Table 1. Effects of crude synthetic juvenile hormone on the metamorphosis of *Aedes aegypti*. For the control, solvent alone was applied to the mature fourth stage larvae.

Stage	Treated animals	
	Number	Emerging adults (%)
Early 4th stage larvae*	75	72
Middle 4th stage larvae†	95	93
Late 4th stage larvae‡	49	31
Mature 4th stage larvae§	99	0
Pupae	12	100
Control	12	100

* Adult eyes invisible. † Eyes linear. ‡ Eyes crescentic. § Eyes with truncate apex; pupal air trumpets invisible.

synthetic juvenile hormone analog on the yellow fever mosquito, *Aedes aegypti* (*5*).

The analog was synthesized (*3*) and used without further purification. One percent (weight/volume) of the hormonally active oil was dissolved in acetone and stored in the refrigerator. In each experiment, a measured volume (0.05 to 0.3 ml) was stirred into the contents of glass jars containing 200 ml of distilled water and a homogeneous group of 10 to 20 mosquito larvae at specific stages in development. In control experiments the water was treated with the solvent alone or with an equivalent solution of farnesol. The temperature was maintained at 21° ± 2°C, and food (pulverized pellets of Purina rabbit chow) was added after the 1st day of treatment.

Larvae at the outset of the final (fourth) larval stage were placed in 200 ml of water containing 3 mg of the crude synthetic material; all underwent pupation, but no adult mosquitoes emerged. Metamorphosis was blocked at stages ranging from pupae to fully formed pharate adults incapable of escaping from the old pupal cuticle. In parallel experiments in which the water was renewed daily and treated with fresh hormone, about one-fourth failed to pupate and, of the remainder which pupated, only a few were able to begin adult development before their development was blocked. The same result was obtained in experiments performed on mature fourth-stage larvae.

Table 2. Effects of 1 day of exposure to crude synthetic juvenile hormone on the metamorphosis of mature fourth stage *Aedes aegypti* larvae.

Dose (mg/200 ml)	Larvae (No.)	Emerging adults (%)
0	54	96
0.05	72	93
0.1	64	59
0.5	80	13
1.0	64	5
3.0	188	0

In control experiments in which larvae were exposed either to the solvent alone or to an equivalent solution of farnesol, virtually all individuals underwent normal metamorphosis.

The effects of the synthetic hormone were studied in further detail. Homogeneous groups of larvae were exposed for 1 day to 3 mg of crude synthetic material in 200 ml of water and then transferred to distilled water. Results show that mature fourth-stage larvae just prior to metamorphosis are most sensitive to the hormone analog (Table 1). Sensitivity at this stage was calibrated by exposure to graded doses for 1 day. Forty percent were killed by about 1 part of the crude synthetic material in 2 million parts of water (Table 2).

When dispersed in water, the material was fully effective for at least 1 day. However, by the end of 1 week, the dispersion became relatively ineffective, presumably because of breakdown of the hormone analog by bacteria and other agents.

Males which survived and emerged as adults in the presence of low doses of hormone were affected in a surprising way. After prior exposure to hormone, many were unable to accomplish the 180-degree rotation of the genitalia which is necessary for successful reproduction. Moreover, we have confirmed the ability of the synthetic hormone to block the embryonic development of mosquito eggs, a phenomenon already reported for three other species of insects (*6*).

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Multiple Sclerosis: Correlation between Immunoglobulin-G in Cerebrospinal Fluid and Brain

Abstract. *There is a positive correlation between the concentration of immunoglobulin-G in plaques of demyelination due to multiple sclerosis (as well as in white matter of normal appearance) and the concentration of this globulin in the cerebrospinal fluid. The tentative conclusion drawn from these results was that, in patients with multiple sclerosis, the increase in immunoglobulin-G in the cerebrospinal fluid is a reflection of an excess of this globulin in the brain.*

Kabat *et al.* (*1*) have reported that the percentage of total protein in the form of γG (immunoglobulin-G) was elevated in the cerebrospinal fluid of most patients with multiple sclerosis. Because the percentage of γG in the serum was normal, they suggested that the increase in the cerebrospinal fluid might be due to "diffusion" from excess γG formed within the tissues of the central nervous system. No further evidence has yet been presented to support this hypothesis (*2*), except for our earlier reports (*3*).

