cooling to 10°C caused significant membrane depolarization, presumably by inhibiting the electrogenic Na^+ pump (*10*), without significant changes in a^i_K. These findings, plus the fact that in the steady state E_K is 20 to 30 mv more negative than E_M, lead to the conclusion that K^+ is actively transported into this cell.

In the steady state, E_{Cl} is more negative than E_M by 2 to 18 mv. The effects of cooling to 1°C and rewarming support the conclusion that Cl^- is actively transported out of cell R2. However, active transport of Cl^- is little affected by ouabain and unaffected by cyanide, an indication that the K^+ and Cl^- transport systems are different.

J. M. RUSSELL

Department of Physiology, University of Utah Medical Center, Salt Lake City 84112

A. M. BROWN

Departments of Physiology and *Medicine, University of Utah Medical Center*

References and Notes

1. A. Mauro, *Fed. Proc.* **13**, 96 (1954); A. L. Hodgkin and R. D. Keynes, *J. Physiol. London* **128**, 28 (1955).
2. R. D. Keynes, *ibid.*, **169**, 690 (1963).
3. The following papers are concerned with K^+ transport: J. C. Keesy, H. Wallgren, H. McIlwain, *Biochem. J.* **95**, 289 (1965); A. V. Escueta and S. H. Appel, *Biochemistry* **8**, 725 (1969); J. S. Kehoe and P. Ascher, *Nature* **225**, 820 (1970). The following papers are concerned with Cl^- transport: F. Strumwasser, *Circadian Clocks* (North-Holland, Amsterdam, 1964), p. 442; D. J. Chiarandini and H. M. Gerschenfeld, *Science* **156**, 1595 (1967); H. D. Lux, *ibid.* **173**, 555 (1971).
4. A. Strickholm and B. G. Wallin, *Nature* **208**, 790 (1965); G. A. Kerkut and R. W. Meech, *Comp. Biochem. Physiol.* **19**, 819 (1966); B. G. Wallin, *Acta Physiol. Scand.* **70**, 419 (1967); A. M. Brown, J. L. Walker, R. B. Sutton, *J. Gen. Physiol.* **56**, 559 (1970); D. L. Kunze and A. M. Brown, *Nature* **229**, 229 (1971). No mention was made in the papers by Strickholm and Wallin and by Kerkut and Meech about how much time elapsed between multiple cellular impalement and internal ionic readings. In the experiments by Brown *et al.* and by Kunze and Brown no more than 30 minutes elapsed.
5. W. T. Frazier, E. R. Kandel, I. Kupferman, R. Waziri, R. E. Coggeshall, *J. Neurophysiol.* **30**, 1288 (1967).
6. J. L. Walker, *Anal. Chem.* **43**, 89A (1971).
7. Concentrations (in millimoles per liter) are: NaCl, 494; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 20; $MgSO_4$, 30; and tris maleate, 10 [see Brown *et al.* (*4*)].
8. We define steady state as that state in which activities and voltages are unvarying with time but the system is not in equilibrium [J. W. Woodbury, in *Physiology and Biophysics*, T. C. Ruch and H. D. Patton, Eds. (Saunders, Philadelphia, ed. 19, 1965), p. 18].
9. S. L. Bonting and L. L. Carravaggio, *Arch. Biochem. Biophys.* **101**, 37 (1963); A. White, P. Handler, E. L. Smith, in *Principles of Biochemistry*, A. White *et al.*, Eds. (McGraw-Hill, New York, ed. 4, 1964), pp. 228–229.
10. D. O. Carpenter, *Comp. Biochem. Physiol.* **35**, 371 (1970).
11. A.M.B. is an Established Investigator of the American Heart Association. Supported by PHS grants HE-10977, HE 13480, and NS 09545.

