Reports

Eastern Equine Encephalitis Virus Isolated from Culex nigripalpus in Trinidad

Abstract. The isolation of the first strain of eastern equine encephalitis virus in Trinidad, West Indies, is described. The virus came from a pool of *Culex nigripalpus* mosquitoes collected from chicken-baited traps in May 1959.

Previous evidence for the presence of eastern equine encephalitis virus in Trinidad was based on the finding of neutralizing antibody to this virus in two separate serum specimens collected in 1954 from the same native donkey (*1*).

In May 1959, a pool of 512 *Culex nigripalpus* yielded eastern equine encephalitis virus. The mosquitoes, of which 498 were gravid and many were engorged, were collected between 2 and 9 May from two chicken-baited traps located on La Fortune and Esperanza cocoa estates, Vega de Oropouche, about 4 miles northeast of Sangre Grande.

The mosquitoes were ground in 3 ml of bovalbumin diluent, subsequently diluted 1:3 after centrifugation, and inoculated intracerebrally into a group of seven 2-day-old white mice. On the 5th postinoculation day one mouse died and the brain of a second moribund mouse was passaged intracerebrally and intraperitoneally to two further groups of suckling mice and intracerebrally to a group of adult mice. The former all sickened or died by the 2nd day, but some of the adult deaths did not occur until the 3rd day. The agent responsible for these deaths passed readily through a bacteria-tight Seitz-EK pad. On further passage the virus killed suckling and adult mice regularly within 2 days of inoculation.

The virus was reisolated from the original mosquito suspension, which was inoculated undiluted into suckling mice by the intraperitoneal route. An attempt to reisolate the virus in chick embryo tissue cultures was unsuccessful.

An acetone-ether-extracted hemagglutinin was prepared from the brains of infected suckling mice. The virus was shown by hemagglutination-inhibition tests to be a member of group A (*2*). Complement-fixation tests were performed with crude saline and acetone-ether-extracted antigens prepared from the brains of infected suckling mice. Various group A antisera were used in these tests, including eastern equine encephalitis and western equine encephalitis hyperimmune mouse sera prepared in the New York laboratories of the Rockefeller Foundation from North American strains of these viruses. With the crude saline antigen, positive reactions were obtained with eastern and western equine encephalitis antisera, and negative reactions with Semliki Forest, Sindbis, Mayaro, and Venezuelan equine encephalitis antisera. The acetone-ether-extracted antigen gave a positive reaction with the eastern equine encephalitis antiserum and a negative reaction with the western equine encephalitis antiserum.

In neutralization tests in mice the virus was neutralized by immune and hyperimmune mouse sera prepared from a North American strain of eastern equine encephalitis virus but not by immune mouse serum prepared from western equine encephalitis virus. It was also neutralized by serum obtained from the native donkey referred to above. No strains of eastern equine encephalitis virus were kept at this laboratory prior to this isolation.

One of the five chickens used as bait in the mosquito traps developed neutralizing and hemagglutination-inhibiting antibodies to the virus between 2 and 16 May (*3*).

Addendum. Since submitting this paper for publication, we have made two more isolations of eastern equine encephalitis virus, both from pools of *Culex (Melanoconion) taeniopus* collected in the traps mentioned above. Strain 1 (TRVL 25780) came from a pool of 249 mosquitoes collected between 5 and 22 Aug. 1959 and suspended in 2 ml of diluent. Strain 2 (TRVL 26263) came from a pool of 142 mosquitoes collected between 1 and 11 Sept. 1959 and suspended in 3 ml of diluent. Both strains were successfully reisolated from the original suspensions. It is noteworthy that the original *nigripalpus* strain was isolated in May, when bird migration was northward. The two *taeniopus* isolations occurred at a time when bird migrations had reversed and were headed southward. Mosquito collections from the same two traps have been made continuously since September 1958. We have no evidence of eastern equine encephalitis activity in Trinidad other than the information provided above.

