non-waxy seeds (Table 1), and both have considerably higher activity than whole seed preparations from *non-waxy* seeds. Table 1 shows also that although the activity of preparations from *waxy* seeds from which the embryos have been removed is significantly reduced as compared with whole seed preparations, there is still measurable activity. Microscopic examination of such preparations after staining shows that 2.5 percent of the starch granules they contain stain blue, and these are sufficient to account for the transferase activity if it is assumed that the remaining blue-staining starch granules have the same activity as those isolated from the embryos. The maternal tissue surrounding the endosperm cannot be cleanly separated from the endosperm and must be pared away by a series of cuts. In a starch-granule preparation made from blocks of endosperm tissue so obtained from *waxy* seeds, 0.4 percent of the granules stained blue because of remnants of maternal tissue; such preparations also showed much less activity (0.25 mμmole ADP released per milligram of starch).

Table 1 shows a greater absolute loss of activity in enzyme preparations from embryectomized *non-waxy* seeds than from embryectomized *waxy* seeds. The embryos of *waxy* and *non-waxy* seeds constitute the same proportion of the seed by wet weight, and the preparations of *non-waxy* embryos have somewhat lower activity than do the preparations from *waxy* embryos. By using mixtures of starch granules from *non-waxy* embryos and *non-waxy* embryectomized seeds, reconstitution experiments were conducted which failed to show any synergistic effects that could account for the greater activity loss in preparations from *non-waxy* embryectomized seeds. It is possible that during the embryectomy of *non-waxy* seeds, tissue (the endosperm) which contains starch granules with enzymatic activity is exposed, and that there is some loss of activity during collection of sufficient material for processing. Since there are apparently no starch granules with enzymatic activity in the endosperm of *waxy* seeds, the only loss of activity in preparations from embryectomized seeds is that directly attributable to the loss of the embryo.

We have not been able to prepare starch granule samples from the endosperms of *waxy* seeds without some contaminating blue-staining granules from the closely adherent maternal tissue. Thus we cannot demonstrate that starch granules from the endosperm are devoid of transferase activity. But it is demonstrable that whole seed preparations of *waxy* stocks have a low percentage of blue-staining granules that have high transferase activity. Methods of preparation that reduce the number of blue-staining granules (embryectomy, for example) reduce transferase activity almost proportionately. We suggest that all transferase activity noted in whole seed preparations from *waxy* seeds is referable to these enzymatically active starch granules from the embryo and maternal tissue and that the endosperm starch granules do not possess the transferase system. Thus, there must be another system or other systems conditioning the formation of α 1–4 linkages in amylopectin as originally suggested (*1*).

This unique distribution of enzymatically active starch granules in the seeds of *waxy* mutants raises an interesting problem in the regulation of enzyme synthesis. If there is a single structural gene for this enzyme, ADP-glucose-starch glucosyl transferase, then in *non-waxy* plants it functions in both gametophytic and sporophytic tissue but in *waxy* plants, only in the sporophytic tissue. Alternatively, there could be two structural genes for transferase, one of which is active in sporophytic tissue but inactive in gametophytic tissue and the other inactive in sporophytic tissue, but active in gametophytic tissue. Then all the 30 known *waxy* mutants represent mutations in the latter structural gene.

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Photoflashes: A Potential New Tool for Control of Insect Populations

Abstract. *Imported cabbageworms,* Pieris rapae (*L.*), *were reared in cans exposed to light from fluorescent lamps for 10 hours daily. When larvae were exposed to daily electronic photoflashes scheduled 3 to 4 hours after the fluorescent lamp was turned off, pupae failed to diapause. Effective wavelengths are lower than those controlling photoperiodism in plants. The effective energies are about 1 joule per square centimeter.*

Reduction of overwintering insect populations is of practical value in the control of the boll weevil (*1*) and has been a part of good farming practice for years. In the temperate zone many insects survive the rigors of winter in a diapausing state which is controlled by hormones. The possible use of hormones in insecticidal formulations has been considered (*2*). However, the use of photochemical reactions to regulate changes in hormones within the insect has not been considered as a means of control. It would be simpler to reach certain insects with light than with insecticidal formulations, and the

