5.80 μ in addition to the band at 5.92 μ. Compound B moved more slowly on silica-gel thin-layer chromatography than did compound A when a mixture of hexane and chloroform (2:1) was used for development. In several other solvent systems on silica-gel thin-layer chromatography or on paper chromatography, compounds A and B were inseparable (*8*).

W. A. Skinner
Department of Pharmaceutical Chemistry, Stanford Research Institute, Menlo Park, California
P. Alaupovic
Cardiovascular Section, Oklahoma Medical Research Institute, and Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City

References and Notes

1. This is a paper IV of a series. The preceding papers are: V. L. Frampton, W. A. Skinner, P. S. Bailey, *Science* **116**, 34 (1952); V. L. Frampton, W. A. Skinner, P. S. Bailey, *J. Am. Chem. Soc.* **76**, 282 (1954); V. L. Frampton, W. A. Skinner, P. Cambour, P. S. Bailey, *ibid.* **82**, 4632 (1960).
2. C. Martius and H. Eilingsfeld, *Ann. Chem.* **607**, 159 (1957).
3. H. H. Draper, A. S. Csallany, S. N. Shah, *Biochim. Biophys. Acta* **59**, 527 (1962).
4. P. Alaupovic, B. C. Johnson, Q. Crider, H. N. Bhagavan, B. J. Johnson, *Am. J. Clin. Nutr.* **9**, II, 76 (1961).
5. D. R. Nelan and C. D. Robeson, *Nature* **193**, 477 (1962); ———, *J. Am. Chem. Soc.* **84**, 2963 (1962).
6. P. Schudel, H. Mayer, R. Rüegg, O. Isler, *Chimia Aarau* **16**, 368 (1962).
7. S. B. Covitt, H. Sarrafizadeh R, P. D. Gardner, *J. Org. Chem.* **27**, 1211 (1962).
8. Supported by grants A-5552 and H-1889 from the U.S. Public Health Service. We thank Dr. Masato Tanabe and R. M. Parkhurst of Stanford Research Institute for valuable assistance.

25 February 1963

Motor Responses of Moths to Low-Intensity X-ray Exposure

Abstract. *A brief burst of x-rays elicited flight activity in moths placed in a darkened x-ray exposure room. Wing-beat activity was recorded as an index of this behavior. Wing-beat activity could be initiated in resting moths or amplitude augmented in active moths by x-ray dose rates of 0.01 to 1.5 roentgens per second, with a latency of less than 1 second after onset of exposure.*

Prompt behavioral repsonses to ionizing radiation have been reported for a number of species. In the rat an arousal reaction from sleep was found to occur within seconds after the start of low-intensity exposure (*1*). Also, an immediate disturbance in lever-pressing behavior upon the onset of exposure has been reported when radiation was used as a conditioned stimulus (*2*). Hug (*3*) has identified some reflex-like reactions in marine invertebrates and insects which have involved tentacle retraction, shell closure, or migration from the exposure area with relatively low-intensity radiation exposure. Born (*4*) observed a closure response of the snail mantle cavity similar to the snail tentacle response described by Hug. With high-intensity beams, prompt behavioral disturbances have been reported in several organisms including daphnids (*5*), turtles (*6*), mosquitoes (*7*), and fish (*8*).

Recent experiments in this laboratory have indicated that the moth is remarkably sensitive to low-intensity radiation exposure. In the initial observations it was found that a brief burst of x-rays would elicit flight behavior in moths placed in a darkened x-ray exposure room. This behavioral response is readily accessible and can be measured by observations of wing-beat frequency. We now describe this reaction and its sensitivity to radiation.

Moths from eight species of Noctuidae and one species of Arctiidae were used in the study. The moths were collected in the San Francisco area and tested within 24 hours after capture. X-rays were generated by a General Electric Maxitron x-ray machine, operated at 250 kv (peak), 25 ma, with a beam half-value layer of 2.3 mm Cu. The exposure interval was controlled by a lead shutter operated manually from outside the x-ray room.

To record the wing beat, the moth was attached to a wire which was cemented to a ceramic crystal transducer (Electrovoice model 53-3). The signal from the transducer was amplified so that vigorous flight movement resulted in a ±10-mm deflection on an oscillographic recording. To attach the moth to the motion transducer, we anesthetized the moth briefly with CO_2 and brushed the scales from the dorsal exoskeleton of its thorax. The moth was then joined to the wire of the transducer with heated Tackiwax (Cenco). The mounted preparation is displayed in Fig. 1.