Approximately 20 ml of fluid was removed from the third ventricle at the time of autopsy from seven of ten multiple sclerosis patients; the cerebrospinal fluid of nine of the ten patients had been examined when the patients were still living. The fluid was cleared of cellular debris by centrifugation (900 relative centrifugal force RCF) for 15 minutes and passage through a Millipore filter (0.45 μ); storage was in a sterile airtight tube at 4°C. The γG was determined by the immunochemical method of Kabat *et al.* (*4*), and the total protein was determined by a colorimetric method—that is, biuret reagent was added to a washed trichloroacetic acid precipitate. For comparison, normal cerebrospinal fluid, obtained from 89 medical students, was processed and examined in the same way.

The mean for total protein (mg/100 ml) and γG (percentage of total protein), plus or minus the standard deviation (SD) for the spinal fluids of 89 medical students was 38 ± 10 mg per 100 ml and 9.9 ± 2.6 percent γG, respectively. For the patients the individual values for cerebrospinal fluid and ventricular fluid total protein and percent of γG are shown in Fig. 1 and Table 1.

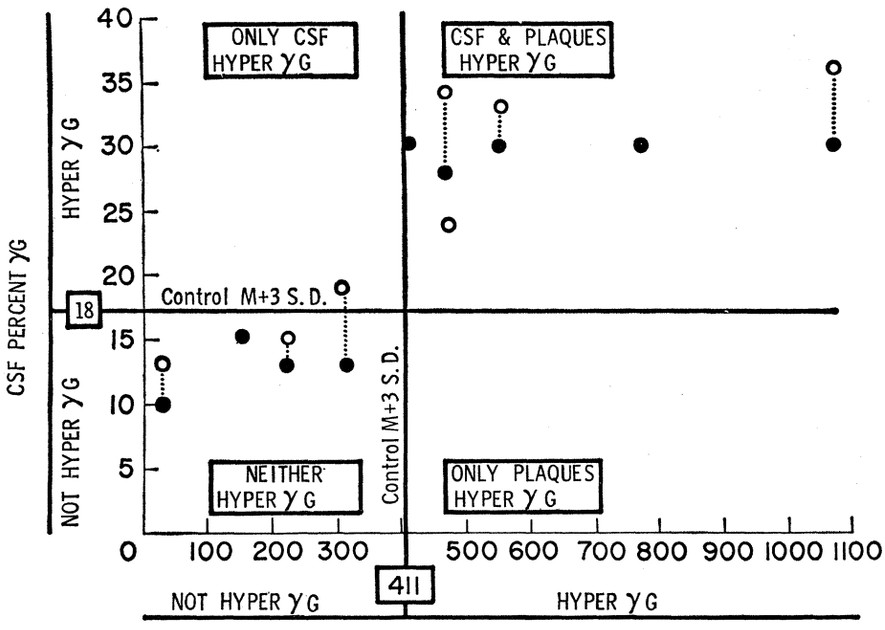

Fig. 1. Positive correlation between the γG in plaques of demyelination due to multiple-sclerosis and that in the patient's cerebrospinal fluid obtained before death (solid circles) or in the ventricular fluid obtained after death (open circles).

The left cerebral hemisphere was obtained at autopsy from the ten patients who died of multiple sclerosis; and from ten patients whose deaths were from other causes (that is, they did not have multiple sclerosis or any other gross or microscopic structural brain damage). The unfixed brain tissue was cut into 3-mm coronal sections at room temperature by an electric slicer. Each section was placed in a plastic bag, labeled, sealed, and frozen; the storage temperature was −90°C.

On a thawed section, bits of normal-appearing white matter were grossly dissected free of demyelinated plaques. Each dissection was accompanied by a punch biopsy, which was used for histological analysis to confirm the presence or absence of myelin (luxol fast blue stain). Then, the dissected bits, from each patient, of normal-appearing white matter were pooled, and the demyelinated plaques were pooled; these pooled dissections were homogenized in enough 0.15*M* sodium chloride to make 20 percent homogenates. A portion was centrifuged (25,000 RCF), and samples of the supernatant were taken for immunochemical assay for γG (*4, 5*) and for the determination of hemoglobin (*6*).

By quantitative immunochemical assay, the amount of γG in the soluble fraction of white matter of brain was 206 ± 84 mg per kilogram of tissue before correction for the residual γG from the blood; after correction this value was 145 ± 89 mg per kilogram of tissue. For both plaques of demyelination and the normal-appearing surrounding white matter, the amount of γG was 2.3 times higher than that of control white matter prior to correction for the residual blood γG, and 2.7 times after correction (*3*).

