Table 1. FRACTIONAL LENGTH OF STYLAR TISSUE PENETRATED BY POLLEN TUBES*

Graft-combinations pollinated	Weeks after grafting 1	2	3	4	5	6	7	8	9	10
1 S_1S_2 grafted on S_1S_2 and selfed	0	0	0	0	0	0	0·1	0	0	0
2 S_1S_2 grafted on S_3S_4 and an S_1S_2 flower selfed	0	0·1	0·1	0·6	0·6	0·3	0·2	0·2	0·2	0
3 S_1S_2 pollinated with S_1S_2 pollen from S_1S_3 grafted to S_2S_4	0	0	0	0·1	0·1	0·2	0·3	0·2	0·2	0·1

* Replication, notation and experimental conditions as for Fig. 1.

sistent with those of Linskens utilizing data from X-irradiated incompatible *Petunia*[5].

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[1] Emerson, S., *Genetics*, **23**, 190 (1938).
[2] Linskens, H. F., *Ber. deutsch. bot. Ges.*, **71**, 3 (1958).
[3] Linskens, H. F., *Ber. deutsch. bot. Ges.*, **72**, 84 (1959).
[4] Lewis, D., *Proc. Roy. Soc.*, B, **140**, 127 (1952).
[5] Linskens, H. F., *Z. Bot.*, **48**, 126 (1960).
[6] Linskens, H. F., *Naturwiss.*, **47**, 547 (1960).
[7] Wilson, J. G., Garnjobst, L., and Tatum, E. L., *Amer. J. Bot.*, **48**, 299 (1961).
[8] Hall, O. L., *Hereditas*, **42**, 261 (1956).
[9] Sulbha, K., and Swaminathan, M. S., *Curr. Sci.*, **28**, 460 (1959).
[10] Naundorf, G., *Gordian*, **59**, No. 1405, 7; No. 1406, 8; No. 1407, 8 (1959).
[11] Hašek, M., *Folia Biol.*, **8**, 73 (1962).

VIROLOGY

Isolation from Man in Slovenia of a Virus belonging to the California Complex of Arthropod-borne Viruses

FOLLOWING observations[1] that in some areas of Slovenia, Yugoslavia, inapparent infections with Central European tick-borne encephalitis virus are common, attempts were made to isolate this agent from clinically inapparent but serologically detectable infections. In the course of this work two strains of another arthropod-borne virus were obtained. Designated Trojica Nos. 1 and 2 after the village where they occurred, these strains have now been identified as belonging to the California complex[2] of arthropod-borne viruses.

The isolations were made from two sera of about 5,000 collected from healthy human beings during the spring and summer of 1960 and 1961 in different localities in Slovenia known as endemic foci of Central European tick-borne encephalitis.

Serum collections were stored for 1–5 weeks at −20° C before being used for virus isolations. About half the specimens were tested individually, the remainder in pools of two or three. Sera were filtered through Seitz *EK* pads and centrifuged at 40,000 r.p.m. for 1 h. Sediments were resuspended in a volume of borate buffer, *p*H 7·2, between 1:10 and 1:20 of the original volume of the serum and inoculated into human embryo kidney cell cultures, chorioallantoic membranes of 12-day-old chick embryos and new-born mice.

Two sera that were pathogenic only for new-born mice yielded agents that could be serially propagated. Re-isolation from the original material kept at −20° C was successful in both cases.

The two strains had similar properties. By intracerebral or intraperitoneal inoculation they were pathogenic for new-born but not adult mice. They were sensitive to ethyl ether and sodium deoxycholate and were not neutralized by immune sera for several agents belonging in groups *A* and *B* of the arthropod-borne animal viruses.

The strains were also poor hæmagglutinin producers. A titre of 1:64 at *p*H 6·0 was obtained on one occasion with a new-born mouse brain antigen from strain No. 1, but this observation could not be repeated. After seven consecutive daily intracerebral passages in new-born mice, antigens with a titre of 1:10 at *p*H 6·0 and 6·1 were obtained. Both strains could be propagated in HeLa cell cultures, and by concentration of the fluid phase with an *LKB* 6300*A* ultrafilter[3], strain No. 1 yielded a low-titre hæmagglutinating antigen.

The strains were identified as belonging to the California complex in a complement-fixation test. Immune sera against both were prepared in adult mice given four intraperitoneal injections of a 10 per cent suspension of live virus. Antigens were prepared by acetone precipitation of a suspension of infected new-born mouse brain tissue in isotonic sucrose.

Neither serum reacted with complement-fixing antigens for the following viruses: eastern and western encephalitis, Semliki forest and Sindbis (group *A*); dengue type 2, Ntaya, Murray Valley encephalitis, Japanese *B* encephalitis, Central European tick-borne encephalitis, Russian spring–summer encephalitis, West Nile, yellow fever, St. Louis encephalitis and Zika (group *B*); and Guaroa, Simbu, Germiston, Bwamba, Chittoor and Neapolitan and Sicilian sandfly fevers. Both sera, however, had antibodies against California encephalitis and Tahyna antigens (Table 1).

Table 1. TITRES OF COMPLEMENT-FIXING ANTIBODIES IN IMMUNE SERA FOR TROJICA STRAINS NOS. 1 AND 2

Antigen	Serum Trojica No. 1	Trojica No. 2
California	16/8*	32/8
Tahyna	8/16	16/8
Trojica No. 1	16/16	32/8
Trojica No. 2	16/8	16/8

* Reciprocal of serum titre/reciprocal of antigen titre

The isolation of the Trojica strains is of interest, because viruses of the California complex have thus far been isolated from mosquitoes in several parts of the world but not from man. Thus, California encephalitis virus was obtained from *Aedes dorsalis* and *Culex tarsalis* in the United States[4], Lumbo from *Aedes pembaensis* in Mozambique[5], Tahyna from *A. vexans* and *A. caspius*[6,7] in Czechoslovakia, Trivittatus from *A. trivittatus* in the United States[4] and Melao from *A. scapularis* in Trinidad and Brazil[8]. Isolation of strains of this complex from man was not unexpected, however, since Bardos and Sevcoviceva[9] have reported the presence of Tahyna neutralizing antibodies in 8 per cent of 50 sera investigated from Slovenia.

Whether the two Trojica strains represent a new virus of this complex or a strain of one of the members already described—perhaps Tahyna—cannot be stated at the moment.

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[1] Likar, M. (unpublished results).
[2] Whitman, L., and Shope, R. E., *Amer. J. Trop. Med. Hyg.*, **11**, 691 (1962).
[3] Likar, M., Buckley, S. M., and Clarke, D. H., *Virology*, **18**, 647 (1962).
[4] Hammon, W. McD., Reeves, W. C., and Sather, G., *J. Immunol.*, **69**, 493 (1952).
[5] Kokernot, R. H., McIntosh, B. M., Worth, C. B., de Morais, T., and Weinbren, M. P., *Amer. J. Trop. Med. Hyg.*, **11**, 678 (1962).
[6] Bardos, V., and Danielova, V., *J. Hyg. Epidem.* (Prague), **3**, 264 (1959).
[7] Casals, J., *Acta Virol.*, **6**, 140 (1962).
[8] Spence, L. P., Anderson, C. R., Aitken, T. H. G., and Downs, W. G., *Amer. J. Trop. Med. Hyg.*, **11**, 687 (1962).
[9] Bardos, V., and Sevcoviceva, L., *J. Hyg. Epidem.* (Prague), **5**, 501 (1961).