donors of these sera had never, so far as was known, been exposed to botulinum antigens and possessed no demonstrable botulinum antitoxins.

The results summarized here are additional evidence of the closer relationship existing between *Cl. botulinum* types C and D than that between types A, B, and C. And they emphasize, in addition, that the lethal toxin and hemagglutinin are not the same.

Reference

1. C. Lamanna and W. I. Jensen, *Soc. Am. Bacteriologists, Proc.* (1952), p. 106.

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Nuclear Emulsions for Electron Microscopy[1]

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The identification of radioactive mineral grains using "Nuclear Research" emulsions and a petrographic microscope is limited to those grains within the resolving power of the microscope. Frequently there is need for the higher resolving power of the electron microscope to make accurate size measurements and study the morphology of these grains.

A technic has been devised to obtain emulsions thin enough to allow transmission of the electron beam, yet containing a sufficiently heavy and uniform concentration of silver bromide crystals to permit the recording of alpha-particle tracks. The technic makes use of Ilford C2 emulsions in gel form. A small quantity of the gel is heated to 50° C in a glass beaker, according to the directions of the manufacturer. When the gel is fluid, approximately 0.025 ml is removed from the beaker with a blood pipet and deposited on a 1 × 3 in. Formvar-coated glass slide. The finely ground sample, the amount used being determined by trial, is mixed with the gel which is then spread uniformly over the surface of the slide by means of a curved stainless steel spatula.[2] An alternative method of adding the sample is to sprinkle the mineral grains over the moist surface of the gel after the slide has been coated. This latter method reduces the possibility of forming silver tracks by pressure of the mineral grains in the silver bromide crystals as the gel is spread over the surface of the glass slide. The volume of gel placed on the slide takes into account loss due to adhesion to the spatula. It is extremely important that the entire procedure, from the removal of the measured amount of gel to the formation of the film on the slide, be carried out rapidly before the gel starts to set or fogging due to pressure of the spatula will result. This time period was found to be less than 1 min. With practice, it is a relatively simple matter to obtain emulsions of consistent thickness. After the emulsion has been prepared it is stored in a light-tight cabinet for a predetermined length of time. For highly active uraninite, an exposure of from 3 to 4 days yielded numerous alpha tracks. The exposed emulsions are developed for 2 min in Eastman Kodak D-8 developer, fixed for 15 min in a standard fixer, and then washed and dried. They are then scored with a sharp blade to divide them into ½-in. squares. The squares of emulsion are floated from the glass slide by placing the slide in a Formvar solvent, ethylene dichloride. After washing in fresh solvent, they are picked up on sections of stainless steel screen and are carefully placed in steam for 1 to 2 min to obtain better adhesion of the gelatin to the screen. At this point suitable areas for the electron microscope may be chosen by examination in an optical microscope and marked. Disks ⅛ in. in diameter are cut from the marked areas by means of a punch. The thickness of an average emulsion prepared in this manner was measured by shadow-casting sections on collodion substrates and was found to be approximately 0.2 μ.

If it is desired to attempt identification of the mineral grains in the emulsions by selected area diffraction in the RCA or Philips electron microscope, it is advisable to remove the gelatin. This can be done in an enzyme if the emulsion is fixed in a 30-percent solution of sodium thiosulphate containing no hardener. Sections of the developed emulsion are picked up on collodion substrates supported on the stainless steel screens. These are carefully immersed in a freshly-prepared 0.5 percent solution of Taka-Diastase at room temperature for 10 min. Some emulsions may require a more concentrated solution. They are then washed

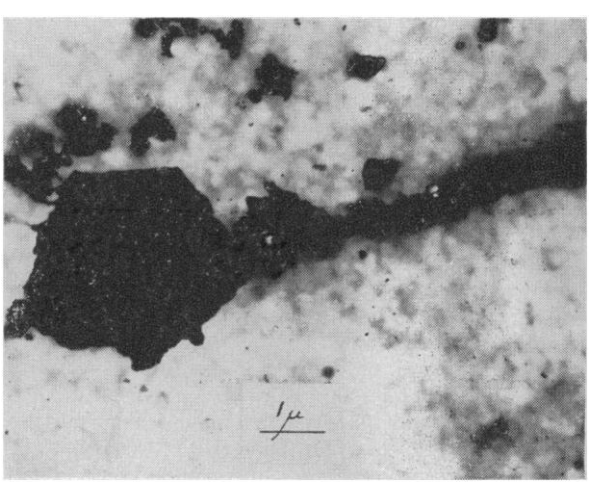

FIG. 1. Electron micrograph of a Carnotite particle with an alpha track. The small opaque particles scattered throughout the field are silver grains caused by normal fogging of the emulsion and are not associated with radioactive particles. The diameter of the Carnotite particle is approximately 3 μ.

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[2] Sold under the trade name, Scoopula, by Fisher Scientific Co., Pittsburgh, Pa.

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

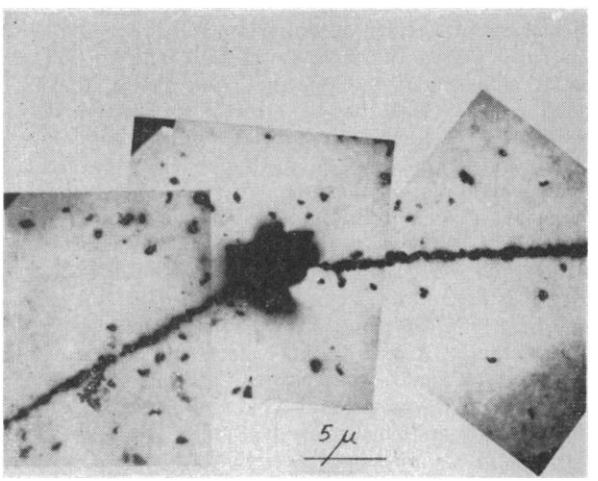

FIG. 2. Electron micrograph of an Uraninite fragment with associated alpha tracks. The mottled background is characteristic of these emulsions and is believed due to variations in the thickness of the gelatin film caused by the silver bromide particles originally embedded within it. The diameter of the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

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

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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.