In Fig. 1 the amount of γG in demyelinated plaques (*7*), corrected for residual blood γG, was plotted against the percentage of γG in the patient's cerebrospinal fluid obtained before death or in ventricular fluid obtained after death (*8*). If 411 mg of γG is the upper limit in the white matter of the control samples (average control value plus 3 times SD; if this value is exceeded $P \leqq .003$) and the percentage of γG in the cerebrospinal fluid is 18 (average value for cerebrospinal fluid of medical students plus 3 times the SD; if this value is exceeded, $P \leqq .003$), then it can be seen that tissue and fluid of three of four patients with multiple sclerosis fell below these cut-off points. If more emphasis is placed on the cerebrospinal fluid, then the values from four of four patients were below. Six of the multiple sclerosis patients' values were above the cut-off points.

The concentration of γG in tissue (fresh) was higher than that of cerebrospinal fluid obtained before death in all the multiple sclerosis cases studied. (Table 1) (*9*). Hence, it is reasonable to assume that the increase in tissue γG underlies the increased concentration in the cerebrospinal fluid. However, the relation is probably not simple since the data showed that the amount in the cerebrospinal fluid did not exceed 36 percent, even though the plaque concentration may be further increased 2.5 times (411 to 1100 mg). This could mean that the homeostasis of γG in the spinal fluid at least in part is the result of a dynamic equilibrium, such that γG enters the spinal fluid faster than it leaves, up to a certain point (36 percent γG).

Furthermore, from these data it appears that γG in cerebrospinal fluid (percentage of the total protein or milligrams per liter) from a patient with multiple sclerosis can be used to predict with a high degree of probability ($P \leqq .003$) that the brain γG concentration will be above 411 mg per kilogram of tissue.

Table 1. Cerebrospinal fluid constituents of the multiple sclerotic patients shown in Fig. 1. All patients had negative serologic reaction (Kahn test) for syphilis and all lumbar punctures were free of contaminating blood. Pre, premortem (1 year before death); post, post mortem.

Patient	Leucocytes (per cm)		Total protein (mg/100 ml)		γG/total protein (%)		Colloidal gold curve	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Si	8		34	398	14	10	0000000000	0000000000
St	1		40		15		0121000000	
Fe	0	38	30	48	14	15	0000000000	0000000000
Wr	2		28	45	14	19	0122100000	0000000000
Bu	10		48		30		5554321000	0000000000
Bo	1		54	82	28	34	2223320000	0012210000
Re*				130		24		1111121100
Ho	23	182	60	273	30	33	5554321000	4455554322
Cr	23		52		30		5542100000	
Wi	13	182	50	85	30	36	5421000000	5554321000

* Lumbar puncture was contraindicated because of sacral decubitus.

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5. Since the procedure of Kabat *et al.* (*4*) has been standardized for body fluids and not for extracts from solid tissues, it was necessary to test its applicability to brain extracts. Standard solutions of γG were recovered quantitatively (90 to 110 percent) when added to homogenates of white matter, as well as to plaques from brains of patients that had died of multiple sclerosis.
6. To determine the extent of contribution of residual blood γG in brain, the carboxyhemoglobin method of M. W. Gordon and J. I. Nurnberger [*J. Histochem. Cytochem.* **4**, 84 (1956)] was used. The amount of hemoglobin (Hb) per kilogram of fresh tissue when divided by the amount per milliliter of patient's blood, and multiplied by (1-hematocrit/100), gives the number of milliliters of serum per kilogram of tissue. The average results and standard error of the mean (SEM) obtained were (control value with multiple sclerosis in parentheses): 2.6 $\pm$ 0.4 (3.2 $\pm$ 1.2), (3.1 $\pm$ 1.3) for white matter and plaques, respectively. No real differences as judged by the number of milliliters of serum per kilogram of tissue exist between the control and multiple sclerosis groups in any brain regions tested. The control average values and SEM used for serum γG and albumin were based on serums from 41 normal medical students: 4.5 g per 100 ml ($\pm$ 0.18) and 1.3 $\pm$ 0.04, respectively; and for 97 multiple sclerosis patients: 4.1 $\pm$ 0.11 and 1.3 $\pm$ 0.04, respectively.
7. Since the plaques of demyelination had, in each case, essentially the same γG concentration as the normal-appearing multiple sclerosis white matter, only the plaque values are shown.
8. A plot of the concentration of γG in cerebrospinal fluid (milligrams per liter) taken from patients 1 year prior to death (based on the data in Table 1) showed a similar correlation.
9. The water content of normal-appearing multiple sclerosis white matter was 716 g per kilogram of tissue; that of plaques was 884 g (Y. Kishimoto, N. S. Radin, W. W. Tourtellotte, J. A. Parker, H. H. Itabashi, *Arch. Neurol.*, in press).
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Light Reaction in Green Plant Photosynthesis: A Method of Study

