Reports

Isolation of Colorado Tick Fever Virus from Rodents in Colorado

Although Colorado tick fever virus has been isolated frequently from man and from the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles (*1*), spontaneous infection of wild mammals has not been previously reported. Species expected to harbor the virus must necessarily be hosts of the immature stages of *D. andersoni,* the only tick that has been incriminated in transmission of the disease to man. We have obtained serologic evidence suggesting the occurrence of natural infections in one such animal species, the Columbian ground squirrel, *Citellus columbianus,* in western Montana and have found this species to be readily susceptible to experimental infection. This report records the first isolations of virus from mammals in nature. The hosts were the golden-mantled ground squirrel, *Citellus lateralis lateralis,* and the porcupine, *Erethizon dorsatum epixanthum,* collected in western Colorado.

Several members of a religious order residing on and operating a large ranch near Snowmass, Pitkin County, Colo., contracted Colorado tick fever in 1956 and 1957. Studies made 10–13 August 1957 revealed that golden-mantled ground squirrels occurred abundantly in pastures adjacent to the residence building and along roadsides on and near the ranch. Twenty were shot and four were captured alive. Two porcupines were captured about 2 miles from the residence. Other animals collected were one chipmunk, *Eutamias* sp., one deer mouse, *Peromyscus* sp., and one woodchuck, *Marmota flaviventris.* Blood samples were taken and refrigerated for transport to the laboratory. Fifteen adult *Dermacentor andersoni* were found on the porcupines, and 97 immature *D. andersoni* and 30 immature *Ixodes sculptus* on the squirrels. These and 33 adult *D. andersoni* collected from vegetation were saved for testing.

Blood samples were tested for presence of virus by triturating the clot in about 2 ml of physiologic saline solution, centrifuging the suspension at moderate speed, and injecting 0.05 ml of the supernatant intraperitoneally into each of a litter of six 4-day-old white mice. Transfers to passage mice were made by inoculation of brain suspensions.

Death resulted in four of six primary test mice injected with blood from one squirrel and in all passage mice. One mouse died in each litter injected with blood from another squirrel and from porcupine 34459, and several mice died in the passage litters. All three isolates were identified by neutralization tests as Colorado tick fever virus.

Blood samples collected 21 August from the four living ground squirrels were tested for virus and for neutralizing antibodies. No neutralizing antibodies were found, but virus was isolated from the blood of two of the animals, one of which had also been positive when it was bled in the field 7 days earlier. These four squirrels were then infested with virus-free laboratory-reared nymphs of *D. andersoni* to determine whether the blood of any of these animals contained sufficient virus to infect engorging ticks. One squirrel died before the ticks had opportunity to feed, but engorged ticks were obtained from the three remaining animals. When tested as adults, the ticks from one squirrel from which virus had been isolated earlier proved to be infected.

Serum samples from 20 ground squirrels, the deer mouse, the chipmunk, and porcupine 34459 did not contain neutralizing antibodies, but the sample from ground squirrel 34444, the woodchuck, and porcupine 34460 neutralized 264, 40, and 576 LD_{50} of Colorado tick fever virus, respectively.

The ticks obtained in the field were tested in separate lots according to host, species, and stage of tick—that is, larvae and nymphs from the same animal were tested separately. Ticks were triturated with sterile sand in physiologic saline solution containing 10 percent rabbit serum, streptomycin, and penicillin. Each sample was injected intraperitoneally into a litter of six 4-day-old mice. On the 8th day after inoculation, unless sickness was noted earlier, two mice of each litter were sacrificed and a brain suspension was passed to another litter. No infection was demonstrated in the adult ticks. By passage, virus was isolated from immature *D. andersoni* from two ground squirrels. One lot consisted of three larvae and the other of four nymphs. The host of the nymphs was one of the squirrels from which virus was isolated (*2*).

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References and Notes

1. C. M. Eklund, G. M. Kohls, J. M. Brennan, *J. Am. Med. Assoc.* 157, 335 (1955).
2. We are indebted to Dr. Gordon Meiklejohn, of the University of Colorado Medical School, for informing us of the occurrence of the Colorado tick fever cases at Snowmass and to Rev. M. Leo Slatterie for permission to collect animals and ticks on the monastery grounds.

28 March 1958

Taxonomic Implication of Actinophage Host-Range

Bacteriophages are known which attack hosts of different species and genera (*1*). However, bacterial viruses attack only closely related hosts. Therefore susceptibility to particular bacteriophages is used to speciate certain bacteria (*2*). Members of the genera *Nocardia* and *Streptomyces* are sensitive to their respective bacteriophages (*3*). Recently we found that two of 12 actinophages, initially isolated on streptomycetes, were able to attack some species of *Nocardia.* We therefore undertook to isolate additional streptomyces phages and nocardia phages in order to determine whether or not other intergeneric susceptibilities existed (*4*).

The strains of *Streptomyces* employed in this investigation have been previously described (*5*). The strains of *Nocardia* used, except for *Nocardia* sp. strain 3403, were supplied by Norman F. Conant, of Duke University. *Nocardia* sp. strain 3403 was obtained from the collection of the New Jersey Agricultural Experiment Station. The cultures of *Actinoplanes* and *Streptosporangium* were supplied by John Couch, of the University of North Carolina. The strains of *Micro-*

Table 1. Host ranges of streptomyces-phages and nocardia-phages with respect to several genera of the Order Actinomycetales. SP- and NP- denote phages initially isolated on streptomycetes and nocardia respectively. Lysis of a host by a standard phage suspension is indicated by a plus sign; no lysis is denoted by a minus sign.

