

## SEROLOGICAL ASPECTS OF VIRUS MENINGO-ENCEPHALITIS

### A Study of the Reactions of Two Viruses Isolated during the 1953 Epidemics in Slovenia and Austria

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#### SYNOPSIS

Two agents isolated in 1953 from patients in epidemics of meningo-encephalitis in Slovenia and Austria were examined in neutralization tests at the Army Medical Service Graduate School in Washington, D.C., USA, and were found to be indistinguishable under the conditions of test from the agents called Russian spring-summer encephalitis virus, Czech encephalitis virus, or louping-ill virus, which are believed at the present time to be strains of the same agent.

Two viral isolates, termed Slovenia and Graz, which were recovered by Dr Vesenjok (see page 513) and Dr Verlinde (see page 565) during the 1953 meningo-encephalitis outbreaks in Slovenia and Austria, respectively, were forwarded to the Army Medical Service Graduate School by Dr Vesenjok for identification. This brief report is concerned with attempts to identify the newly recovered viruses by appropriate serological tests.

Information available to us from the Yugoslavian reports (see pages 491, 503, and 513) indicated many clinical and epidemiological similarities between the epidemics in Slovenia and earlier outbreaks of meningo-encephalitis in western Russia and in Czechoslovakia, which were shown to be caused by the Russian spring-summer encephalitis (RSSE) or louping-ill (LI) viruses. Moreover, the Yugoslav authors reported a significant increase during the

course of illness of neutralizing antibody against LI virus in the sera of three patients involved in the Slovenian epidemic.

Our first step was to determine the relationship of the Slovenia and Graz agents to other viruses known to cause encephalitis or related febrile illnesses. In this procedure, the two newly recovered agents were used in neutralization tests in mice against rabbit antisera previously prepared against the viruses of Japanese B encephalitis (Jap. B), West Nile (WN), St. Louis encephalitis (SLE), Murray Valley encephalitis (MVE), western equine encephalomyelitis (WEE), eastern equine encephalomyelitis (EEE), and RSSE. The EEE immune serum was obtained from rabbits which had received five intraperitoneal injections of EEE virus in the form of an infected mouse-brain suspension over a period of 37 days. All other antisera mentioned above were the same ones as those described in experiments already published.<sup>1,2</sup> These reports also describe the technique of the intracerebral neutralization test which was employed in the present study.

The results of this test, which are summarized in table I, show that the Slovenia and Graz agents were not neutralized by six of the specific immune rabbit sera and therefore bear no evident antigenic relationship to the viruses of Jap. B, WN, SLE, MVE, WEE, or EEE. However, the marked neutralization of Slovenia and Graz viruses by the RSSE rabbit antiserum clearly establishes the relationship of both newly recovered agents to RSSE virus.

In order to determine more exactly the relationship of the Slovenia and Graz viruses to the RSSE and LI viruses, which had previously been shown

**TABLE I. PRELIMINARY IDENTIFICATION BY INTRACEREBRAL NEUTRALIZATION TESTS IN MICE OF THE SLOVENIA AND GRAZ AGENTS ISOLATED DURING MENINGO-ENCEPHALITIS EPIDEMICS IN YUGOSLAVIA AND AUSTRIA IN 1953**

Immune rabbit serum	Logs of homologous virus neutralized	Slovenia virus			Graz virus		
		log titre of virus	logs of virus neutralization by antiserum	neutralization index of antiserum	log titre of virus	logs of virus neutralization by antiserum	neutralization index of antiserum
Normal (control)	—	8.5	—	—	8.9	—	—
Jap. B	4.7	8.0	0.5	3	8.5	0.4	2
WN	3.5	8.5	0	0	8.5	0.4	2
SLE	2.4	8.4	0.1	0	8.5	0.4	2
MVE	3.6	8.4	0.1	0	8.4	0.5	3
WEE	4.1	8.4	0.1	0	8.5	0.4	2
EEE	3.7	8.4	0.1	0	8.5	0.4	2
RSSE	3.8	5.3	3.2	1,600	5.2	3.7	5,000

to be serologically indistinguishable,<sup>2</sup> the agents under study were tested in intraperitoneal neutralization tests in mice, using rabbit antisera previously prepared against four RSSE viruses isolated in the USSR, a LI virus isolated in Scotland, and a virus isolated in Prague, Czechoslovakia, in 1950 and designated the Czech strain. These antisera were the same ones as those described in a recent publication,<sup>2</sup> which also sets forth the technique of the intraperitoneal neutralization test employed in the current study. The results of this test, presented in table II, indicate that the Slovenia and Graz agents are neutralized to the same extent, for all practical purposes, by sera from rabbits immunized against each of four typical RSSE viruses, LI virus, or Czech encephalitis virus. Furthermore, the neutralization indices of the antisera are essentially the same with the two new viruses as with the homologous agents, which had already been shown to be identical by this procedure.

**TABLE II. RELATIONSHIP BETWEEN THE SLOVENIA - GRAZ AGENTS AND THE RSSE - LI GROUP OF VIRUSES AS DETERMINED BY INTRAPERITONEAL NEUTRALIZATION TESTS IN MICE**

Immune rabbit serum	Logs of homologous virus neutralized	Slovenia virus			Graz virus			
		log titre of virus	logs of virus neutralization by antiserum	neutralization index of antiserum (millions)	log titre of virus	logs of virus neutralization by antiserum	neutralization index of antiserum (millions)	
Normal (control)	—	8.2	—	—	8.9	—	—	
RSSE	Moscow	6.5	1.5	6.7	5	1.5	7.4	25
	Spring N4	7.1	1.6	6.6	4	2.0	6.9	8
	Sophy East	6.5	1.5	6.7	5	2.3	6.6	4
	Spring West	6.3	1.5	6.7	5	2.0	6.9	8
Louping-ill	6.9	1.5	6.7	5	2.4	6.5	3	
Czech	6.6	1.6	6.6	4	2.0	6.9	8	

It is therefore concluded, as a result of the neutralization tests described in this report, that both the Slovenia and Graz agents are strains of the Russian spring-summer—louping-ill—Czech encephalitis group of viruses.

RÉSUMÉ

Cet article est consacré à l'étude sérologique de deux souches de virus — Slovénie et Graz — isolées au cours des épidémies qui ont sévi en 1953 dans les régions du même nom.

Les renseignements reçus de Yougoslavie indiquaient diverses similitudes cliniques et épidémiologiques entre les épidémies de Slovénie et d'autres manifestations épidémiques

en Russie occidentale et en Tchécoslovaquie, causées par le virus de l'encéphalite verno-estivale russe (RSSE) et celui du louping-ill (LI).

L'auteur a comparé les deux souches à l'étude, au moyen des tests de neutralisation intracérébrale, aux souches de virus suivantes: encéphalite japonaise B, West Nile, St. Louis, Murray-Valley, encéphalomyélite américaine des équidés (souche Est et souche Ouest), encéphalite verno-estivale russe. Ces réactions n'ont révélé aucune parenté entre les virus Slovénie et Graz d'une part et les six premiers virus qui viennent d'être énumérés, d'autre part. En revanche, les virus à l'étude ont été neutralisés, de façon pratiquement identique, par les sérums anti-RSSE (quatre souches de provenances différentes), anti-LI et anti-encéphalite tchèque. Ces résultats permettent d'affirmer que les virus Slovénie et Graz appartiennent au groupe « encéphalite verno-estivale russe — louping-ill — encéphalite tchèque ».

## REFERENCES

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