Reports

Cache Valley Virus, a Previously Undescribed Mosquito-Borne Agent

Abstract. Cache Valley virus was isolated from *Culiseta inornata* mosquitoes collected in Utah. The newly isolated agent causes signs of encephalitis in mice inoculated intracerebrally. It has been classified with the Bunyamwera group of anthropod-borne (Arbor) viruses.

During routine testing of wild-caught mosquitoes for the presence of western and St. Louis encephalitis viruses, an agent was recovered which appears to be unlike previously described mosquito-borne viruses of North America. The new isolate has been named Cache Valley virus for the locality from which the infected mosquitoes were collected.

Cache Valley virus was isolated from a pool of 50 *Culiseta inornata* mosquitoes collected on 16 Aug. 1956 under a bridge near the town of Wellsville in Cache Valley, northern Utah. Signs of encephalitis were observed in each of six weanling mice used for the initial isolation attempt (*1*). Most of the test mice were found in a moribund state 8 to 10 days after inoculation, and all had died by the 13th day. Three weeks later the virus was again recovered in mice inoculated with a stored portion of the original mosquito suspension. The agent obtained from these tests possessed the usual characteristics of viruses: it passed readily through a Seitz filter; no growth was obtained on common laboratory media inoculated with mouse-brain suspensions containing the agent; and its infectivity was not reduced by the addition of antibiotics.

Cache Valley virus was adapted to growth in weanling mice with considerable difficulty. The LD_{50} titer of a fourth-passage mouse-brain suspension was only $10^{-4.6}$. Significantly higher titers were not obtained through 11 passages in mice of this age. After the 12th passage, the agent was propagated in 2- to 3-day-old mice. The 14th mouse-passage virus stock, prepared from infant mouse brain, had an LD_{50} titer of $10^{-7.7}$ in weanling mice.

In further studies of Cache Valley virus the following results were obtained. (i) The virus was immunogenic, but not pathogenic, for rabbits inoculated via the intramuscular route. (ii) It was pathogenic for infant mice inoculated either by the intraperitoneal or intracerebral route. (iii) It was not pathogenic for weanling mice inoculated intraperitoneally or subcutaneously. (iv) It apparently was not infectious for 0.5-day-old chicks; chicks inoculated subcutaneously with large doses of Cache Valley virus did not develop a viremia, and, furthermore, neutralizing antibodies against the agent were not demonstrable in their serum 30 days later. (v) The virus produced cytopathogenic effects in tissue-culture cells grown from hamster kidney. (vi) Evidence was found suggesting that horses may become infected with Cache Valley virus under natural circumstances. Serum specimens from four horses living in Cache Valley were considered positive in mouse-neutralization tests (*2*). In these tests, neutralization indexes of 50, 80, >320, and >500, respectively, were obtained. Negative results were obtained in similar tests on serums from three other horses and five human beings from the same vicinity.

Cache Valley virus in third mouse passage was sent to the Rockefeller Foundation Virus Laboratories in New York, where Casals and Whitman found it to be serologically distinct from the viruses of Arbor groups A, B, and C and found that it belongs, instead, to their newly designated "Bunyamwera group" (*3*). In addition to Cache Valley virus, this group includes the following viruses: (i) *Bunyamwera,* isolated by Smithburn *et al.* (*4*) from a mixed pool of *Aëdes* mosquitoes collected in Uganda, British East Africa; (ii) *Wyeomyia,* isolated by Roca-Garcia (*5*) from *Wyeomyia melanocephala* mosquitoes collected in Colombia, South America; and (iii) Kairi virus (TR8900), isolated from *Aëdes scapularis* mosquitoes collected in Trinidad, British West Indies (*6*).

Apparently, the distribution of Cache Valley virus is not confined to Utah. Immune serum prepared against an agent isolated from *Culiseta inornata* in the National Institutes of Health's Rocky Mountain Laboratory, Hamilton, Montana (*7*), protected against three logs of Cache Valley virus in mouse-neutralization tests. Casals and Whitman (*3*) report that an agent indistinguishable serologically from Cache Valley virus was isolated by O. R. Causey from *Aëdes scapularis* mosquitoes collected near Belém (Pará), Brazil. Another virus apparently identical to or very closely related to Cache Valley virus has been recovered by Aitken (*8*) from *A. scapularis* collected in Trinidad, British West Indies.