Hosts	Actinophages					
	SP-3	SP-4	SP-8	NP-3	NP-4	NP-5
Streptomyces griseus S34	−	+	−	+	−	−
Streptomyces griseus S104	+	+	+	+	+	+
Streptomyces griseus 1945	−	−	+	+	+	−
Streptomyces olivaceus S11	+	+	+	+	+	−
Streptomyces venezuelae S13	+	+	+	+	+	+
Streptomyces cyaneus S45	−	−	−	+	−	−
Nocardia sp. 3403	−	−	−	−	−	+
Nocardia paraguayensis	+	+	+	+	−	−
Nocardia madurae	−	−	−	−	+	−
Nocardia brasiliensis	−	−	−	−	−	−
Nocardia asteroides	−	−	−	−	−	−
Actinoplanes sp.	−	−	−	−	−	−
Streptosporangium sp.	−	−	−	−	−	−
Mycobacterium phlei	−	−	−	−	−	−
Micromonospora sp.	−	−	−	−	−	−

monospora and *Mycobacterium* were from the culture collection maintained at the University of Minnesota. The general precedures for isolating and purifying actinophages followed those previously published (*6*). The nutritive substrate used in earlier work did not support rapid growth of all the organisms studied here. Therefore a modified medium was devised, containing the following ingredients: Difco peptone, 5 g; Difco yeast extract, 3 g; glucose, 1 g; Difco casamino acids, 1 g; Armour beef extract, 2 g; Difco agar, 15 g; deionized water, 1 liter.

Eleven soil samples, collected in Minnesota, have yielded three streptomyces phages and five nocardia phages. Of the three newly isolated streptomyces phages, one attacked *Nocardia paraguayensis* as well as several members of the genus *Streptomyces* (Table 1). Of the five newly isolated nocardia phages, three attacked streptomycetes. The strains of *Actinoplanes, Streptosporangium, Micromonospora,* and *Mycobacterium* used in this investigation were resistant to all streptomyces phages and nocardia phages tested.

At the present time, the genera *Actinomyces* and *Nocardia* are taxonomically placed in the family Actinomycetaceae, whereas *Micromonospora* and *Streptomyces* are placed in the family Streptomycetaceae. In view of the results reported here, it seems that the genera *Nocardia* and *Streptomyces* are closely related and should not be separated into different families. This interpretation is supported by the recent discovery that *Nocardia asteroides* produces conidiospore-like structures (*7*). The formation of chains of conidiospores is one of the fundamental criteria for differentiating *Nocardia* and *Streptomyces*. In fact, it has been recently pointed out that there is no clear-cut distinction between the genera *Nocardia* and *Streptomyces* and that the separation of *Streptomyces* from *Nocardia* is invalid (*8*).

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4. This investigation was supported in part by research grants from the National Institute of Allergy and Infectious Diseases, Public Health Service, and from the Medical Research Fund of the University of Minnesota. We are indebted to Dr. Norman F. Conant of Duke University for the strains of *Nocardia* used, except strain 3403, and to Dr. John Couch of the University of North Carolina for the cultures of *Actinoplanes* and *Streptosporangium*.
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21 March 1958

Detection of Ultraviolet Absorbing Spots on Paper Chromatograms by Blueprint Paper

Many organic compounds having strong ultraviolet absorption at 250 to 260 mμ can be detected on paper chromatograms by the photographic method of Markham and Smith (*1*) or Haines and Drake (*2*). The chromatogram is used as a "negative" over a sheet of contact printing paper. The light source is a quartz mercury arc, used with a filter that transmits primarily the intense 253.7-mμ line. Substances having high extinction at this wavelength show as white spots on a black background when the print is developed. Several refinements of this technique have been proposed (*3, 4*), but it requires a darkroom and the usual developing and fixing baths which are not conveniently available in many laboratories.

This report (*5*) describes the use of ferric ferricyanide blueprint paper, which is relatively insensitive to visible light and can be handled without special precautions in any well-lighted laboratory (though direct sunlight must be avoided). It can be developed in a few seconds by rinsing with cold tap water. Excellent "prints" are obtained even with an inexpensive unfiltered mercury arc light. The cost of blueprint paper is less than one-fifth that of photographic printing paper.

Blueprint paper is available from several manufacturers and in various grades. All will probably give satisfactory results, although there will be minor differences in speed, depth of color, and contrast. In this work, Dietzgen XL, a thinly-coated paper, has been used. It seems to give somewhat greater exposure latitude, lighter blue background, and better-defined spots than some other papers.

Two kinds of low-wattage ultraviolet lamps are useful. One is a quartz "germicidal" mercury arc emitting over 90 percent of its energy at 254 mμ. The other is a "black light" fluorescent lamp, whose phosphor emits most of its energy in the long-wave ultraviolet near 360 mμ. Both lamps also emit visible light, but filtering is unnecessary because of the low sensitivity of blueprint paper to wavelengths above 400 mμ. The lamps are mounted on a frame about 2 inches above the paper. For one-dimensional chromatograms up to 2 inches wide and 12 inches long, one lamp is sufficient; for wider chromatograms several parallel lamps must be used (or a single high-wattage lamp set 12 inches or higher above the paper). Complete equipment and materials needed for ultraviolet blueprinting of chromatograms are obtainable from Microchemical Specialties Company, Berkeley, Calif.

The technique has been used so far primarily with test strips and one-dimensional chromatograms of Whatman No. 4 paper (available in 600-foot rolls and widths of ½, 1, 1½, . . . inches). Blueprint paper is taken from a 2-inch wide roll in a dispenser and laid on a thin, flexible-plastic, 12-inch rule. The filter paper strip is laid on the sensitized surface of the blueprint paper, and paper clips are used to attach the ends of the papers to the plastic rule. If better contact is desired, more clips may be used at intervals of 2 inches; the clips show