in two to three changes of distilled water and dried, care being taken not to move the screens any more than is necessary. If the proper care is exercised, the gelatin is completely removed by the enzyme, thus leaving on the substrate the mineral grains and any tracks emanating from them which may have been there before removal of the gelatin. Success in obtaining single crystal patterns will be limited to those particles thin enough for transmission by the electron beam. Ring patterns should be of value if fields can be obtained in which there are high enough ratios of active grains to inactive ones to make identification possible.

Electron micrographs (Figs. 1 and 2) indicate that

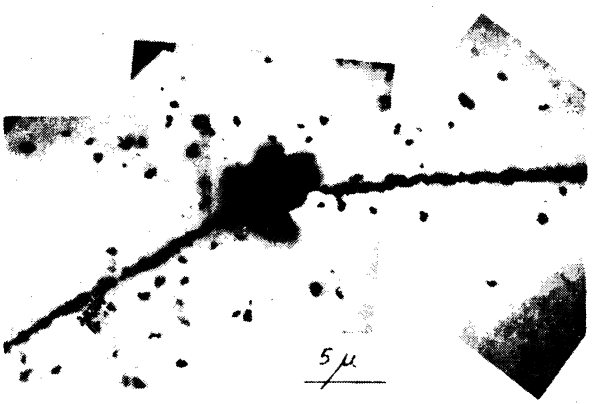

FIG. 2. Electron micrograph of an Uraninite fragment with associated alpha tracks. The mottled background is characteristic of these emulsions and is believed due to variations in the thickness of the gelatin film caused by the silver bromide particles originally embedded within it. The diameter of the Uraninite fragment is approximately 5 μ.

the emulsion contains a sufficient concentration of silver bromide crystals to yield continuous tracks. It is seen that there is some lack of sharpness around the edges of the mineral grains because of the surrounding film of gelatin. This occurs even when the grains are dispersed on top of the soft gelatin rather than within it.

The present technic allows one to study the size and shape of radioactive minerals identified by their alpha tracks. It does not, however, permit one to distinguish nonradioactive grains from the radioactive ones because the thinness of the film prevents the recording of all alpha particles emitted from a given grain. The maximum angle an alpha particle from a grain resting on the Formvar base may make with the surface of the emulsion and produce a discernible track, about 2 μ in length, may be as low as 5 to 6°. If it is assumed that most of the mineral grains are at a depth of 0.1 μ in the emulsion, this angle is reduced to about 3°. Because of this consequent reduction in the number of alpha tracks recorded for a given grain, it is not feasible to regard the usual lower limit of at least two radial tracks from a single grain as the criterion for judging a grain to be radioactive.[3]

[3] Yagoda, H. *Radioactive Measurements with Nuclear Emulsions.* p. 177. New York: Wiley, 1949.

Received January 11, 1954.

Temperature Dependence of Rattling Frequency in the Rattlesnake, *Crotalus v. viridis*

L. E. Chadwick[1] and Hermann Rahn

Department of Physiology and Vital Economics, The University of Rochester, School of Medicine and Dentistry, Rochester, New York

It has been generally appreciated that the rate of rattling of a poikilotherm snake is likely to depend upon the temperature of the animal. However, although isolated records of this rate at a particular temperature can be found in the literature, exact comparisons over a large temperature range are missing except for the report of Klauber (*1*), who observed in *Crotalus v. viridis* an average increase of 93 cpm/° C between external temperatures of 11 and 22° C. In Klauber's measurements, the motion was recorded on a smoked drum by means of a pin attached to the rattle.

In the fall of 1941 we had an opportunity to study 18 adult male and female *C. v. viridis* recently removed from a hibernating den located near Cheyenne, Wyoming. These were collected and sent through the kindness of Dr. George L. Baxter of the University of Wyoming.

Rattling frequency was determined with the aid of a mercury arc stroboscope light. The snakes were placed in an icebox at 5° C or in an incubator at 37° C and left there 1–2 hr. Immediately after these exposures, a thermometer was inserted at least 6 cm into the cloaca. Temperature and frequency readings were then made simultaneously while the animal slowly warmed or cooled.

The grouped results of 226 observations at various body temperatures are shown in Fig. 1. The line fitted to the averages by the method of least squares describes an empirical relationship where the rattle frequency in cpm is equal to $155T - 283$, T being the temperature in degrees centigrade.

Of interest is the linear nature of the relationship over the large temperature range, as well as the high frequencies with which a poikilotherm vertebrate is able to respond not only at temperatures approaching those of warm-blooded animals but also at normal environmental temperatures. By extrapolating the curve 5°, a frequency of 100 cps at 41° C is obtained. This may be compared with the frequency of the humming bird wing beat of about 75 cps at a similar body temperature, or with the wing-beat frequencies of various insects (*2*).

[1] Present address: Medical Laboratories, Army Chemical Center, Maryland.

