cose-6-phosphate and 6-phosphogluconate dehydrogenases (*19, 20*). The enzyme selectivity shown by octanoate in our study is in line with the lack of inhibition described for lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and fructose-1,6-diphosphatase by palmityl-CoA (*20*). In addition, our studies showed that another gluconeogenic enzyme, glucose-6-phosphatase, was also not affected by octanoate under the conditions used in this study.

Further work also has demonstrated that the inhibition of glucokinase by octanoate can be prevented by glucose, and the effect was dependent on the glucose concentration (*2a*). However, phosphofructokinase is not protected by glucose, but is protected by its own substrate, fructose-6-phosphate. Glucose is not protective for pyruvate kinase, and this enzyme is not protected by its substrate, phosphoenolpyruvate. In a fortified system of supernatant from a rat-liver homogenate there was a dose-dependent inhibition by octanoate of the conversion of glucose to lactate. This confirms the findings that the key enzymes of glycolysis, glucokinase, hexokinase, phosphofructokinase, and pyruvate kinase, were inhibited by this fatty acid, and it indicates that these enzyme inhibitions were operative in decreasing overall glycolysis in vitro. The specificity of fatty acid action was further supported by our investigation of enzymes of the Krebs cycle. These data show that octanoate inhibited isocitrate dehydrogenase and fumarase from rat liver, whereas under the same conditions the activities of the malic enzyme and malic dehydrogenase (assayed from malate to oxaloacetate and also from oxaloacetate to malate) were not affected (Fig. 3). Our findings provide an explanation for the observation that fatty acids result in a decreased functioning of the Krebs cycle (*10*).

It is noteworthy and it is much in line with our proposed concept that the malic enzyme which provides the gluconeogenic pyruvate from the Krebs cycle intermediate malate is not inhibited by the fatty acid. Since it was shown that a number of free fatty acids were capable of inhibiting glucokinase and hexokinase (*21*) and blocking lipogenesis in a cell-free supernatant system (*22*) prepared from a rat-liver homogenate, it appears that fatty acids may function physiologically as feedback inhibitors of glycolysis, the direct oxidative pathway, and lipogenesis and may be capable of decreasing the activity of the Krebs cycle.

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Photoperiodism in Lemna: Reversal of Night-Interruption Depends on Color of the Main Photoperiod

Abstract. *With main photoperiods of red or white light, the inhibition of flowering in* Lemna perpusilla *6746 caused by interruptions of the night with red light cannot be reversed by far-red light, since far-red light itself is highly inhibitory. However, with a main photoperiod of blue light, far-red light is much less inhibitory and partially reverses the effect of red night-interruptions. If the main blue photoperiod is terminated by a brief red exposure, reversibility is abolished, as the far-red light is again fully inhibitory. This latter effect can be reversed by far-red light. These results add light quality to the already known characteristics of the main light period which affect reversibility in the dark period, and are consistent with the idea that the effects of blue light on photoperiodism in* L. perpusilla *are mediated exclusively by phytochrome.*

Demonstrations of the role of phytochrome in the photoperiodic control of flowering depend on the fact that effects caused by interruptions of the dark period with red light can be reversed by subsequent far-red illumination (for example, *1*). Exceptions to such reversibility, however, have been found in two photoperiodic plants, *Lemna perpusilla* 6746, a duckweed, and the Japanese morning glory, *Pharbitis nil* (*2, 3*). Partial reversibility has been obtained in *Pharbitis* by the use of very short intervals between the treatment with red light and the treatment with far-red (*4*), but this technique is hardly applicable to the *Lemna* system, in which the failure of reversal is due to the inhibitory action of far-red as a night-interruption (*3*).

A new approach to this problem was suggested by the fact that, though *L. perpusilla* 6746 is relatively indifferent to the day length under light schedules consisting of blue or far-red light alone (5), a brief red night-interruption in conjunction with a blue main

photoperiod is nevertheless highly inhibitory (*6*). One might thus expect that a far-red interruption of the night would itself be noninhibitory in conjunction with a blue photoperiod and might thus be able to reverse the effect of red light.

Cultures of *L. perpusilla* strain 6746 were started from colonies raised in continuous light and were grown in test tubes with one-half strength Hutner's medium and 1 percent sucrose at 26°C (*6*). The blue and red light sources were at one-half the standard intensity (*6*). The source of far-red light consisted of five 30-watt incandescent lamps about 70 cm above the cultures and separated from them by 8 to 10 cm of water and a 3-mm thickness of Rohm and Haas "black" FRF-700 "Plexiglas." The percentage of flowering was evaluated (*3*) after the cultures had been exposed to the schedules in question for 7 and 9 days.

