generated by the visual receptors. The amplitudes of the early receptor potentials can be hundreds to thousands of times larger for a given flash energy (and hence their threshold flash energies are correspondingly lower), their action spectra correspond to the appropriate visual pigments (*2*, *9*), they saturate at high flash energies (*2*), and they are completely photolabile (*2*, *4*). However, these differences do not rule out the possibility that a similar mechanism may generate all of these fast electrical signals.

We expect these new electrical responses in skin will help reveal the mechanisms that generate the fast electrical signals in the eye. Moreover, the late response in skin may prove helpful in investigating reactions of organisms to light which are mediated by non-optic receptors, such as the dermal light sense (*10*).

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Vesicular Stomatitis Virus Replication in Human Leukocyte Cultures: Enhancement by Phytohemagglutinin

Abstract. *The replication of vesicular stomatitis virus in human leukocyte cultures shows that virus yields can be enhanced 6- to 180-fold by treating the leukocyte cultures with phytohemagglutinin prior to virus inoculation. Data suggest that a substance is produced in phytohemagglutinin-treated leukocyte cultures which is capable, on transfer to fresh leukocytes, of inducing blast cell formation and enhancing virus replication.*

A variety of viruses have been demonstrated to replicate to low titer in human leukocyte cultures (*1*). As an approach to the investigation of the role of the human leukocyte in the host response to viral infections, we have developed in the laboratory a model system consisting of vesicular stomatitis virus (VSV) and human leukocyte cultures. Vesicular stomatitis virus produces a mild disease in animals and man (*2*). It was chosen because it replicates in a wide range of cell culture systems (*3*, *4*) and, though interferon-sensitive, does not itself induce interferon in infected cells (*4*). Phytohemagglutinin (PHA), an extract of the red kidney bean, *Phaseolus vulgaris*, was employed in these studies because it has been demonstrated to induce an interferon in human leukocytes (*5*) and thus might have a potential role in augmenting host resistance to the virus infection. We thought that PHA might also stimulate VSV replication because of its stimulatory effect on macromolecular synthesis in normal leukocytes (*6*) and its ability to induce replication of herpes simplex (*7*) and mumps (*8*) viruses in human leukocyte cultures.

Whole blood was obtained by venapuncture from healthy adults and placed in sterile tubes containing 0.2 ml of heparin per 12 ml of blood. Leukocytes were obtained by incubating the blood at 37°C for 1 to 2 hours, permitting sedimentation of cellular elements. The leukocyte-rich plasma supernatant was aspirated and centrifuged at 1000 rev/min for 10 minutes. The plasma was removed and the pellet, consisting of leukocytes and a small number of red cells, was resuspended in warm growth medium consisting of Eagle's minimum essential medium supplemented with tryptose phosphate broth (4 percent), unheated fetal calf serum (10 percent), sodium bicarbonate (1.75 g/liter), penicillin, and streptomycin. All cell culture vessels were gassed with 5 percent CO_2 in air and incubated at 37°C. Immediately after being placed in culture, the leukocytes were counted in a hemocytometer, employing an erythrosin B vital dye exclusion technique (*9*) which showed cell viability consistently greater than 99 percent. The procedures employed in obtaining leukocyte cultures did not significantly alter the cell differential count from that of the donor's whole blood. The leukocytes were then diluted in growth medium to a final concentration of approximately 1×10^6 cells per milliliter.

Lyophilized commercial phytohemagglutinin-P (Difco Co.) was dissolved in phosphate-buffered saline (10 mg/ml) at room temperature, sterilized by filtration, and stored at 4°C. Each fluid portion of PHA was discarded after 3 weeks because there was a gradual depletion of activity with longer storage. Fifty μg of PHA in a 0.05-ml volume was added to each milliliter of cultured leukocyte suspensions containing 1×10^6 cells per milliliter; control cultures received identical volumes of phosphate-buffered saline. The PHA-treated and control cultures were then incubated either stationary or in a roller drum at 37°C for intervals varying between 1 minute and 3 days before VSV inoculation.

Vesicular stomatitis virus, Indiana strain, was added to screw-cap tubes containing leukocyte cultures at a virus-to-cell multiplicity ratio of approximately 1 : 1. The culture tubes were placed in a roller drum at 37°C for 1 hour to allow cellular adsorption of virus. The leukocytes were then washed by one cycle of centrifugation, and the cell pellet and leukocytes adhering to the glass were resuspended by pipetting in growth medium at the original volume. Portions of each culture were quickly dispensed into screw-cap culture tubes, 1 ml per tube, gassed, and rolled at 37°C. Cultures collected at intervals thereafter were frozen and thawed rapidly three times to disrupt the cells, and the virus yield in each tube was assayed in monolayers of mouse L cells. Virus titers were calculated

by the 50 percent end-point method of Reed and Muench (*10*). Experiments indicated that PHA had no effect on the sensitivity of L cells to VSV. Interferon was assayed by a cytopathic protection test in monolayer cultures of human fetal lung fibroblasts against a Sindbis virus challenge (*5*).

