Table 1. Inhibition of demyelination by antiserum to IgA. The proportion of cultures undergoing demyelination when exposed to sensitized lymph node cells is compared to the proportion undergoing demyelination when exposed to cells plus rabbit antiserum to IgA.

Day node cells taken after immunization	Cultures demyelinated	
	Node cells plus rabbit antiserum to rat IgA	Node cells only
7	*0/2	3/3
8	1/2	2/2
9	0/3	3/3
10	2/2	1/1
11	0/2	3/6
12	0/2	2/2
12	1/3	3/6
12	1/3	1/3

* One culture showed incipient degeneration in two fibers.

(Table 1). This difference is significant at 0.05 by the chi-square test. The incidence of demyelination in the controls corresponds closely with our accumulated experience to date: 37 out of 57 (65 percent) cultures that had been given lymphocytes taken from animals 7 to 12 days after immunization have demyelinated. Demyelination was first evident at 72 hours and was extensive by 96 hours when the experiment was terminated (Fig. 1*b*).

Data with other specific antiserums suggest that this effect is specific. Addition of specific rabbit antiserum to rat IgG to the feeding medium has failed to block the myelin-destructive capacity of sensitized cells. Addition of a rabbit antiserum to rat α_2-macroglobulin serum has similarly been ineffectual.

Lymph node cells that have been treated with high-frequency sound do not demyelinate the cultures (*9*); this suggests that some function of the intact cells is necessary for demyelination to occur. They could elaborate an IgA class antibody to a peripheral nerve constituent. This antibody might attack the myelin directly or become firmly attached to lymphocytes, making them active cells. Alternatively, the sensitized cells might have had a cytodestructive antibody of the IgA class attached to their surfaces in the lymph nodes. In any of the above-mentioned cases, an antiserum to IgA would act by interposing itself between the IgA antibody to nerve and the peripheral nerve antigen.

A third possibility is suggested by the experiments of Sell and Gell (*10*). They found that antiserum to allotype (directed against genetically determined antigenic sites on rabbit immunoglobulins) added to rabbit lymph node cell cultures would trigger morphologic alteration and proliferation of the type seen after addition of specific antigen to sensitized cells. Possibly our rabbit antiserum to IgA triggered a similar response in the cells added to our cultures, directing them away from the cytodestructive response they make ordinarily. Finally, it is possible that antiserum to IgA binds to receptor sites on the peripheral nerve cultures impeding access of the sensitized cell or its active product to the peripheral nerve antigen.

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Polysomes and Protein Synthesis in Cells Infected with a DNA Virus

Abstract. *In HEp-2 cells infected with herpes simplex virus the rate of protein synthesis at first decline, is stimulated between 4 and 8 hours after infection, and progressively and irreversibly declines from 9 to 16 hours later. The increase and decrease in rates coincide with corresponding changes in the amounts of cytoplasmic polysomes and amounts of labeled amino acids in nascent peptides bound to polysomes. The data indicate that (i) early and late inhibition and intervening stimulation of protein synthesis are due to the corresponding breakdown and formation of polysomes, and (ii) the bulk of viral proteins is probably made on cytoplasmic polysomes.*

Herpes simplex virus inhibits the uptake of precursors into DNA, RNA, and proteins (*1*) immediately after infection. With regard to protein synthesis in infected HEp-2 cells we now report two findings. First, the pattern of protein synthesis in the cytoplasm of infected cells consists of (i) an initial decline lasting approximately 3 hours, (ii) a period of stimulated synthesis from about 4 through 8 hours after infection, and (iii) a progressive, irreversible decline. This pattern corresponds to the pattern of amino acid uptake into peptides of whole infected cells reported previously (*1*). Second, the stimulation and inhibition of protein synthesis coincide with increases and decreases, respectively, in the amounts of cytoplasmic polysomes and with corresponding changes in the uptake of labeled amino acids into nascent peptides. The data thus indicate that breakdown and formation of cytoplasmic polysomes account for the pattern of protein synthesis in infected cells.