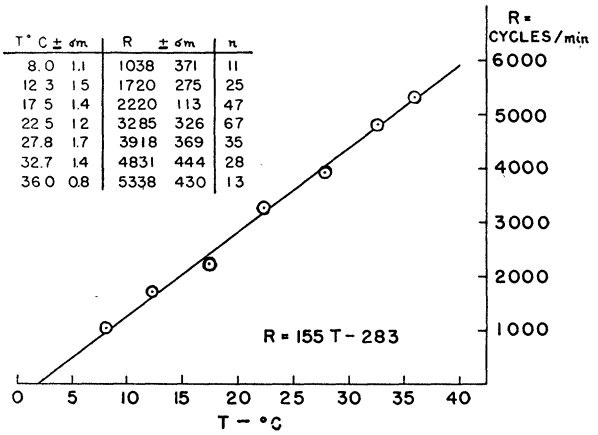

FIG. 1. Relationship between body temperature and rattling frequency in *Crotalus*.

The to and fro movement of the rattle is accomplished by 3 large muscle groups on each side Their fibers run diagonally, the origin and insertion connecting successive tail vertebrae. All 3 muscle groups on each side contract simultaneously during rattling. This has been determined by recording the action potentials of the several muscle groups simultaneously. The potentials were led off insulated needle electrodes that were inserted into the muscle tissue and isolated by an indifferent ring electrode. Frequencies of these electrical recordings were the same as those of the actual rattle movements observed simultaneously under the stroboscope. These observations, which were made in collaboration with Dr. E. B. Wright, suggest that there is no sequential alternation of contractions in the 3 muscle groups which might account for the high frequencies of rattling observed.

It is also of interest to point out that these tail muscles are dark red to brown in color, in distinct contrast to the other skeletal muscles of the body, which are white to light pink. This suggests the possibility of a high content of muscle hemoglobin or other respiratory pigment (*3*) which might be essential not only for the high frequencies but also for the long duration of uninterrupted rattling that is sometimes observed. Continuous rattling for periods as long as 20 min has been reported (*1*).

References

1. KLAUBER, L. M. *Occasional Papers San Diego Soc. Nat. Hist.* No. **6**, 13 (1940).
2. CHADWICK, L. E. *In*: Roeder, K. D. *Insect Physiology*, pp. 591–604. New York: Wiley, 1953.
3. SACKTOR, B., and BODENSTEIN, D. *J. Cellular Comp. Physiol.* **40**, 157 (1952).

Received January 11, 1954.

A Technic for Shipping Live Mosquitoes with Particular Reference to *Culex tarsalis*

James M. Brennan[1] and G. Allen Mail[2]

Department of Health, Education, and Welfare, USPHS

Experience in isolation of viruses from mosquitoes has demonstrated the desirability of fresh material for test purposes. Even with the resistant western equine encephalitis virus a drop in titer may occur during prolonged storage of infected *Culex tarsalis* in dry-ice chests. In connection with virus encephalitis investigations at the Rocky Mountain Laboratory, our attention was centered on an adequate and practical method of transporting living female mosquitoes from considerable distances afield to the virus laboratory.

The first attempt to transfer mosquitoes over a long distance to our laboratory was made in the summer of 1952 when specimens freshly collected in Glasgow, Montana, were conveyed by automobile to Hamilton, Montana, a distance of some 500 mi. The mosquitoes, in the bags in which they were collected, were carried in a portable icebox, but out of direct contact with the ice. The elapsed time from packing to unpacking was about 28 hr. Mortality was negligible, and the mosquitoes were as active as when collected. The next attempt was made by shipping *C. tarsalis* females by Railway Express from Bismarck, North Dakota, to Hamilton, Montana, some 800 mi. The bags of mosquitoes were packed in a small carton within a large carton. Refrigeration was provided by Mason jars of water frozen solid by CO_2. Sawdust and newspapers furnished the necessary insulation. The elapsed time from packing to unpacking was about 39 hr. Again, mortality was negligible and the mosquitoes were active and vigorous on arrival.

Encouraged by the results of these trials, we have developed the shipping technic described below.

The dual-purpose collecting and shipping bag is a type widely used and is simply a cylindrical muslin bag with a window of bobbinet forming the closed end. The dimensions of the bag are accommodated to a supporting inner wire frame that forms the outlines of a right cylinder. This frame, of 3/32-in. Monel wire, consists of two 4½-in. rings, with parallel planes and on the same axis, held in position by a single vertical wire 8½ in. long. The bag is closed by rubber bands. For shipping purposes a bag of this size will hold up to 300 mosquitoes without crowding. It is desirable that a schedule be arranged whereby mosquitoes may be collected shortly before packing and shipping.

One or two bags of mosquitoes are placed in a substantial paper carton of a size that will permit the inclusion of a refrigerating medium. We have found Sno-Gel Refreezants,[3] model R10-B, to be an adequate

[1] National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.
[2] Communicable Disease Center, Technology Branch, Water Projects Section, Mitchell, Nebraska.

[3] Manufactured by Sno-Gel, Inc., 334 Magnolia St., Oakland 20, Calif. "Refreezants are a harmlesss, odorless substance holding liquid in colloidal suspension." Model R10-B is such a substance in a cylindrical plastic skin about 10 by 2 in., sausage-like in appearance, freezing at 30° F. These can be frozen either in deep-freeze units or more quickly with dry ice, and can be reused indefinitely.