radioactive progesterone from *Holarrhena floribunda* leaves treated with pregnenolone-4-C^{14}.

Pregnenolone-4-C^{14} (New England Nuclear, 46 μc/μmole) was applied in acetone solution (20 μl, 280,000 count/min) to several leaves of a *Holarrhena floribunda* plant (*7*) growing in soil. The leaves were then sprayed with a solution of silicone oil in petroleum ether to promote absorption of the pregnenolone (*8*). A total of ten such treatments were given, three per week. Three days after the last treatment, the leaves were removed and lyophilized. The dried leaves (0.5 g) were homogenized with water at *p*H 9 to 10, the solution was separated by centrifugation, and the residue was reextracted with water. The aqueous solutions were combined and extracted with dichloromethane. The leaf residue was extracted with boiling acetone, and the two organic extracts were combined and freed of alkaloids by extraction with 0.5*N* HCl (*9*).

The neutral fraction (1,490,000 count/min), weighing 37 mg, was examined by thin-layer chromatography on a silica-gel G plate (50 × 200 mm), 0.3 mm thick, which was developed with a mixture of dichloromethane and methanol (97 : 3). A scan of the radioactivity showed four peaks, the major one being associated with unchanged pregnenolone (R_F, 0.32). The other three were located at R_F of 0.00, 0.14, and 0.56, the last coinciding with progesterone. Since neither pregnenolone nor progesterone was present in the extract in quantity sufficient for detection by spraying with 50-percent sulfuric acid, cochromatographed standards were used to locate their positions on the chromatogram.

By preparative thin-layer chromatography of one-fourth of the neutral fraction in the same system, the radioactive zone corresponding to progesterone was isolated (8800 count/min). The material in this zone was subjected to thin-layer chromatography on a plate, as described, with a mixture of hexane and ether (3 : 7). Two radioactive peaks were located by a scanning procedure, the major peak having the same mobility as progesterone (R_F, 0.25) and the other having an R_F of 0.14. The zone corresponding to progesterone was removed and eluted (4800 count/min). Thin-layer chromatography of a portion of this material on a silica-gel G plate, developed continuously (*10*) with a mixture of hexane and ether (4 : 1) for 8 hours, showed only a single radioactive peak, coinciding with progesterone.

The remainder of the progesterone zone was treated with 2,4-dinitrophenylhydrazine in methanol. The product was chromatographed on a plate, as above, with a mixture of carbon tetrachloride and dichloromethane (1 : 9) being used for development. No radioactivity was associated with the zone of the chromatogram corresponding to progesterone (R_F, 0.08), and the only peak revealed by scanning coincided with a cochromatographed sample of progesterone bis(2,4-dinitrophenylhydrazone) (R_F, 0.48) prepared from authentic progesterone (*11*). This zone was removed and eluted (3500 count/min). A portion of this was diluted with pure progesterone bis(2,4-dinitrophenylhydrazone) and recrystallized from a chloroform-ethanol mixture. The crystals, having a specific activity of 34.9 ± 1.7 count min^{-1} $\mu mole^{-1}$ (*12*), were recrystallized from the same solvent with no change in specific activity (35.2 ± 1.7 count min^{-1} $\mu mole^{-1}$). The specific activity also remained constant after recrystallization from a chloroform-benzene mixture (34.8 ± 1.7 count min^{-1} $\mu mole^{-1}$). Finally, hydrolysis to progesterone with chromous chloride and HCl (*13*), followed by recrystallization from hexane, did not alter the specific activity (34.3 ± 1.7 count min^{-1} $\mu mole^{-1}$).

The incorporation of pregnenolone into progesterone was low (<1 percent), and a further experiment demonstrated that the latter is rapidly metabolized by the plant. Progesterone-4-C^{14} was administered to a second *H. floribunda* plant, exactly as in the pregnenolone experiment above, and the leaves were worked up in the same manner. Only 3 percent of unchanged progesterone was recovered. Thus, the conversion of pregnenolone to progesterone must have been much higher than the incorporation rate indicates.

This experiment, together with previous work on the biosynthesis of steroids from acetate, mevalonic acid, and squalene (*8*, *14*), indicates that the pathway of biosynthesis of progesterone is probably the same in plants as in animals. However, its physiological function, if any, in plants remains to be determined.

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Aggressive Mimicry in Photuris: Firefly Femmes Fatales

Abstract. *Firefly females of the genus* Photuris, *long known to be carnivorous, attract and devour males of the genus* Photinus *by mimicking the flash-responses of* Photinus *females. Although suspected, this behavior had not been observed previously.*

While observing firefly behavior, several naturalists have noted that females of the genus *Photuris* are carnivorous. Many, including myself, have discovered this by trying to keep groups of fireflies alive overnight in the same container. In the morning one usually finds one *Photuris* female and bits and pieces of all the rest. Barber (1) observed *Photuris* females in spider webs eating glowing fireflies that had been captured and wrapped by spiders. He also observed courting males of the genus *Photinus* receiving flashed responses from perched *Photuris* females. He asked: "Does she lure him to serve as her repast?" During the past three summers while working in the field on flash-communication in the firefly genus *Photinus*, I have made several observations

which have a bearing on Barber's question.