W. G. Downs
T. H. G. Aitken
L. Spence
Trinidad Regional Virus Laboratory, Port-of-Spain, Trinidad, West Indies

References and Notes

1. W. G. Downs, C. R. Anderson, M. Theiler, *Am. J. Trop. Med. Hyg.* **5**, 626 (1956).
2. J. Casals and L. V. Brown, *J. Exptl. Med.* **99**, 429 (1954).
3. The studies and observations on which this report is based were conducted with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme, and the Rockefeller Foundation.

22 July 1959

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)].

The Clock Paradox

Abstract. In Minkowski space, a coordinate system can always be chosen so that the straight line (geodesic) joining two events (not on a null line) is either parallel to the time axis or parallel to the space axis. In either case the geodesic has maximum length (time dilatation and Lorentz contraction).

In a recent report [*Science* **129**, 1359 (1959)] C. C. MacDuffee shows that, in special relativity, the equation of motion of a one-dimensional unaccelerated particle (that is, the equation of a geodesic in Minkowski space) is a straight line $x = at + b$ (where $|a| < 1$ in units such that the velocity of light is 1). He then proves that a geodesic joining two points P_1 and P_2 has maximum length (time dilatation or clock paradox).

The latter proof is considerably simplified by noting that there is no loss of generality in choosing $a = 0$. Physically this amounts to choosing an inertial system whose t-axis is parallel to the P_1P_2 geodesic; this is always possible if, as assumed, $|a| < 1$. Mathematically,

it amounts to observing that $x = at + b$ can always be reduced to $x =$ constant by a linear transformation for which $dt^2 - dx^2$ is invariant.

Then in the coordinate system for which the geodesic P_1P_2 is $x =$ constant, the space-time interval ds is a pure time interval dt (since $dx = 0$). The elapsed proper time is

$$\int_{t_1}^{t_2} dt = t_2 - t_1$$

The concept of the same place at two different times *is* meaningful in this reference frame; P_1 and P_2 are the same place at two different times. Now for any other arc joining P_1 and P_2, say $x = x(t)$, with

$$\left|\frac{dx}{dt}\right| < 1$$

(the latter condition makes the arc a possible path of a particle), the proper time is given by

$$ds = \sqrt{dt^2 - dx^2} = \sqrt{1 - (dx/dt)^2}\, dt < dt$$

$$s_2 - s_1 = \int_{P_1}^{P_2} ds < \int_{t_1}^{t_2} dt$$

—that is, the proper time is largest for a geodesic.

Exactly the same proof, for the case slope of $P_1P_2 < 1$, gives $t = ax + b$, $|a| < 1$ (or $t =$ constant in an inertial system whose x-axis is parallel to P_1P_2), and shows that the distance between two points is longest along a geodesic. This is the Lorentz contraction: an object measures longest in its rest system.

MARY L. BOAS
Department of Physics, DePaul University, Chicago, Illinois

29 June 1959

Serological Procedure for the Detection of Antibodies to Penicillin

Abstract. A hemagglutination technique for demonstrating antibodies to penicillin in sera from penicillin-allergic subjects is described. Erythrocytes coupled to penicillin T by means of bis-diazotized-benzidine constitute the "antigen" used to measure the antipenicillin antibody. The hemagglutination reaction is highly specific, as indicated by hemagglutination-inhibition studies. The clinical significance of these antibodies remains to be elucidated.

The antigenic property of penicillin is recognized by such in vivo methods as the skin scratch and intradermal tests and the conjunctival test. However, little is known of the nature of the antibody to penicillin. It has been demonstrated by passive transfer techniques (*1*), and recently an in vitro demonstration of circulating antibody has been reported by Ley *et al.* (*2*). Watson and his colleagues (*3*) describe an attempt to characterize the penicillin antibody by electrophoretic studies in starch gels.

Table 1. Hemagglutination* titers of sera from penicillin-sensitive subjects.

Patient's serum	Serum dilutions (in NRS 1:100)						
	1:4	1:8	1:16	1:32	1:64	1:128	1:256
JH-2	4+	4+	4+	4+	3+	±	0
DMP-2	4+	3+	2+	±	0	0	0
BV-1	2+	2+	2+	0	0	0	0
AV-2	0	0	0	0	0	0	0

* Erythrocytes sensitized with penicillin T by means of BDB.