It seems reasonable to assume that the recovery of Cache Valley virus represents a bona fide isolation from mosquitoes. None of the other viruses in the "Bunyamwera group" has ever been in our laboratory. Additional evidence was the reisolation of Cache Valley virus from the original mosquito pool, and the finding that Cache Valley virus was "neutralized" by sera obtained from horses living in the same area from which the mosquitoes were collected.

The possible importance of Cache Valley virus to the health of human beings or animals is not known. However, it is recognized that the causal agents in many cases of disease of the central nervous system among various animal species remain unidentified each year. Therefore, further studies are planned to determine the pathogenesis of Cache Valley virus in experimentally infected animals, the host range of the virus in nature, its natural mode of transmission, and whether or not naturally acquired Cache Valley virus infections may result in disease in man or other animal species.

PRESTON HOLDEN*
A. D. HESS
Communicable Disease Center,
U.S. Public Health Service,
Greeley, Colorado

References and Notes

1. Mosquitoes were suspended in 33⅓-percent normal inactivated serum-saline diluent containing antibiotics. The resulting suspension was inoculated into 3- to 4-week-old mice via the intracerebral route.
2. In mouse-neutralization tests, constant amounts of serum were mixed with serial tenfold dilutions of virus. After incubation at 37°C for 2 hours, the serum-virus mixtures were inoculated into weanling mice via the intracerebral route.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

3. J. Casals and L. Whitman, *Am. J. Trop. Med.*, in press.
4. R. C. Smithburn, A. J. Haddow, A. F. Mahaffy, *Am. J. Trop. Med.* **26**, 189, 208 (1946).
5. M. Roca-Garcia, *J. Infectious Diseases* **75**, 160 (1944).
6. C. R. Anderson, T. H. G. Aitken, L. Spence, W. G. Downs, *Am. J. Trop. Med.*, in press.
7. The serum was provided through the kindness of Carl M. Eklund of the Rocky Mountain Laboratory.
8. T. H. G. Aitken, personal communication.

* Present address: Division of Research Service, National Institutes of Health, Bethesda, Maryland.

2 July 1959

Resistance to Deformation of Axial Structures in Living Guinea-Pig Spermatozoa

Abstract. Rotation of living guinea-pig spermatozoa can be effected by drawing the shaft of a long microneedle of diameter 0.5 to 1.0 μ across the tail; the special characteristics of this rotation demonstrated in the tail a polygonal structure and high resistance of the peripheral fibrils to deformation by pressure of the microneedle.

In the course of research on fragility (in immune reactions) of guinea-pig spermatozoa (*1*) during which use was made of micromanipulation to effect dissection of living spermatozoa, it was observed that it was easily possible to rotate an immobilized spermatozoon by drawing a long glass rod (or long shank of a microneedle) at right angles to the length of the spermatozoon across the tail, approximately halfway between the midpoint and the body. This was effected by making the diameter of the needle close to the diameter of the tail (Fig. 1) (in general about 0.5 to 1.0 μ) and about 200 μ or more long.

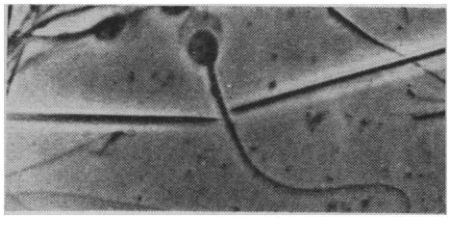

Fig. 1. Living guinea-pig spermatozoon with two microneedles showing diameter and position for rotation with relation to tail. Phase contrast. (×1150)

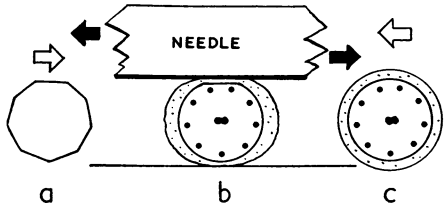

Fig. 2. (*a*) Polygonal structure effective for rotation by movement of needle across surface. (*b*) Tail of spermatozoon on section with suggested deformation of surface protoplasm on pressure of needle; resistance of peripheral fibrils and central protoplasm. (*c*) Normal structure and relative disposition of fibrils in tail (schematic).