22 October 1971; revised 6 December 1971

Vesicular Stomatitis Virus (Indiana Serotype): Transovarial Transmission by Phlebotomine Sandflies

Abstract. *Transovarial transmission of vesicular stomatitis virus (Indiana serotype) by experimentally infected* Lutzomyia trapidoi *and* Lutzomyia ylephiletrix *to their progeny was demonstrated. Virus was recovered from all developmental stages; mean virus titers from egg to first generation adult showed a four-log increase, indicating that virus multiplication occurred during development of the sandflies. Virus titers in first generation adult females were comparable to those found in their parents. These infected female sandflies transmitted vesicular stomatitus virus Indiana by bite to susceptible animals and transmitted the virus transovarially to their offspring (second generation). Results demonstrate a possible mechanism for transmission and maintenance of this virus in nature without a vertebrate (heat) host reservoir.*

Because of its clinical similarity to foot-and-mouth disease, vesicular stomatitis is of economic and veterinary importance. The disease occurs in both epizootic and enzootic forms. Immunologic surveys indicate that vesicular stomatitis virus (VSV) naturally infects many species, including man (*1, 2*). Antibodies are frequently found among humans living in areas where VSV is endemic, an indication that the virus may be of public health importance (*1, 2*).

The recovery of VSV Indiana from wild phlebotomine sandflies (*3, 4*) along with the evidence of virus multiplication in, and bite transmission by, experimentally infected sandflies (*4*), suggests that these blood-sucking insects may be vectors of the virus. However, a susceptible vertebrate species that develops a viremia sufficient to infect a biting arthropod (*5*) has not been found. Although VSV is classified as an arbovirus (*6*), available data suggest that it does not follow the conventional insect-vertebrate cycle of mosquito-borne viruses; the natural source of VSV has also remained a mystery (*5*). In view of earlier reports suggesting "transovarial transmission" of sandfly (*papatasi*) fever virus (*7, 8*) together with recent isolation of several other viral agents from male sandflies (*9–12*), we investigated the possibility of insect-to-insect transmission of VSV Indiana. We report here generation-to-generation transmission of VSV Indiana by experimentally infected phlebotomine sandflies, and we thus demonstrate a possible mechanism for transmission and maintenance of the virus without a vertebrate host reservoir.

Infant hamsters were inoculated subcutaneously with 10^4 plaque-forming units of a third-passage strain of VSV Indiana. Approximately 24 hours later, one of the hamsters was exposed for 3 hours in a cage with wild sandflies. The hamster was then bled, and the extent of viremia was determined. Blood-engorged flies were removed from the cage, were placed individually in numbered clay pots (*13–15*), and were maintained at 26° to 28°C until oviposition occurred. After oviposition, females were removed from the pots and were frozen at −60°C for subsequent virus titration. Decomposing leaf material was added to the pots as a food source for emerging larvae. At various intervals during development, eggs, larvae (first, second, third, and fourth instar), pupae and F_1 adults were removed from the pots and tested for the presence of virus.

All sandfly specimens except eggs and first instar larvae were triturated individually in sterile 2-ml tissue grinders (Ten Broeck) containing 1.0 ml of medium 199 with 10 percent fetal bovine serum inactivated by heat, penicillin (10,000 unit/ml), streptomycin (16 μg/ml), and amphotericin B (2.5 μg/ml). Because of their tiny size, eggs and first instar larvae were processed under a dissecting microscope. These specimens were placed in separate wells of a microplate containing 0.1 or 0.2 ml of the aforementioned diluent and were triturated individually with a metal probe. Suspensions of parent female sandflies and specimens of hamster blood were prepared in serial, tenfold dilutions and titrated in Vero cell (a continuous line of African green monkey kidney) microplate monolayer cultures; four wells were used for each dilution (*16*). Microplates were incubated at 37°C and were examined after 48 hours for VSV cytopathic effect under an inverted microscope (*16*); results were recorded as the tissue culture infectious dose, 50 percent effective ($TCID_{50}$) per insect specimen or per milliliter of hamster blood (*17*).

Table 1. Infection rates of VSV Indiana in F_1 progeny of experimentally infected *L. trapidoi* females.

Developmental stage	No. positive/ No. tested	Percent positive	Virus titers of positive insects*		
			No. tested	Range	Mean
Egg	10/154	6.5	7	0.3 — 0.8	0.7
First instar	25/108	23.1	22	0.3 — 2.3	1.1
Second instar	23/143	16.1	15	1.3 — 3.6	2.2
Third instar	28/142	19.7	20	1.3 — 2.8	2.3
Fourth instar	24/132	18.2	22	1.6 — 4.8	3.2
Pupa	56/154	36.4	19	1.8 — 4.0	2.5
F_1 adult male	48/225	21.3	27	2.0 — 4.0	3.0
F_1 adult female	61/248	24.6	36	2.8 — 6.1	5.1

* Titers expressed as $\log_{10}$ of $TCID_{50}$ units per insect.