In the test procedure, the animal preparation was mounted on a stand in the exposure room. The x-ray tube was turned on and the beam attenuated to a background radiation level by closing the shutter. The moth was allowed to dark adapt for 4 minutes prior to the exposure tests. Each animal was tested at least three times with dose rates of 0.33, 0.13, 0.10, and 0.07 r/sec. The stimulus intensity at the site of action is presumed to be related to the dose rate. For a closer determination of the minimum effective exposure intensity, additional dose rates were usually required. Differential dose rates were achieved by varying the distance from the subject to the source. The thimble chamber of a Philips dosimeter was placed adjacent to the moth to record the dose rate during exposure. The duration of the exposure varied from 1 to 15 seconds and the time between exposures varied from several seconds to 3 minutes.

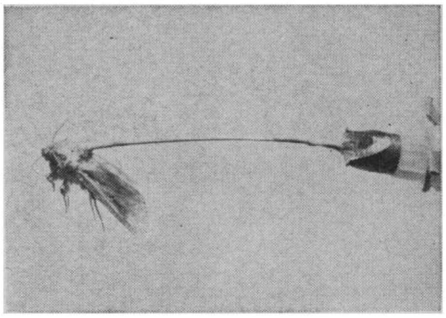

Fig. 1. Moth preparation for the study of activation of wing beat by low-intensity x-rays. The attachment of the subject to the crystal transducer can be seen.

To demonstrate that the moth was reacting to the x-ray beam and not to shutter manipulation or some other stimulus, several types of controls were used. (i) Each animal was given several "sham" exposures in which all conditions were the same except that the power to the x-ray tube was turned off. (ii) A tympanic nerve preparation, made according to the method of Roeder (*9*), was used to test for the presence of auditory stimulation from

Table 1. X-ray dose rates effective in eliciting prompt motor activity for each specimen tested. All species are classified in the family *Noctuidae* except the last specimen (family *Arctiidae*).

Species	Minimum effective dose rate (r/sec)
Crymodes devastator (Brace)	0.50
Protorthodes rufula (Grote)	0.37
Agrotis ypsilon (Rottemberg)	0.01
A. ypsilon (Rottemberg)	0.01
A. ypsilon (Rottemberg)	0.12
A. subterranea (Frabricuis)	0.20
Acronicta mormorota (Smith)	0.10
Catocala irene (Behr)	0.17
C. irene (Behr)	1.50
Prodenia praefica (Grote)	0.17
Mamestra configurata (Walker)	0.33
Halisidota maculata (Harris)	0.03

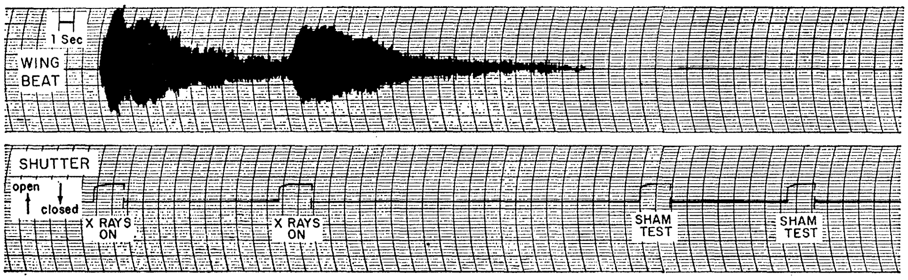

Fig. 2. Initiation and augmentation of wing beat in a moth (*Agrotis ypsilon*) on exposure to x-rays at 0.17 r/sec. For the sham exposure test the shutter was opened but power to the x-ray tube was turned off.

high-frequency sounds produced during the shutter operation and exposure. Any sounds which were within the moth's frequency and intensity range could be observed on an oscilloscope as spike potentials in the nerve preparation. (iii) This control was provided to detect possible effects of the x-ray beam on the motion transducer or the lead wires, which might in turn evoke flight activity in the moth. For this purpose, additional preparations were made with animals attached to wires which were fastened directly to the ring stand. Under dim red illumination and through a window from the control room, visual observations were made of the initiation of flight at the onset of the exposure to the x-ray beam.