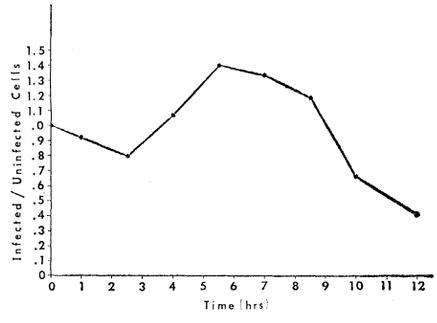

Fig. 1. Variation in specific activity of trichloroacetic acid–insoluble peptides extracted from the cytoplasm of cells pulse-labeled with C^{14}-amino acids at different times after infection with herpes simplex virus. The specific activity is corrected for that obtained from uninfected cells.

The pattern of protein synthesis in HEp-2 cells infected with herpes simplex virus strain mP (*2*) was determined as follows: At 1 to 2 hour intervals after infection, portions containing 2×10^7 infected HEp-2 cells in suspension were pulse-labeled for 15 minutes in 4 ml of a medium containing 5 μc of reconstituted C^{14} amino acid mixture from labeled protein hydrolyzate (Schwarz BioResearch Incorporated, Orangeburg, New York). Incorporation was stopped by pouring the cell suspension onto a frozen slurry of phosphate-buffered saline. The cells were washed, resuspended in 2 ml of reticulocyte standard buffer (RSB) (*4*) for 10 minutes, and then disrupted in a tight-fitting Dounce homogenizer selected and calibrated to break no more than 2 percent of nuclei. The cytoplasmic extract was separated from nuclei and membranes by centrifugation. Portions were removed for determination of protein by the Lowry method and of radioactivity of the fraction insoluble in trichloroacetic acid. The specific activity of pulse-labeled cytoplasmic extract follows a pattern (Fig. 1) similar to that of whole-cell homogenate (*1*); that is, there is an initial decline until 3 hours after infection, a period of increased specific activity between 4 and 8 hours after infection, and finally a progressive, irreversible decline. This pattern is highly reproducible. As indicated (*1*), the decrease in amino acid incorporation during the first 3 hours of infection is a characteristic feature of herpes virus infection of HEp-2 cells.

The variation in specific activity of cell extracts after pulse labeling with amino acids is due to a corresponding increase or decrease in the rate of cytoplasmic protein synthesis and not to variation in the specific activity of the amino acid pool. This conclusion is based on the following experiment. At intervals after infection, suspensions of 8×10^7 infected HEp-2 cells were labeled for 15 minutes and treated as described. A portion of whole-cell homogenate was removed for determination of radioactivity. The remainder was centrifuged and the supernatant containing the cytoplasmic extract was layered on sucrose gradients (15 to 30 percent by weight) made in RSB and centrifuged at 25,000 rev/min for 65 minutes in a Spinco SW-25 rotor. Fractions (1 ml) were collected through a flow cell monitored for 260-mμ absorbance in a Gilford recording spectrophotometer. The fractions were mixed with equal volumes of 15-percent trichloroacetic acid, and precipitates were collected on Millipore HA filters. These were washed with trichloroacetic acid, 75-percent alcohol containing 2 percent potassium acetate, and 75-percent alcohol; they were then air-dried and immersed in toluene-base scintillation mixture for counting C^{14} disintegrations. The optical densities (OD) and radioactivities of the density-gradient fractions are shown in Fig. 2. The total amount (OD) of polysomes