Virus infection of sandfly progeny was detected by inoculating 0.1 ml of each triturated egg or insect suspension into single tubes of Vero cells. Cultures were incubated at 37°C and examined for 2 days for VSV cytopathic effect. Positive cultures were harvested and identified as VSV Indiana in a plaque neutralization test on microplates (*18*), in which specific guinea pig immune serum was used. Virus titers of most of the VSV-positive eggs, larvae, pupae, and F_1 adults were determined by inoculating serial tenfold dilutions of the original insect suspension into Vero monolayer cultures in microplates as described above.

Transovarial transmission of VSV Indiana was attempted in four anthropophilic species, *Lutzomyia trapidoi, L. ylephiletrix, L. sanguinaria* and *L. gomezi.* The blood of hamsters used to infect parent sandflies contained $10^{5.5}$ to $10^{8.0}$ $TCID_{50}$ of VSV Indiana per milliliter. Most of the flies feeding on viremic hamsters became infected. The mean titer of virus of 130 positive *L. trapidoi* was $10^{5.3}$ $TCID_{50}$, and of 14 infected *L. ylephiletrix* was $10^{5.7}$; mean titers of infected females of the two other species were significantly lower, $10^{4.2}$ for 36 *L. sanguinaria*, and $10^{3.9}$ for 6 *L. gomezi.* Progeny of noninfected female sandflies were discarded.

A total of 1716 sandfly eggs, larvae, pupae, and F_1 adults were tested. Of 1306 offspring from infected *L. trapidoi* that had fed on viremic hamsters, 21 percent yielded VSV Indiana, and of 96 *L. ylephiletrix* offspring, 20 percent were also positive. In contrast, none of the 269 progeny from infected *L. sanguinaria* nor of the 45 offspring from *L. gomezi* yielded virus. This indicates species differences in the ability to transmit the agent.

Table 1 is a summary of results obtained in offspring from infected *L. trapidoi.* The virus was recovered from all developmental stages. Mean virus titers of infected F_1 adult females were about 10^4 higher than the mean titers observed in infected eggs and first instar larvae, indicating that virus multiplication occurred during development of the sandflies. Of 473 F_1 adults tested, VSV Indiana was recovered from both male (48/225) and female (61/248) flies. Virus titers of 36 infected *L. trapidoi* F_1 adult females ranged from $10^{2.8}$ to $10^{6.1}$ (mean $10^{5.1}$) $TCID_{50}$, titers comparable to those found in wild and experimentally infected sandflies (*4*).

To determine whether the F_1 adult female *L. trapidoi* were infectious, 50 flies were allowed to feed on clean suckling hamsters. These hamsters were bled 24 hours later; blood specimens were inoculated into tube cultures of Vero cells. The virus was recovered from the blood of 9 of 12 bitten hamsters, indicating that infected F_1 adult females can transmit the virus by bite to susceptible animals.

After feeding on clean hamsters, these F_1 females were allowed to oviposit, and their progeny were reared and were tested for virus. Of 86 F_2 larvae from infected F_1 adults, 34 percent yielded VSV Indiana; this result shows that the virus can be passed from generation to generation, and suggests that there may even be an increase in transmission rate from the F_1 to F_2 generation.

Our results also suggest that the mechanism of VSV Indiana transmission from parent to offspring is transovarial; however, to rule out the possibility that transmission occurs by absorption of the virus to the exterior of the egg or that emerging larvae may become infected by ingesting secretions left in the rearing pots by their parents [as has been demonstrated with certain of the nuclear polyhydrosis and granulosis viruses (*19*)], we performed the following experiment: After oviposition, about 200 eggs from infected *L. trapidoi* females were removed from rearing vessels, were soaked in 1 percent NaOH for 10 minutes, were rinsed twice in 70 percent ethanol, and were washed in distilled water (*20*). The eggs were then transferred to clean pots. Virus was recovered from both triturated suspensions of sterilized eggs and from larvae emerging from them. As controls, approximately 400 eggs from noninfected *L. trapidoi* were soaked overnight in a concentrated suspension (10^8 $TCID_{50}$ per milliliter) of VSV Indiana. Half of these eggs were then sterilized on their surfaces as described above. Triturated suspensions of unsterilized eggs yielded virus ($10^{5.0}$ $TCID_{50}$), while sterilized eggs gave negative results. None of the larvae emerging from either of these two control groups yielded VSV, thus demonstrating that it is possible to sterilize the surface of eggs and that successful transmission depended on presence of the virus within the egg.