The replication of VSV in human leukocyte cultures is illustrated in the control culture curves presented in Figs. 1 and 2, although each of these figures describes other experiments to be presented below. These growth curves represent 2 of 70 different experiments in which VSV replication took place in control leukocyte cultures. They illustrate the variability of VSV replication in this system. In Fig. 1 new virus appeared between 6 and 8 hours after inoculation and peak virus titers of $10^{4.5}$ were reached at 24 hours. In Fig. 2 new virus appeared between 4 and 6 hours and peak virus titers of $10^{6.2}$ were reached at 24 hours.

When PHA was added to leukocyte cultures 16 hours prior to VSV inoculation (Fig. 1), the subsequent period of eclipse before the detection of new virus was extended by 2 hours. Moreover, virus replication in PHA-treated cultures failed to plateau at 24 hours but continued beyond this point and reached peak titers of $10^{6.8}$ at 48 hours. The PHA-treated cultures illustrated in Fig. 2 also produced higher titers of virus than their controls, but the enhancement was only sixfold as compared with the 180-fold enhancement illustrated in Fig. 1. These values represent the lower and upper limits of virus enhancement found in many experiments. A 30- to 40-fold enhancement is the mode. Similar virus growth curves are obtained irrespective of time of addition of PHA (1 minute to 72 hours before VSV).

Phytohemagglutinin is a known mitogenic agent (*11*). Thus the possibility existed that PHA induced an increased number of cells in cultures and that virus enhancement was due to virus replication in this increased number of cells. In point of fact, it was found that there was a 33- to 50-percent reduction of viable cells over a 4-day period in noninfected cultures compared with noninfected control cultures. In addition, peak virus yields in PHA-treated cultures occurred 24 to 48 hours before any demonstrable mitotic activity. These findings eliminated the possibility that the observed

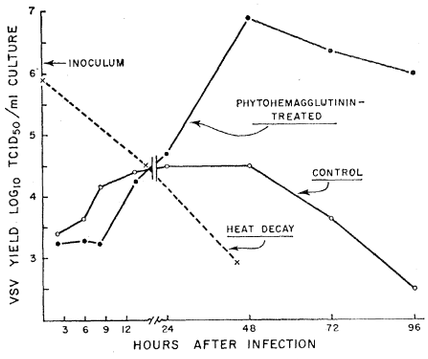

Fig. 1. The effect of phytohemagglutinin on VSV replication in human leucocyte cultures. The heat decay curve represents thermal inactivation of VSV at 37°C in (i) growth medium containing PHA and nonviable leukocytes and (ii) growth medium alone. Virus yield is expressed in terms of 50 percent tissue culture infective doses ($TCID_{50}$) per milliliter of culture medium containing approximately 1×10^6 leukocytes at the time of virus inoculation.

virus enhancement was due to replication in an increased number of cells in cultures treated with PHA.

The following results suggest that the extension of the virus eclipse period noted in PHA-treated cultures is mediated by an interferon. Interferon was not detected in control cultures, but was detected in those PHA-treated cultures in which the virus eclipse period was extended; in those few experiments where PHA did not elicit detectable interferon in the culture media, early virus replication was identical to that found in control cultures. There was a similar extension of the eclipse period when exogenous Newcastle disease virus–induced human interferon (*5*) was added to

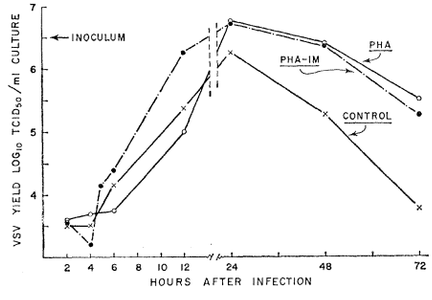

Fig. 2. Enhancement of VSV replication in human leukocyte cultures by a factor produced in and released from PHA-treated leukocytes. Culture treated with PHA, ○———○ (PHA); culture treated with medium from untreated incubated leukocytes, X——X (control); culture treated with medium from PHA-treated incubated leukocytes (washed free of residual PHA), ●–·–·–● (PHA-IM).

leukocyte cultures prior to virus inoculation.