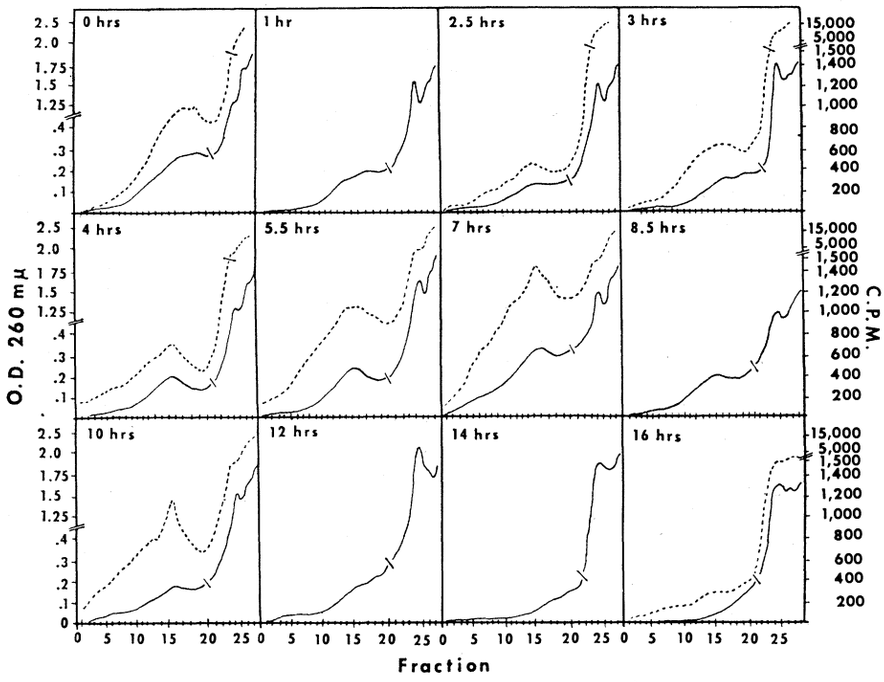

Fig. 2. Optical density (*OD*) and radioactivity (*CPM*) of cytoplasmic extracts sedimented in sucrose density gradients (15 to 30 percent by weight) immediately after 15-minute-pulse labeling and extraction from HEp-2 cells at times (indicated) after infection. Fraction 1 is the bottom of the gradient. Solid line, optical density; dashed line, counts per minute.

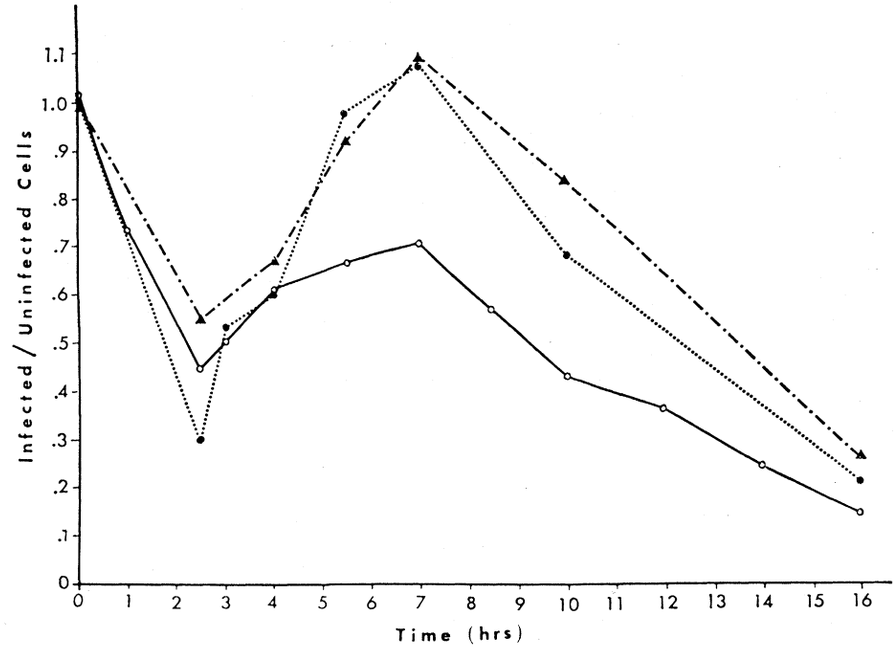

Fig. 3. Variation in total optical density of the polysome region (○——○), total radioactivity of nascent peptides bound to polysomes (●- - - -●), and total radioactivity incorporated into nascent peptides found in whole-cell homogenates (▲- - - -▲) at different times after infection. Corrected for activity in uninfected cells.

and the total radioactivity of amino acids in nascent protein bound to polysomes at different times after infection are shown in Fig. 3. The pattern of labeled amino acid incorporation into acid-insoluble peptides of whole-cell homogenates is also shown in Fig. 3. The striking feature of these data is the remarkable correlation between the specific activity of peptides from homogenates of pulse-labeled cells (*1*), the specific activity of peptides extracted from the cytoplasm of pulse-labeled cells (Fig. 1), the amounts of cytoplasmic polysomes recovered, and the amounts of pulse-labeled peptides bound to these polysomes at different times after infection. These data indicate that inhibition of protein synthesis early and late after infection is due to a corresponding breakdown of cytoplasmic polysomes, that the increase in protein synthesis between 4 and 8 hours after infection is due to stimulation of cytoplasmic polysome formation, and that the bulk of viral proteins is probably made in the cytoplasm. In this connection it is noteworthy that the polysomes formed after 4 hours of infection sediment more uniformly and slightly faster (Fig. 2) than those of uninfected cells.