The isolation of VSV Indiana from naturally infected *L. trapidoi*, along with the experimental evidence of virus multiplication and bite transmission by this same species, suggests that it may serve as a vector of VSV Indiana (*4*). The demonstration of transovarial transmission of VSV Indiana through two consecutive generations of *L. trapidoi* is therefore significant, for it provides a mechanism for infection of this species and for maintenance of the virus in nature. It is also the first conclusive evidence for transovarial transmission of a vertebrate virus vectored by a dipteran insect. Our results further indicate that progeny of infected females can transmit VSV Indiana by bite to a susceptible animal. However, transovarial transmission rates of 20 to 30 percent would not sustain the virus for long in the insect population in the absence of one or more of the following: (i) selective survival of infected sandflies, (ii) virus transmission to many females during insemination by infected males, or (iii) the existence of another virus source that occasionally replenishes the transovarial cycle.

ROBERT B. TESH
BYRON N. CHANIOTIS
KARL M. JOHNSON
U.S. Public Health Service, National Institute of Allergy and Infectious Diseases, Middle America Research Unit, Balboa Heights, Canal Zone

References and Notes

1. R. B. Tesh, P. H. Peralta, K. M. Johnson, *Amer. J. Epidemiol.* **90**, 255 (1969).
2. R. P. Hanson and L. H. Karstad, *Proc. U.S. Livestock Sanit. Ass.* **61**, 300 (1957).
3. P. H. Peralta and A. Shelokov, *Amer. J. Trop. Med. Hyg.* **15**, 369 (1966).
4. R. B. Tesh, B. N. Chaniotis, K. M. Johnson, *Amer. J Epidemiol.* **93**, 491 (1971).
5. R. B. Tesh, P. H. Peralta, K. M. Johnson, *ibid.* **91**, 216 (1970).
6. R. M. Taylor, *Catalogue of Arthropod-Borne Viruses of the World* (Government Printing Office, Washington, D.C., 1967).
7. H. E. Whittingham, *J. State Med.* **32**, 461 (1924).
8. S. D. Moshkovski, N. A. Diomina, V. D. Nossina, E. A. Pavlova, J. L. Livchitz, H. L. Pels, V. P. Roubtzova, *Med. Parazitol. Parazit. Bolez.* **6**, 922 (1937).
9. H. C. Barnett and W. Suyemoto, *Trans. N.Y. Acad. Sci.* **23**, 609 (1961).
10. J. R. Schmidt, M. L. Schmidt, M. I. Said, *Amer. J. Trop. Med. Hyg.* **20**, 483 (1971).
11. T. H. G. Aitken, personal communication.
12. R. B. Tesh and B. N. Chaniotis, in preparation.
13. M. Hertig and P. T. Johnson, *Ann. Entomol. Soc. Amer.* **54**, 753 (1961).
14. P. T. Johnson and M. Hertig, *ibid.*, p. 764.
15. B. N. Chaniotis, *J. Med. Entomol.* **4**, 221 (1967).
16. E. J. Sullivan and M. J. Rosenbaum, *Amer. J. Epidemiol.* **85**, 424 (1967).
17. L. J. Reed and H. A. Muench, *Amer. J. Hyg.* **27**, 493 (1938).
18. E. Earley, P. H. Peralta, K. M. Johnson, *Proc. Soc. Exp. Biol. Med.* **125**, 741 (1967).
19. K. M. Smith, *Insect Virology* (Academic Press, New York, 1967), pp. 128–145.
20. V. G. Dethier and N. Goldrich, *Science* **173**, 242 (1971).

