not only resist viral infection but also cannot be immediately restimulated to produce more interferon. Thus, although PHA is needed to produce virus-susceptible cells, at the same time it may render them partially resistant. To resolve this problem, it would be essential to find potent inducers of mitosis and blast forms which neither stimulate per se nor block virus-induced interferon synthesis.

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LITERATURE CITED

1. BERG, R. B. 1961. Multiplication of ECHO 9 virus in suspensions of human leukocytes. *Proc. Soc. Exptl. Biol. Med.* **108**:742-744.
2. BERG, R. B., AND M. S. ROSENTHAL. 1961. Propagation of measles virus in suspensions of human and monkey leukocytes. *Proc. Soc. Exptl. Biol. Med.* **106**:581-585.
3. CANTELL, K., AND K. PAUCKER. 1963. Quantitative studies on viral interference in suspended L cells. IV. Production and assay of interferon. *Virology* **21**:11-21.
4. GRESSER, I. 1961. Production of interferon by suspensions of human leukocytes. *Proc. Soc. Exptl. Biol. Med.* **108**:799-803.
5. GRESSER, I., AND C. CHANY. 1964. Multiplication of poliovirus type I in preparations of human leukocytes and its inhibition by interferon. *J. Immunol.* **92**:889-895.
6. HENLE, G., F. DEINHARDT, V. V. BERGS, AND W. HENLE. 1958. Studies on persistent infection of tissue cultures. I. General aspects of the system. *J. Exptl. Med.* **108**:537-560.
7. LEE, S. H. S., AND R. L. OZERE. 1965. Production of interferon by human mononuclear leukocytes. *Proc. Soc. Exptl. Biol. Med.* **118**:190-195.
8. MOORHEAD, P. S., P. C. NOWELL, W. J. MELLMAN, D. M. BATTIPS, AND D. A. HUNGERFORD. 1960. Chromosome preparation of leukocytes cultured from human peripheral blood. *Exptl. Cell Res.* **20**:613-616.
9. NAHMIA, A. J., S. KIBRICK, AND R. C. ROSAN. 1964. Viral leukocyte interrelationships. I. Multiplication of a DNA virus—Herpes simplex—in human leukocyte cultures. *J. Immunol.* **93**:69-74.
10. NOWELL, P. C. 1960. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* **20**:462-466.
11. PAUCKER, K. 1965. The serologic specificity of interferon. *J. Immunol.* **94**:371-378.
12. RODRIGUEZ, J., AND F. DEINHARDT. 1960. Preparation of a semipermanent mounting medium for fluorescent antibody studies. *Virology* **12**:316-317.
13. WALKER, D. L., AND H. C. HINZE. 1962. A carrier state of mumps virus in human conjunctiva cells. I. General characteristics. *J. Exptl. Med.* **116**:739-750.
14. WHEELOCK, E. F. 1965. Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. *Science* **149**:310-311.