Since the early delay in the virus eclipse period is probably mediated through an interferon produced in PHA-treated leukocyte cultures, we next investigated the possibility that another substance, distinct from PHA, but produced in leukocyte cultures treated with PHA, mediated VSV enhancement. We added PHA (50 μg per milliliter of culture) to leukocytes (4×10^6 cells/ml) and incubated the mixture for 1 hour at 37°C. The cells were then washed free of PHA by repeated cycles of centrifugation, and after the fourth cycle one-half of the supernatant of the growth medium was removed. It comprised the "wash" that would be used to test the effectiveness of cell washing in removing PHA. The leukocytes (viable numbers now reduced to approximately 1×10^6 cells/ml) were resuspended in the remaining growth medium and incubated for 20 hours at 37°C. The centrifuged supernatant medium was then collected; it comprised the "incubation medium" (PHA-IM). "Incubation medium" from non-PHA–treated leukocyte cultures (control) was prepared similarly. Portions of these three medium specimens were then added to fresh leukocyte cultures at a 1 : 2 dilution and incubated for 16 hours at 37°C. A fourth portion of leukocytes received PHA, and VSV was then inoculated into all four cultures. After a single cycle of centrifugation at 1 hour, the cells were resuspended in fresh volumes of their respective test mediums, and virus growth curves for all four cultures were determined as previously described.

Figure 2 depicts the results of one representative experiment. Both control and "wash" treated cultures produced nearly identical titers of virus; thus only the control curve is shown. Both PHA- and PHA-IM–treated cultures produced higher titers of virus than the control and "wash" treated cultures, and this enhancement was best seen at 48 hours. It should be noted that the ultimate degree of enhancement was nearly identical in both the PHA- and PHA-IM–treated cultures.

Evidence that the enhancement of virus replication noted above is due to a substance or substances produced by leukocytes in response to treatment with PHA, and not to PHA itself,

consists of the following: (i) In contrast to PHA, PHA-IM must be added back to cultures after virus inoculation and wash in order to enhance virus replication. This finding makes unlikely, but does not totally exclude, the possibility that small amounts of PHA were present in the PHA-IM preparation and caused virus enhancement when added back. (ii) In order to exclude the possibility that PHA-IM contained PHA, experiments were conducted which indicated that PHA-IM enhanced virus replication but did not agglutinate leukocytes; in a series of dilution experiments we were unable to separate the leukoagglutinating and enhancing properties of PHA. (iii) Experiments in collaboration with T. C. Merigan indicate that PHA-IM at high concentrations reversed the PHA-interferon-induced plaque reduction of VSV in a primary human foreskin fibroblast culture assay system; the mechanism of reversal remains to be determined. PHA alone had no effect on VSV plaque formation in this system.

In the search for the mechanism of PHA-induced enhancement of VSV in human leukocyte cultures, we have focused our attention on the lymphocyte transformed by PHA into blast cells (*11*). This approach is due in part to the repeated observation of a close correlation between blast cell formation and virus enhancement, which to our knowledge is a relationship not satisfactorily demonstrated before. Recent experiments indicate that PHA-IM, as well as PHA, induces blast cell formation in uninfected leukocyte cultures. Morphologic studies of PHA- or PHA-IM–treated cultures have revealed that VSV infection produces a tenfold reduction in the number of blast cells and marked damage to many of the remaining blast cells as compared with uninfected treated cultures. The observation that blast cells undergo cytopathic effects in infected cultures suggests that VSV replicates in this cell type. This conclusion is further supported by our recent finding that cultures of lymphocytes (99 percent homogenous) prepared in siliconized glass bead columns (*12*) are incapable of supporting significant VSV replication, but the addition of PHA induces both blast cells and VSV replication. The cell type supporting VSV replication in buffy coat cultures in the absence of PHA has not yet been determined. Thus it appears that VSV enhancement is intimately tied to blast cell formation and to those biochemical alterations implicit in this cellular transformation.

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Protein Synthesis during First Cleavage of Sea Urchin Embryos

I was not surprised to find contradictory results, published a month apart, concerning the rate of protein synthesis during the first cleavage in the sea urchin embryo. Gross and Fry (*1*) found no decline in the rate of protein synthesis in fertilized eggs of *Strongylocentrotus purpuratus* through the first cycle of cell division. Sofer, George, and Iverson (*2*), working with the fertilized eggs of *Lytechinus variegatus*, found a decline in the rate of protein synthesis as the cells began the first cell division.

For the past 2 years I have been working on this same problem with the eggs of *Lytechinus pictus* and *Strongylocentrotus purpuratus*, and, much to my bewilderment, I have obtained both results (that is, the continuation and the decline of protein synthesis as the eggs entered the first mitotic division). As far as I have been able to determine, the disparity in my own results is not due to (i) species used; (ii) the amino acid used (both Gross and Fry, and Sofer, George, and Iverson used C^{14}-leucine; I have used C^{14}-valine and C^{14}-phenylalanine); or (iii) the time of year or the region in which the eggs were collected.

After 2 years of repeating experiments and wondering how many more times they should be repeated, and refraining from publishing either of these conflicting results, I find that I have been scooped on both counts.

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10 October 1966