Abstract. *Frozen green plants* (Chlorella) *carry out a photoreaction which can be assayed by measuring the light produced when the sample is heated. Heating at a rate of several degrees per second gives a measurable signal which shows that green plants can store energy at very low temperatures. The absorption cross section for the unit which carries out the photoreaction is several hundred times larger than that for one chlorophyll molecule, an indication that these are the "photosynthetic units" of photosynthesis.*

Photosynthesis is the process in which green plants reduce carbon dioxide to carbohydrate and oxidize water to oxygen. The energy comes from sunlight absorbed by chlorophyll. Apparently carbon dioxide reduction consists of a series of enzyme reactions, none of which are photochemical (*1*). This series, called the Calvin cycle, is driven by electrons at −0.4 volt and by ATP (adenosine triphosphate). It is not understood how the chlorophyll apparatus uses light energy to lift an electron from the level of water (+0.8 volt) to that of the reductant (−0.4 volt) and makes ATP at the same time.

A procedure which apparently allows us to study the photochemical part of photosynthesis is described here. The experiment consists of four simple steps: (i) a *Chlorella* suspension (or leaf plug) is held in the dark at room temperature (20° to 25°C) for 5 minutes to "relax"; (ii) the sample is frozen while still in the dark, at temperatures from −10 to −200°C; (iii) the sample is illuminated while cold (we believe that the photochemical reaction takes place, but that enzyme reactions do not); and (iv) the sample is rapidly heated in the dark to 100°C.

The light emission (the signal) from the sample as it is heated measures the amount of photoreaction that has taken place. It is the fast heating that makes the signal large compared to the background, which is the light emitted on heating a sample that had zero illumination in step (iii). The background is larger than the signal when the sample is heated slowly, for example 20°C per minute.

I have previously presented an electron-hole picture of photosynthesis (*2*) in an attempt to explain the transfer of electrons from water to the reductant (−0.4 volt). In this picture the electron transfer is carried out by photosynthetic units, each made up of 500 chlorophyll molecules. Each unit has two reaction centers separated in space. A light quantum absorbed by any one of the 500 chlorophyll molecules forms an exciton which runs over the whole unit. An exciton hits reaction center A, breaks up to form a free hole in the chlorophyll, and binds an electron to A. This electron, at −0.4 volt, can go to the Calvin cycle. A second exciton cannot react with A until the electron has moved out of the trap. Similarly, an exciton can react at B to form a bound hole and a free electron in the chlorophyll. Again, a second exciton cannot react with B until the hole has been used in the oxidation of water. The movement of the free electron and hole constitutes the electronic conductor between A and B needed to prevent a reverse reaction between the reducing and oxidizing power. The recombination of the free electron and hole is the mechanism for the production of delayed light (*3*).

If one assumes that, at low temperatures (−100°C), the electron in the reaction center A and the hole in reaction center B were stable, then this explains the fact that at low temperatures green plants emit delayed light after a short flash, but do not emit light after a period of continuous illumination (*4*). This had been observed in Calvin's laboratory during a study of delayed light at low temperatures (*5*). Although the results of Müller and Lumry (*6*) show that the ratio of delayed light to fluorescence in green plants is much less than would be predicted by the electron-hole picture outlined above, this picture will still be used as a matter of convenience to describe the present experiments.

These observations were repeated with a phosphoroscope constructed for the study of delayed light. This instrument is of the Becquerel type and consists of two shutters on a rotating shaft. The first shutter is open for 140 degrees, allowing the exciting light to hit the sample; both shutters are closed for the next 40 degrees, then the second shutter is open for 140 degrees, allowing light from the sample to fall on a photomultiplier, and finally both shutters are again closed for 40 degrees. The signal from the photomultiplier was displayed on an oscilloscope. The sample cavity in this phosphoroscope is 3.2 cm in diameter. This is large enough to allow the cooling of samples by heat conduction down a large aluminum bar, the outer end being cooled with liquid nitrogen. Samples can be held at any temperature between 0° and −130°C.

Samples of *Chlorella* were frozen on the bar and put in the phosphoroscope under the dimmest illumination possible. After the speed of the phosphoroscope was set at 100 cy/sec and the temperature of the bar adjusted to the desired value, the exciting light