Figure 2 shows the response of one moth (*Agrotis ypsilon*) to two exposures at a dose rate of 0.17 r/sec. Each exposure was 2 seconds in duration. The first stimulus was presented when the animal was inactive and the second exposure was given during evoked flight. In each moth tested, the exposure to the x-ray beam initiated wing beat or caused a change in the amplitude of the beat during flight. The dose rate required to initiate this action varied from subject to subject. Table 1 gives the minimum rate to which each of the subjects responded. The response occurred early within the first second of exposure. If the moth was inactive, sometimes a suprathreshold intensity of x-rays for that preparation did not elicit the flight pattern. The response to subsequent radiation exposures could be restored if the moth were first exposed to a brief flash of light to establish a state of heightened excitability.

The control tests indicate that the response of the moth is to the x-ray beam. When subjected to the "sham" trials the animals gave no evidence of initiation of the flight pattern. The record of spike potentials from the tympanic nerve preparation gave no indication of the presence of auditory stimuli during shutter operation and exposure. Animals not attached to the transducer were observed to exhibit vigorous flight movement at the onset of exposure, which indicates that the response could not be attributed to radiation-induced changes in the electrical detection system.

The complex motor reaction demonstrated in these moths occurs at a radiation intensity below that reported for any other organism (*1–8*). Several specimens exhibited responses to radiation intensities in the range of 0.01 to 0.12 r/sec. The latency of reaction was less than 1 second on most records. The differences in effective dose rates among specimens may reflect differences in radiosensitivity among species, the variations in physiological state among specimens, and the level of excitation upon which radiation is imposed.

It is difficult to ascribe the response to radiation action on muscle fibers in view of the low exposure dose and the short latency. It is significant that the motor response could be obtained only when the specimen had been in the darkened room for several minutes. Electroretinograms are being made of a large series of moths by using both beta radiation plaques and x-rays as the source of stimulation. The preliminary data indicate that the threshold intensity for visual activation may be comparable to that required for initiation of wing beat in the moth. It is suggested that the induction of flight activity may be the behavorial consequence of visual stimulation through low-intensity radiation (*10*).

JAMES C. SMITH
Department of Psychology, Florida State University, Tallahassee

D. J. KIMELDORF
E. L. HUNT
Division of Biological and Medical Sciences, U.S. Naval Radiological Defense Laboratory, San Francisco, California

References and Notes

1. E. L. Hunt and D. J. Kimeldorf, *Science* **137**, 857 (1962).
2. J. Garcia, N. A. Buchwald, B. H. Feder, R. A. Koelling, *Nature* **196**, 1014 (1962).
3. O. Hug, *Intern. J. Radiation Biol., Spec. Suppl.* **2**, 217 (1960).
4. W. Born, *Strahlentherapie* **112**, 634 (1960).
5. E. R. Baylor and F. E. Smith, *Radiation Res.* **8**, 466 (1958).
6. A. T. Krebs, *ibid.* **15**, 372 (1961).
7. G. Goldhaber and B. Feldman-Muhsam, *Nature* **153**, 528 (1944).
8. B. B. Scarborough and R. G. Addison, *Science* **136**, 712 (1962).
9. K. D. Roeder and A. E. Treat, *Am. Scientist* **49**, 135 (1961).
10. Supported by AEC contract No. AT-(40-1)-2903 with one of us (J.C.S.), the Institute of Molecular Biophysics, Florida State University, and the Bureau of Medicine and Surgery, U.S. Navy. The work was done at the U.S. Naval Radiological Defense Laboratory.

18 February 1963

■

Strychnine: Its Action on Spinal Motoneurons of Cats

Abstract. *In cats strychnine does not alter measurably the potential, threshold, after potentials, or refractoriness of the membrane of the spinal motoneurons. Increased reflexes probably result from increase of excitatory impingement upon motoneurons. Spikes recorded during a rapid succession of nerve impulses produced by strychnine ("strychnine burst") suggest that soma membrane resistance is appreciable during stimulation.*

Many attempts have been made to explain the mechanism by which strychnine influences spinal cord activity. A decrease in the size of inhibitory postsynaptic potentials has been shown (*1*), but strong descending inhibitory influences from vestibular stimulation occur in the cord treated with strychnine (*2*). Wall *et al.* (*3*) have shown that the drug decreases the excitability and presumably increases the level of polarization of dorsal root terminals, but does not change motoneuron excitability to extracellular stimulation. It prolongs the repolarizing phase of the antidromic spike in crayfish stretch receptors with concomitant repetitive axonal firing which presumably results from the maintained depolarization of the soma. Inhibitory influences can still be detected in this structure after strychnine poisoning (*4*).