Our results bear on two aspects of virus multiplication in animal cells. First, the inhibition of host protein synthesis with herpes simplex, a DNA virus, is the immediate result of breakdown of cytoplasmic polysomes. The mechanisms of inhibition of protein synthesis in cells infected with DNA virus shown here and with RNA virus (*5*) appear similar. However, in both instances the cause of breakdown of host polysomes is unknown.

Second, the data suggest a discrepancy in the time and site of synthesis of viral proteins and their utilization. Our data indicate that viral proteins are mostly made in the cytoplasm between 4 and 12 hours after infection. On the other hand, more than 95 percent of the final yield of virus is made between 12 and 17 hours after infection (*6*). The discrepancy in the time of synthesis of virus constituents and their utilization may be due to a physical separation of the sites of viral DNA and protein synthesis. Although mature virus accumulates in the cytoplasm, viral DNA is made and acquires at least one coat in the nucleus (*7*). The time required to transport protein subunits into the nucleus may account for the interval between the time of synthesis and utilization of viral components.

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Calibration of β Gauges for Determining Leaf Water Status

Abstract. *The recent development of β gauges for the indirect determination of the water status of plants has had a widespread impact on plant ecological-physiological research. Calibration of these instruments has, however, been tedious and cumbersome, involving repeated simultaneous measurements of β absorption and leaf water content. This procedure can be much simplified, as long as the β absorption at a single known relative water content is measured, together with the turgid and dry weights of a sample of leaf discs.*

Beta-particle gauging is being used increasingly to provide continuous, nondestructive measurement of the water status of plant leaves (*1*). A source of β particles is arranged on one side of a leaf and a radiation detector on the other. The absorption of particles by the leaf depends on the energy of the β particles and on the mass per unit area of the leaf. In experiments lasting only a few hours, changes in the mass per unit area, the "effective thickness," are largely the result of changes in the water content of the leaf, since changes in dry-matter content cause only very small changes in effective thickness. Changes in actual leaf thickness closely parallel changes in effective thickness, but direct measurements of the former quantity lack the accuracy and sensitivity of a β gauge and disturb the microenvironment around the leaf.

Leaves differ one from another in effective thickness according to species, age, position on the plant, and environmental conditions during development. Even if the area of the lamina occupied by main veins is excluded, differences in effective thickness also occur from point to point on the leaf surface. Hence, every time a β gauge is used, it should be calibrated for that area of the leaf between source and detector, and, strictly speaking, the results obtained are valid only for that area. Clearly, if this method is to be used extensively, a simple and reliable method of calibration is required which involves as few measurements as possible.

Calibration in terms of leaf water potential (*2*) is most desirable, but is cumbersome because of the methods at present available for determining water potential. Relative water content (also called relative turgidity) (*3*) can be interpreted as water potential with an acceptable degree of accuracy by means of sorption curves relating the two (*4*). Relative water content also provides a suitable basis for calibration because it can be related to effective thickness, in the following manner.

If R is the relative water content (percent) defined as

$$R = \frac{100\,(W_f - W_d)}{W_s - W_d} \qquad (1)$$

and D is the effective thickness (mg cm^{-2}) given by

$$D = \frac{W_f}{A} \qquad (2)$$

Eq. 2 can be substituted in Eq. 1 to give

$$R = 100\left(\frac{AD - W_d}{W_s - W_d}\right) \qquad (3)$$

where W_f, W_s, and W_d are the weights (mg) of a tissue sample of area A (cm^2), when sampled, fully turgid ($R = 100$), and oven dried, respectively. (In fact $W_s \geqq W_f \geqq W_d$.) The slope of the relationship between relative water content and effective thickness, dR/dD, is then obtained by differentiation, giving

$$\frac{dR}{dD} = \frac{100\,A}{W_s - W_d} \qquad (4)$$

Thus the slope is determined by the water content at $R = 100$, and, knowing the slope, the intercept can be obtained from one estimate of D at a