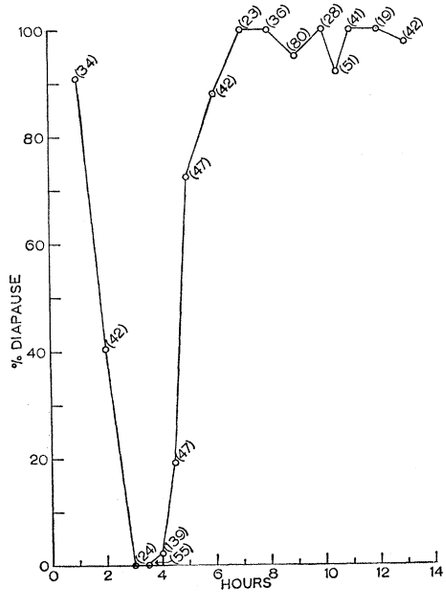

Fig. 1. The incidence of pupae in diapause when photoflashes are applied daily to *Pieris rapae* larvae at varied schedules after 10 hours of light. "Hours" are measured from the end of the 10-hour light period. The parentheses enclose numbers of insects tested at each flash schedule. Larvae were held at 17° to 20°C and fed collard leaves.

use of light should also circumvent residue problems. Intense, mobile (even airborne) sources of light of narrow frequency ranges have been made available as a result of new advances in light technology. These and inexpensive mercury or xenon lamps deserve serious consideration for field tests designed to control insect pests selectively by inhibiting their diapause.

Interruption of the darkness by a 5-minute exposure to light has been reported to inhibit diapause in the imported cabbageworm, *Pieris rapae* (L.) (*3*). This 5-minute interruption is considerably shorter than times found to be effective on other species (*4*), but it is not the limit. When photoflashes from a xenon lamp (~5600°K) with a discharge half-life of 0.0008 second (*5*) were applied to larvae of *P. rapae* (*6*) 4.0 hours after the end of a 10.0-hour light period in a 24-hour cycle, only 3 pupae of 139 entered diapause. Without the photoflashes, 100 of 105 pupae diapaused. Data in Fig. 1 indicate that daily photoflashes are most effective when applied 13 to 14 hours after the onset of a 10-hour light period. Supplementary flashes applied 13 to 14 hours after daybreak to larvae of the generation which normally overwinters should prevent diapause in the cabbageworms. At 20°C, 10 hours of light and a flash 14 hours after the onset of light are almost as effective as 14 hours of light (which gave no diapause in 126 insects) or continuous 24-hour light (which gave no diapause in 49 insects).

Data now available strongly suggest that the wavelengths which are most effective for *P. rapae* (Table 1) and other insects (*7*) will not interfere with phytochrome effects in plants. Furthermore, the time that is effective differs from the most effective period in plants (*9*). The total energy employed at the wavelengths effective for *P. rapae* (Table 1) seems lower than that which has given responses in reproduction of birds and mammals (*10*). Since a watt is 1 joule/sec, 5 mw for 300 seconds is 1.5 joules (Table 1). We have found that light from ultraviolet fluorescent lamps (F4T5/BL) works as well as that from white lamps (F4T5/CW) for 10-hour photoperiods with interrupting light under conditions reported (*3*). It should be possible then to influence diapause induction in some insects without adverse reactions in birds, mammals, or plants.

Table 1. Energy required for inhibition of diapause in *Pieris rapae*. Larvae were exposed daily to 55 to 275 lu/m² of cool-white fluorescent light for 10.0 hours. This was followed by dark. Then larvae were exposed, in a rack of 14 glass tubes, to the spectra of wedge interference filter (*11*) which was projected in the slide holder of a projector (*11*), the source light being either a 100-w or 500-w tungsten lamp. Focus was adjusted upon the rack, and wavelengths were calibrated with emission bands from a mercury lamp. Wavelengths were rechecked and intensities were measured with a simple spectroradiometer designed, constructed, and calibrated by K. H. Norris (*8*). Individual tubes of larvae were exposed to spectral bandwidths of about 30 nm. Since the second order of interference was employed in the filter, the blue end of the spectrum is overlapped by the far red from the first order interference, and the red end is overlapped by blue from the third order. The paired numbers represent the two orders transmitted to a particular tube. The light from the spectra was scheduled to turn off at 4.0 hours after the end (or 14 hours from the start) of the 10.0-hour daily photoperiod..