The special feature of the rotation of the spermatozoon (shown by rotation of the head) was its positive nature (in that the needle did not slip and rotation was directly proportional to the speed of movement of the needle). This indicated, first, that the tail cannot be round or flat but must possess a basic polygonal structure on section (Fig. 2*a*) in order that the tail may be engaged between the needle and the slide surface (Fig. 2*b*); this polygonal structure is shown by electron microscopy of the tail where a set of nine peripheral fibrils surrounds a double axial thread (Fig. 2*c*). Second, micromanipulation demonstrated that the basic fibrillar structure must be highly resistant to deformation; here electron microscopy shows a thin outer protoplasmic sheath (which contains microsomes) smoothly rounded in outline with a spiral layer outside the peripheral fibrils; a light structureless protoplasm occupies the central region around the axial fibril (Fig. 2*c*).

This second conclusion on the physical properties of the spermatozoa shown by micromanipulation appears to confirm an early observation on micromanipulation of spermatozoa by Terni (*2*); in addition, observations by Duryee (*3*) on microdissection of *Triturus pyrrhogaster* spermatozoa revealed similar properties in this species.

Deformation of the thin surface protoplasmic layer must take place in order that the needle may be brought in close contact with the fibrils. The question then arises as to why the internal protoplasm, greater in amount than that between and around the peripheral fibrils, does not also deform; if any displacement of fibrils takes place it must be slight since rotation requires persistence of the polygonal structure; these observations also concern the forces which maintain the fibrils in position; the forces not only resist pressure from the surface but resist tension on their long axes without evidence of elasticity; such lack of elasticity is to be expected from the characteristics of the surface resistance.

It would be desirable, and it should be possible, to obtain quantitative data on these forces. The observations presented indicate that the forces will be found to be high among biological materials of fibrillar structure.

C. A. ERSKINE

School of Anatomy, Trinity College, University of Dublin, Dublin, Ireland

References

1. C. A. Erskine, *Proc. Soc. Study Fertility*, in press.
2. T. Terni, cited by W. Duryee (personal communication).
3. W. Duryee, personal communication.

13 May 1959

Induction of the Respiration-Deficient Mutation in Yeast by Various Synthetic Dyes

Abstract. Series of triphenylmethane and xanthene dyes were found to be effective as inducers of respiration-deficient mutation in yeast. The quantitative difference in the mutagenic effect appeared to be in close relationship to the chemical structure of the respective dyes. This survey may provide a useful clue for elucidation of the mechanism of mutagenic induction.

Among various inducers of the respiration-deficient (*petite colonie*) mutation in yeast, several synthetic dyes show pronounced effects (*1*). The work under discussion deals with a comparative survey of the *petite-colonie*-inducing effects of various dyes in an attempt to seek the relationship, if any, between the chemical structure and the efficacy of the dye as an inducer.

A strain of baker's yeast (Fleischmann) mentioned in an earlier report (*2*) was cultured in a liquid medium

Table 1. Production of mutants by various dyes. Minimal concentration is required to produce more than 3 percent mutants, optimal to produce more than 90 percent.

Dye (Manufacturer)	Concentration (mg/lit)		
	Minimal	Optimal	For entire suppression
Crystal violet* (Merck)	0.2	0.6	2.4
Ethyl violet* (Bayer)	0.2	0.4	2.0
Methyl violet* (Merck)	2.0	4.0	8.0
Malachite green* (Japan)	1.0	3.0	10.0
Pararosaniline* (Japan)	2.0	8.0	50.0
Rosaniline* (Japan)	3.0	8.0	60.0
Victoria blue B* (Japan)	6.0	20.0 (<40%)	30.0
Victoria blue 4R* (Japan)	6.0	10.0 (<60%)	30.0
Pyronine Y† (Bayer)	2.0	6.0	50.0
Pyronine B† (Bayer)	2.0	6.0	50.0
Acridine red 3B† (Japan)	12.0	40.0	100.0
Acriflavine (Japan)	0.4	1.5	12.5
Caffeine (Japan)	1500	2500	3500

* Triphenylmethane dye; † xanthene dye. Acridine red 3B is a xanthene dye in spite of the name.