In order to study the flash-communicative systems of fireflies it is essential to have females of the species being studied. Unfortunately, these are usually at a premium. An hour or two of searching may yield but one, more frequently none. The best method is to walk about the area flashing a pocket flashlight in a manner which simulates the flash-pattern of the males of that particular species. Although in competition with dozens or hundreds of male fireflies, the flashlight will often draw flash-responses from females 6 to 12 m away, while male fireflies are seldom answered at distances greater than 3 m. While searching in this manner for female *Photinus* fireflies, I have on five occasions received flash-responses from *Photuris* females.

1) *Fife, Goochland County, Virginia, 13 June 1963.* While searching in the site of *Photinus ignitus* Fall, I received a single flash-response to a quick flash of the flashlight after a delay of 5.5 seconds at 14°C. This is the delay time and flash of *P. ignitus* females. When collected after several more similar flash-responses, this female was found to be *Photuris* (*2*).

2) *Red Hills State Park, Lawrence County, Illinois, 24 July 1963.* During the early period of activity of *Photinus pyralis* (Linné) I located a *Photuris* female in a *P. pyralis* site by her flash given 2.2 seconds after a flash from my flashlight at 21°C. This is the time delay Buck (*3*) found for *P. pyralis*.

3) *Gainesville, Alachua County, Florida, 24 May 1964.* In the site of a large population of a species in the *Photinus collustrans* LeConte complex, two *Photuris* females repeatedly answered my single flash with a single long pulse, 1 second in duration after a delay of about 1 second (the flash-and-delay-characteristics of this *Photinus* species). No *Photuris* males were seen.

4) *Gainesville, Florida, 6 April 1965.* The flash pattern of males in one species of the *Photinus consanguineus* LeConte complex consists of two short pulses separated by about 2 seconds. This phrase is repeated every 4 to 7 seconds. While searching for females I received a response from the direction of a low weed along a stream. The flash appeared greener and brighter than usual and upon investigation I found a large (14 mm), black *Photuris* female. One 11-mm black *Photuris* male was later caught which emitted single, ragged, flickering flashes at intervals from 3 to 5 seconds in duration. I watched this female for the next half-hour, and during that time she responded to twelve passing males of the *Photinus* species with a single flash-response similar to that of the females of this species—a single pulse about 1 second after the second male pulse. All of these males were at least partially attracted to her. One flew into the stream. Two flew into the grass below her and then she stopped answering them; presumably she couldn't see their flashes. Eight of the males were attracted to within 1 m of her and then she stopped answering them. While answering, she would occasionally flash after the first male pulse and then again after the second pulse. Usually she answered only after the second pulse. I also noted that, as the males neared her, she greatly reduced the intensity of her flashes. The last male attracted, after three or four flash-exchanges, landed about 7 cm from her. After another flash sequence I turned on my light and found him 15 cm from her. Following the next flash exchange, after a pause of 10 to 15 seconds, I checked and found she was clasping him and chewing on his pronotum.

5) *Gainesville, Florida, 15 April 1965.* The flash pattern of the males in one species in the *Photinus consimilis* Green complex consists of two or three pulses delivered at 1.2- to 1.4-second intervals; the flash pattern is repeated every 10 to 14 seconds. Being unable to find females of this species, I tried unsuccessfully to attract the males, using a variety of different flashlight techniques. Later, while again searching for females, I received a response at 5.0 seconds delay after the last stimulus pulse at about 22°C. The response flash consisted of two, long, single pulses about two seconds apart. This female responded with a similar delay several times and, when collected, was found to be a *Photuris*. Using this flash-response I was able to attract several, although not all, of the *Photinus* males tested.

The answer to Barber's question has precipitated a deluge of new questions, not the least of which concerns the males of the genus *Photuris*. Is the female *Photuris* predaceous before she has mated? If so, how does her mate avoid the fate of attracted *Photinus* males? Also of interest is the question of how this interspecific signaling developed in evolution. The most logical beginning would have the female *Photuris* preying on *Photinus* males of species with signal systems similar to their own. Finally, can a single *Photuris* species prey upon more than one *Photinus* species with different signal systems? In other words, how many flash patterns do *Photuris* females have in their "repertoires," and is predation on *Photinus* fireflies in any sense obligatory? Certainly, this kind of predation must have had effects on the evolution of the signal systems and other behavior of members of the genus *Photinus*.

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Rhythmic Enzyme Changes in Neurons and Glia during Sleep

Abstract. *Rhythmic changes occur in the activities of enzymes in both the neurons and neuroglia isolated from the caudal part of the reticular formation of rabbits killed during sleep and wakefulness. During sleep, enzyme activity is high in the neurons and low in the glia; during wakefulness, this situation is reversed. In the oral part of the reticular formation rhythmic enzyme changes occur only in the neurons. No rhythmic changes occur in the hypoglossal and trigeminal mesencephalic neurons and glia. These findings indicate that the caudal part of the reticular formation reflects in metabolic changes the biological clock behind the sleep rhythm.*

We reported previously (*1*) that rhythmic changes in enzyme activity occur during sleep and wakefulness in both neurons and neuroglia in part of the brainstem. We studied the succinate-oxidizing enzyme system in the large nerve cells and glia of the