Wavelength (nm)	100-w Projection lamp with no filter				500-w Projection lamp with water filter		
	Irradiance (mw/cm²)	Ratio of insects in diapause exposed for:			Irradiance (mw/cm²)	Ratio of insects in dispause exposed for	
		15 sec	60 sec	120 sec		300 sec	1800 sec
433	0.2	10/10	10/12		0.8	0/2	0/9
823	0.3				1.4		
458	0.3	6/7	6/8	2/4	4.3	0/3	0/11
883	0.3				1.2		
485	0.8	6/6	8/8	0/2	5.5	0/4	0/9
942	0.4				0.8		
509	1.6	10/13	7/8	1/1	12.1	1/1	0/9
992	0.6				0.7		
535	1.9	5/6	5/5	8/8	15.7	1/3	0/12
1046	0.5				2.0		
557	2.2	3/3	3/3	6/6	20.4	1/5	0/9
1088	0.5				1.6		
579	2.3	3/3	11/11	5/5	48.0	0/1	1/7
1124	0.2				0.4		
599	2.3	3/3	12/12	1/1	18.1	2/4	1/12
622	2.6	3/3	8/8		16.9	0/4	0/12
642	2.6	3/3	6/6	1/1	13.3	0/2	0/5
447	0.6	8/8	6/6	1/1	3.2	0/2	0/5
664	2.7				10.8		
461	1.1	4/4	9/10		4.4	1/5	1/8
685	2.9				9.4		
476	1.5	3/3	1/4		2.6	0/1	0/6
705	2.3				3.9		
485	1.0	5/5	0/5	1/2	1.4	0/3	0/6
715	2.2				2.2		

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Inhibition of Specific Binding of Antibody to the $Rh_0(D)$ Factor of Red Cells in Antibody Excess

Abstract. *The uptake by red cells of I^{131}-labeled nonagglutinating antibody to the $Rh_0(D)$ factor was dependent on the proportion of cells to antibody during sensitization. Inhibition of antibody binding by red cells in the region of antibody excess occurred with $Rh_0(D)$ positive red cells that were both papain modified and untreated; the inhibition was independent of the Rh phenotype.*

The relation between the quantity of erythrocyte-bound antibody and the ratio of antigen to antibody at the time of reaction is of both practical and theoretical importance in immunohematology and blood grouping. The detection of blood-group antibodies depends on conditions optimum for demonstrating an antigen-antibody reaction. Occasionally in serological investigations a hemagglutinating serum fails to give visible reactions when tested with red cells until the serum is diluted. This phenomenon, which has been referred to as a prozone or zoning, is poorly understood and undoubtedly plays an important role in the hemagglutination reaction and other antigen-antibody systems in which antigenic determinants are found on large, particulate, multimolecular structures such as cells, bacteria, phage, and so forth.

This report deals with the binding of I^{131}-labeled antibody to red cells containing the $Rh_0(D)$ antigen when the amount of either antibody or antigen is constant. The $Rh_0(D)$ factor represents a genetically determined antigenic configuration on the red cell stroma which chemically is poorly defined and is present in approximately 85 percent of Caucasian blood samples (*1*). Individuals who are $Rh_0(D)$ negative, if exposed to erythrocytes containing $Rh_0(D)$ by either transfusion or pregnancy, may respond by forming an antibody to the $Rh_0(D)$ antigen.