This paper reports a new serological technique for detecting antibodies to penicillin. The principle of coupling the antibiotic to the erythrocyte by means of bis-diazotized-benzidine (BDB) is used to prepare the "antigen" (*4*). The bis-diazotized-benzidine provides a stable bond between the penicillin and the red cell. Hemagglutination occurs when cells sensitized in this fashion are mixed with the serum from certain individuals who are hypersensitive to the drug. The pattern of agglutination obtained resembles that seen in the virus hemagglutination test (*5*).

Successful coupling of penicillin to erythrocytes has been accomplished with penicillins T (*p*-aminobenzyl penicillin) and V (phenoxymethyl penicillin). Rabbit, sheep, or human group O, Rh-negative erythrocytes may be used. If erythrocytes of heterologous species are employed, absorption procedures are carried out to eliminate nonspecific agglutination. After preliminary experiments, the human red cell was selected as a matter of convenience.

It is essential to determine the optimal ratio of BDB to penicillin for the sensitization of the erythrocytes. This is accomplished by a "checkerboard" titration of a known positive antipenicillin serum and a negative serum with (i) erythrocytes exposed to a constant amount of penicillin and varying volumes of a 15-fold dilution of BDB and (ii) erythrocytes exposed to varying amounts of penicillin and a constant volume of the diluted BDB. That combination of the two reagents producing the highest titer with the positive serum and no reactions with the negative serum and in the controls is used for the performance of the test proper.

The sensitized erythrocytes are washed once in 1-percent normal rabbit serum in a phosphate buffer of *p*H 7.3 (NRS 1:100). The same medium (NRS 1:100) has to be used to prepare the 2-percent erythrocyte suspension, as the sensitized cells are unstable in saline. For the same reason NRS 1:100 is also used to prepare the twofold dilutions of the test sera.

The reaction between the serum and the sensitized erythrocytes is allowed to take place at room temperature. The results of the hemagglutination may be read in 3 to 5 hours, and observation may be repeated after overnight incubation on the bench.

Nine of 20 sera from persons with reported allergic manifestations to penicillin have produced positive hemagglutination reactions with titers from 1:4 to 1:64. Representative titrations are shown in Table 1.

Hemagglutination-inhibition experiments with decreasing concentrations of penicillin solutions and a constant dilution of a serum known to produce a 4+ reaction indicate that this technique is highly specific and sensitive. Eight units of penicillin V and four units of penicillins G and T completely prevented agglutination of erythrocytes linked to penicillin T. Moreover, the cross reactions exhibited by the hemagglutination-inhibition test appear to lend support to the assumption that the penicillin molecule per se, rather than the side chain, is primarily concerned in eliciting antibody formation.

The clinical significance of the antibody to penicillin is being investigated (*6*).

MARGUERITE EPP
Department of Bacteriology, University of Saskatchewan, Saskatoon, Canada

References and Notes

1. G. A. Peters, L. L. Henderson, L. E. Prickman, *Ann. Allergy* **15**, 135 (1957); M. Coleman and B. B. Siegel, *J. Allergy* **26**, 253 (1955); B. B. Siegel and M. Coleman, *ibid.* **28**, 264 (1957).
2. A. B. Ley *et al.*, *Science* **127**, 1118 (1958).
3. K. C. Watson, S. M. Joubert, M. A. E. Bennett, *Nature* **183**, 468 (1959).
4. A. B. Stavitsky and E. R. Arquilla, *J. Immunol.* **74**, 306 (1955); J. Gordon, B. Rose, A. H. Sehon, *J. Exptl. Med.* **108**, 37 (1958).
5. J. E. Salk, *J. Immunol.* **49**, 87 (1944).
6. I wish to thank A. L. Tosoni, Connaught Medical Research Laboratories, University of Toronto, for the penicillin T and C. W. Pettinga, Eli Lilly and Company, Indianapolis, Ind., for the penicillin V used in this work. Thanks and appreciation are extended to G. Dempster, professor and head of the department of bacteriology, College of Medicine, University of Saskatchewan, Saskatoon, for suggestions and help in this investigation.

17 July 1959