Data from experiments with a 10-hour main photoperiod composed of red, or cool white, fluorescent light confirmed previous reports that far-red interruptions inhibit flowering under these conditions and fail to reverse the inhibitions caused by red light. When a 10-hour blue photoperiod was used, however, clear reversals were obtained. An interruption consisting of 15 minutes of far-red followed by 15 minutes of red causes complete inhibition (Table 1), while the opposite order of presentation allows considerable flowering. Thus a reversal is demonstrated. Substantial reversal is still possible by 1 hour after the red exposure, though very little occurs 2 hours after the red exposure.

The introduction of a relatively small proportion of red light into the main blue photoperiod was sufficient to change the situation drastically. The reversal obtainable with the 10-hour blue main photoperiod is completely abolished when the main photoperiod consists of 9 hours of blue followed by 1 hour of red (Table 2). This effect of red light is, in turn, far-red reversible (Table 2, Experiment 2). That is, the effect of far-red given as a brief interruption of the dark period is dependent on the quality of the light closing the preceding photoperiod. With a main blue photoperiod, the far-red is relatively innocuous. If a brief exposure to red is given at the end of the main blue photoperiod, the far-red interruption now is completely inhibitory. However, if the brief red exposure is itself followed by far-red, the initial situation is restored.

Table 1. Effects of red (R) and far-red (F) night-interruptions on the flowering of *L. perpusilla* 6746 exposed to 10-hour, blue main photoperiods. Treatment was begun 6 hours and 45 minutes after the beginning of the dark period. The numbers in parentheses are the time in minutes of each treatment.

Treatment	Fronds flowering on day 9* (%)
	49.0 ± 1.8
F (15), R (15)	0.0
R (15), F (15)	21.0 ± 2.9
R (15); dark (30), F (15)	12.6 ± 1.9
R (15); dark (60), F (15)	10.2 ± 1.2
R (15); dark (120), F (15)	0.4 ± 0.2

* Means of five cultures, ± standard errors.

Earlier studies on the reversibility of night-interruption in both *Pharbitis* and *Xanthium* indicate that the responses to various night-interruptions depend on the length and intensity of the main photoperiod (*4*, *7*). Both factors are considered to affect the amount of Pfr—the far-red absorbing and presumably active form of phytochrome—as well as the amount of a postulated substrate for Pfr action. The rate and extent of the postulated reaction between Pfr and its substrate during the light period are regarded as modifying the effect of the same reaction during the dark period, but the hypothesis is not explicit enough that its precise relationship to my data can be determined.

Table 2. Dependence of the effect of far-red (F) during the night on the addition of red (R) or far-red to the main blue (B) photoperiod. Interruptions were made 6 hours and 45 minutes after the beginning of the dark period. The numbers in parentheses are the times in minutes of each treatment.

Interruptions	Fronds flowering on day 9* (%)
Experiment 1, 10 hours B	
None	42.0 ± 3.6
R (15)	0.0
R (15, F (15)	16.4 ± 2.2
Experiment 1, 9 hours B, 1 hour R	
None	47.8 ± 4.8
R (15)	0.0
R (15), F (15)	0.0
Experiment 2, 9½ hours B	
None	60.6 ± 4.6
F (15)	26.0 ± 1.6
Experiment 2, 9½ hours B, ¼ hour R	
None	70.0 ± 2.6
F (15)	0.0
Experiment 2, 9½ hours B, ¼ hour R, ¼ hour F	
None	63.2 ± 2.4
F (15)	24.0 ± 1.8

* Means of five cultures, ± standard errors.

Previous work with etiolated seedling tissues indicates that, if the red light is considered as holding phytochrome at a state of equilibrium of roughly 100 percent Pfr and the far-red maintains it at 1 to 5 percent Pfr, then the blue light maintains it at about 50 percent Pfr (*6*). Restating the present results in these terms, maintaining a high concentration of Pfr (with red light) for 10 hours results in subsequent sensitivity during the dark period to even the small amount of Pfr produced by far-red light. Maintaining an intermediate amount of Pfr with blue light during the main photoperiod sets up a condition during the dark period in which only a high amount of Pfr is totally inhibitory, and the smaller amounts produced by blue or far-red are less so. Thus the far-red can reverse the effects of red. Superficially then, the condition in which there is an intermediate amount of Pfr resembles that of the long, high-intensity photoperiods used in the *Pharbitis* and *Xanthium* work cited, while the condition in which there is a large amount of Pfr resembles the short, low-intensity photoperiod (*4*, *7*). However, as already noted, the situations may not be precisely similar, particularly since other experiments with *L. perpusilla* suggest that white or red photoperiods, no matter how long or intense, will not reduce the inhibitory effect of far-red as a night-interruption as a blue main photoperiod will (*3*, *8*). A further complication is provided by the evidence in Table 2, suggesting that the state of the phytochrome immediately before the dark period has a profound effect on the subsequent effects of far-red.