A globulin fraction was obtained from high-titered antiserum by the ethodin (Rivanol) precipitation procedure (*2*). The globulin fraction was labeled with I^{131} by the monochloride technique (*3*), and antibody containing eluates were obtained by heat elution from $Rh_0(D)$-positive stroma which had been sensitized with the labeled globulin fraction with techniques described previously (*4*, *5*). Table 1 lists the properties of the labeled globulin and the eluate which contained the I^{131}-labeled antibody to the $Rh_0(D)$ factor. The radioactivity of sensitized red cells and antibody solutions was determined by gamma-ray spectrometry with a well-type crystal scintillation detector.

Portions of a red cell suspension (approximately 10 percent) were incubated at 37°C for 60 minutes with the eluate containing the labeled antibody to the $Rh_0(D)$ antigen at *p*H 6.5, ionic strength 0.26. After reaction, the sensitized cells were washed four times with 0.15*M*, *p*H 6.5 phosphate buffer, and the I^{131} bound to the red cells was determined. Papain-modified cells were prepared as described previously (*5*).

Figure 1 shows the results obtained when a constant volume of antibody-containing eluate (0.38 μg of nitrogen per milliliter of eluate) was added to a series of tubes containing different volumes of a 10.5-percent suspension of red cells containing $Rh_0(D)$ (type A, Rh phenotype R_1R_1). The total volume was adjusted with buffer to 4 ml for all tests. As the red cell concentration was increased, the cell-bound nitrogen increased rapidly and began to approach a plateau in the region of antigen excess. When 3 ml of a 10.5-percent cell suspension was incubated with 1 ml of eluate (0.38 μg of nitrogen), a total of 0.109 μg of nitrogen or 28.6 percent of the total I^{131} was bound to the red cells. An average of 29.9 percent of the radioactivity of this eluate preparation was specifically absorbed by $Rh_0(D)$-containing red cells when a given volume of the eluate was repeatedly absorbed with different portions of red cells. This value is in good agreement with the value of 28.6 percent (Fig. 1) obtained in marked antigen excess (approximately 10 times optimum) when a constant volume of antibody was used to sensitize progressively increasing volumes of red cells.

Figure 1 also presents the data as nitrogen bound per milliliter of packed red cells (defined by 5 minutes centrifugation at 12,000*g* or about 10^{10} red cells). The maximum I^{131} bound per milliliter of packed red blood cells occurred when 0.021 ml of packed cells was incubated with the 1 ml of the eluate containing labeled antibody to the $Rh_0(D)$ antigen and amounted to 1.45 μg of nitrogen per milliliter of packed cells. As the cell concentration was increased with a constant volume of antibody the nitrogen bound per milliliter of packed cells decreased, so that in antigen excess there was only 0.35 μg of nitrogen per milliliter of packed cells. When the red cell concentration was reduced below the concentration which gave optimum binding of antibody, 0.021 ml, there was a suggestion that the quantity of antibody bound to the red cells decreased (1.45 to 1.33 μg of nitrogen per milliliter of cells) even though under these conditions excess antibody was present.

To determine whether the decreased

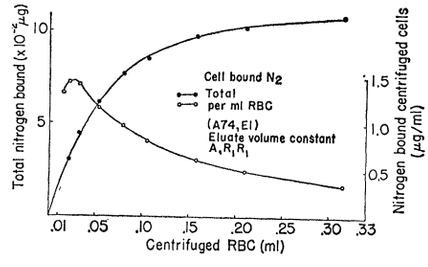

Fig. 1. Red cell binding of I^{131}-labeled antibody to the $Rh_0(D)$ red cell factor when the reaction is carried out with constant antibody concentration and varying red cell concentration (antigen). Results are shown as total nitrogen bound to all the red cells present in the reaction mixture and as nitrogen bound per unit volume of red cells (ml). The red cells were group A, and phenotypically were homozygous for the Rh factors $Rh_0(D)$, rh'(C), and rh''(e). This information is designated in shorthand form on the figure as A, R_1R_1. The reaction volume was adjusted to 4 ml for all tests by the addition of 0.15*M*, *p*H 6.5 phosphate buffer. A control suspension of $Rh_0(D)$ negative red cells bound only 0.11 percent of the I^{131} added to the cells.