29 September 1971; revised 27 December 1971 ■

GABA Catabolism: Localization of Succinic Semialdehyde Dehydrogenase in Brain Motor and Sensory Nuclei

Abstract. *The localization of brain succinic semialdehyde dehydrogenase, a specific gamma-aminobutyric acid degradative enzyme, could potentially yield valuable information concerning the function of the enzyme. Application of a new histochemical technique for this enzyme has revealed characteristic patterns of neuronal staining that are consistent within embryologically and functionally similar nuclei of the brainstem of the rat.*

Cytochemical procedures for localizing neurotransmitters and their metabolic enzymes provide important morphological clues to the chemical circuitry of the central nervous system. At the light microscopic level, such work has been limited almost exclusively to the monoamines (*1*) and to acetylcholinesterase (*2*). In an attempt to develop methods that might be useful in locating synapses mediated by gamma-aminobutyric acid (GABA), we modified standard tetrazolium methods for oxidation reactions [see (*3*)] to localize succinic semialdehyde dehydrogenase (SSADH) in the brain of the rat (*4*). We now report that this staining method for SSADH produces a characteristic pattern of staining among cranial nerve nuclei; neurons of predominantly motor function can be clearly distinguished from neurons with predominantly sensory function. This result suggests that the cytochemical differences among these functionally distinguishable neurons may reflect similar characteristic patterns of their neurochemical innervation.

Sprague-Dawley rats (250 g) were decapitated, and the whole brain or the spinal cord was removed; the handling of the tissue and the histochemical procedure for SSADH were performed as previously described (*4*). The histochemical procedure utilizes direct reduction of nitroblue tetrazolium by reduced nicotinamide adenine dinucleotide at *p*H 9.0; deposition of formazan has been shown to be independent of tissue diaphorase activity (*4*). Coronal sections (14 μm) were taken serially at 56-μm intervals beginning at the midcervical spinal cord and extending through the telencephalon. Sagittal sections were similarly processed from midline to the lateral extent of the hemisected brain; the caudal limit of the sagittal sections was high thoracic spinal cord, but all rostral brain structures were included. Frequently, 14-μm sections were stained with toluidine blue to assess the population of neurons present in sections adjacent to those used for the SSADH staining reaction.

Comparison of sections reacted histochemically for SSADH with seriatim sections stained with toluidine blue reveals that only a small population of neurons present within the brainstem are stained for SSADH. Specific nuclear groups stained for SSADH uniformly exhibit either cell body staining, or neuropil staining, or both. Staining comparisons are based on comparative analysis of serial sections through the various nuclei; intranuclear morphological differences require that ranking of neuropil staining represents an overall as-

Table 1. Histochemical activity of SSADH in the brainstem of the rat. Staining intensity is based on a scale from 0 to 4+. Neuropil staining is compared in relation to other neuropil areas; that is, it is not judged relative to adjacent cellular staining.

PRIMARY MOTOR NUCLEI*

(A) *Somatic efferent column*			(B) *Special visceral efferent column*			(C) *General visceral efferent column*		
Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil
Oculomotor	++++	++	Trigeminal motor	++++	++	Edinger-Westphal	++++	+++
Trochlear	++++	++	Facial	++++	++			
Abducens	++++	++	Ambiguus	++++	++	Dorsal motor nucleus of vagus	++++	+++
Hypoglossal	++++	++	Spinal accessory	++++	++			

SENSORY NUCLEI†

(D) *General somatic afferent column*			(E) *Visceral afferent column*			(F) *Special somatic afferent column*‡		
Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil
Mesencephalic root	++++	++	Tractus solitarius	0	++++	Ventral cochlear	++++	++
Principal trigeminal	0	++++				Dorsal cochlear	+	++++
Spinal tract trigeminal	0	++++	Parasolitarius	0	++++	Inferior vestibular	+	++++
Gracilis	0	++++				Medial vestibular	+	++++
Cuneatus	0	++++				Lateral vestibular	++++	+
Substantia gelatinosa	0	++++				Superior vestibular	+++	++

* Large (25 to 50 μm) multipolar, intensely stained neurons predominate as the cell type observed in columns A and B. Column C neurons are 15-by 40-μm fusiform or ovoid cells surrounded by homogeneous strong staining neuropil (see Fig. 1). † All sensory nuclei shown are secondary sensory nuclei with the notable exception of the mesencephalic root trigeminal nucleus. Cells of the mesencephalic root are primary sensory afferent cells analogous to the first order ganglion cells of the other sensory nuclei listed. ‡ The special afferent system also includes olfaction (visceral) and vision (somatic).