The significance of these findings is at least threefold: (i) under appropriate conditions, reversal by far-red light of a red night-interruption is now obtainable in *L. perpusilla* 6746; (ii) the quality of the light during and at the close of the main photoperiod must be added to the previously known factors controlling such reversibility in other plants; and (iii) these results bear on the idea (*9*) that the interactions of red, blue, and far-red in *Lemna* indicate the activity of a second pigment system in addition to phytochrome. While the data presented

do not disprove this idea, they do suggest that it needlessly confuses the matter, since a general explanation in terms of different amounts of Pfr is consistent with all available evidence (*10*).

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Thalidomide Solutions

In a report in *Science*, entitled "Intravenous injection of thalidomide in pregnant rabbits" by Fox *et al.* (*1*), the authors claimed to have produced a supersaturated solution of thalidomide (1 mg/ml) in a medium containing carboxymethylcellulose (CMC, 5 mg/ml) and glucose (50 mg/ml). In brief, their preparation was as follows: 0.5 g of CMC was heated with 100 ml of water until the mixture became a clear solution (about 10 minutes). Five grams of glucose was dissolved in the solution, and 100 mg of thalidomide was added. The mixture was then boiled until all of the thalidomide was dissolved (about 20 minutes). The solution was immediately filtered, cooled in a water bath to 50°C, and then infused, at a temperature of about 40°C, into pregnant rabbits.

Since thalidomide undergoes spontaneous hydrolysis in neutral and alkaline solutions even at room temperatures (*2*), the drug might be rapidly converted to its hydrolytic products under the conditions described by the authors. Using their method, we prepared solutions of tritiated thalidomide and then assayed them for thalidomide by a method developed in our laboratory. A portion of the solution was shaken with ten volumes of ethylene dichloride (EDC). Over 90 percent of the thalidomide, but less than 2 percent of the hydrolytic products, was extracted into the organic phase. Part of the extract was assayed for radioactivity in a liquid-scintillation spectrometer; another was subjected to radio-chromatography. Our results indicated that the amount of thalidomide in the solution after it cooled to 40°C ranged from 4.5 to 30 percent of the amount added. The variability depended on the vigorousness of boiling; intense boiling caused more hydrolysis than slow boiling did.

The possibility that tritiated thalidomide is hydrolyzed more rapidly than the unlabeled compound does not seem likely, since virtually identical values for the amounts of unchanged thalidomide were obtained when the EDC extracts were assayed by both the radiochemical method and that of ultra violet spectroscopy of Green and Benson (*3*).

Boiling the solution of CMC and glucose with thalidomide caused the *p*H to drop from an initial value of about 7.1 to a value of about 5.3, as the acidic metabolites were formed. Since hydrolysis of the drug proceeds more rapidly in neutral than in slightly acidic solutions, the presence of buffers would obviously influence the rate of hydrolysis. Carboxymethyl cellulose might have served as a weak buffer in the authors' medium, for this substance contains from 7 to 8.5 percent sodium (*4*). Indeed, when 100 ml of distilled water (*p*H 6.82) was boiled with 100 mg of thalidomide, only about 10 percent of the drug was hydrolyzed. Since different batches of CMC might be expected to have different buffering capacities, we also tested a sample of the batch of CMC used by Fox and his co-workers. We found that from 70 to 95 percent of the thalidomide was hydrolyzed by the solubilizing technique.

Because the degree of hydrolysis which we have found in the preparation of thalidomide solutions is variable, the amount of thalidomide Fox and his co-workers administered to the pregnant rabbits cannot be determined. However, our results indicate that it probably was not greater than 30 percent of the stated values. Hence, their failure to observe teratogenic effects in rabbits after intravenously administering the thalidomide solution might be partially caused by inadequate dosage.

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We do not disagree with the guarded conclusion put forth by Schumacher, Blake, and Gillette and regret that we did not pay more attention to the hydrolizing effect of carboxymethylcellulose (CMC) on thalidomide at the start of our experiments.

We also agree that the method of preparing the solutions gives various results in the hands of different experimentors, variations mainly caused by the vigorousness of the boiling. Using the same batch of CMC we sent to Schumacher *et al.* and the same extraction method (ethylene dichloride) followed by determination of the thalidomide content of the residue with the method of Green and Benson, we obtained at the laboratory of Sloan-Kettering Institute values of 35 to 55 (compared to 5 to 30 percent obtained by Schumacher *et al.*).

The intravenous injection of thalidomide to pregnant rabbits plays an important part in the thalidomide problem. We have started a new series of experiments eliminating CMC and shall report the results in due time.

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