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ANTIGENIC VARIATION IN RABIES VIRUS STRAINS

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Summary. — Several rabies virus isolates from small wild rodents, one strain isolated from a fox and another from a cat, as well as the CVS strain were compared in cross-protection and virus--neutralization tests. Antigenic variations between the strains and between different batches of individual strains were found. These antigenic differences could not be explained by denaturation caused by UV irradiation or deep-freez storage, by the presence of "incomplete" particles or by passage in immune organism. An antigenic difference was batch-specific and was only demonstrable on comparison with a relatively large number of strains: it has probably developed during the assembly of antigenic determinants of the virion. There was no correlation between protective and virus-neutralizing activities.

Key words: rabies virus; antigenic variation; cross-neutralization test

Introduction

Prior to the discovery of rabies-related viruses, the rabies virus was considered antigenically uniform (Wiktor, 1982) and hardly any significance was attributed to antigenic variation among individual strains demonstrable by common methods (Schneider and Schoop, 1972). However, antigenic differences between some rabies strains have been recently demonstrated by monoclonal antibodies (Wiktor and Koprowski, 1978). The rabies strains isolated from small wild rodents in 1968—71 differed mutually in some biological properties (Sodja *et al.*, 1982). Our attention was, therefore, paid to their antigenic relationships. Some of our preliminary results obtained in the cross-protection test were reported in 1970 at a meeting of the WHO/ /FAO research programme on wildlife rabies in Europe. We have pursed this problem further and our findings are presented below.

Materials and Methods

Virus strains. CVS, an isolate from the brain of a fox (street-F) and one from that of a cat (street-C), and a set of isolates (1968—71) from the brains, salivary glands and brown fat of various small rodents trapped in different localities: strains 297BF, 301B, 598SG, 648SG, 656BF, 66B, 454B, 460SG, 502BF, 703B, 544SG (*Microtus arvalis*), 472B and 482 SG (*Mus musculus*); and 638SG (*Apodemus flavicollis*). All strains were used as 10 % brain suspensions from subadult outbred mice (Velaz, Prague). The passage numbers are indicated in the text.

Cross-protection test (*CPT*) was based on the method of Habel (1973). Intraperitoneal (i.p.) immunization was mostly done with 6 consecutively increasing nonlethal doses of the virus suspension, 0.2 ml of each, injected during two weeks. On day 14 from starting immunization, the strains were titrated intracerebrally (i.c.) in immunized and non-immune mice; the MLD_{50} values were determined according to Reed and Muench (Lorenz and Bögel, 1973) and the protection indices (PI) were calculated.

Virus cross-neutralization test (*VCNT*) was performed according to Johnson (1973). Hyperimmune and control hamster sera inactivated at 56 ° for 30 min and diluted 1 : 20 or 1 : 100 were mixed 1 : 1 with individual dilutions of the strains under study and incubated at 37 °C for 90 min. The mixtures were then inoculated i.c. into 9—10 g mice. On completion of the experiment, the virus neutralization index (VNI) was calculated.

The serum cross-neutralization test (*SCNT*) was done in a similar way (Atanasiu, 1973). The results are expressed as serum-neutralization titres (SNT) (the reciprocal values of serum dilutions neutralizing 30—50 MLD_{50}).

Protection by humoral immunity correlation. Groups of mice were i.p. immunized three times a week for two weeks with 0.2 ml virus. After 14 days, a part of the animals was bled off. Ten per cent brain suspensions in physiological saline and sera diluted 1 : 10 were inactivated at 56 °C for 30 min. VCNT and CPT were performed and the VNI and PI were determined.

Passages in immunized mice. (1) Groups of mice were i.p. immunized with 6 doses of strain 297BF/M-22 and after 14 days the same strain was titrated in them. Brain suspensions of mice dying after the highest virus dilution were then passaged 5 times in animals immunized in the same way. (2) Mice were immunized i.p. with one 0.2 ml dose of CVS (10^2 i.c. MLD_{50}/0.03 ml); CPT and CNT were done by days 7, 14 and 21 later. For VCNT, the mice were immunized with two doses of CVS (10^2 and 10^3 i.c. MLD_{50}/0.03 ml) given at one week intervals. Strains street-C, street-F, 66B and CVS were tested. These strains were then passaged on CVS-immunized mice in the same way as in the case (1). After 5 and 10 passages CPT was performed with the passaged and non-passaged viruses.

Abbreviations used: $\bar{x}_1$ = mean VNI (PI) of strain; $\bar{x}_2$ = mean VNI (PI) of antiserum (immunized animals); $\bar{x}_3$ = mean antigenic difference (AD) of a given strain; $\bar{x}_4$ = mean AD of antiserum (immunized animals); $\bar{x}_5$ = mean VNI (PI); $\bar{x}_6$ = mean AD.

Results

In the first series of experiments we compared the antigenic relationships in the CPT between four strains at a higher mouse-passage level (297BF/M-12, 301B/M-13, 472B/M-10 and 482SG/M-10) and between another four at a lower (598SG/M-5, 638SG/M-4, 648SG/M-3 and 656BF/M-4) (Table 1). The PI values ranged from —0.5 to 4.82 (the negative values were mainly found after immunization with week antigen); mean PI ($\bar{x}_5$) and AD ($\bar{x}_6$) were 1.53 ± 0.75 and 1.42 ± 0.75, respectively. It is evident from the Table 1 that the animals were repeatedly better protected against challenge by certain strains (297BF, 301B) and not so well against others (598SG, 482SG, 648SG). Arrangement of the strains to a particular order from lowest to highest PI gave a weak-to-strong virulence „strain-sequence" (SS). The differences in PI values were designated "antigenic difference" (AD). The differences in arithmetic means between the PI of the "immunization" strain and of the strains under comparison express the degree of their "antigenic resemblance". The mean PI values calculated (a) from three CPT values obtained in animals immunized with high, intermediate and weak virulent strains and (b) from all CPT values were essentially the same. An exception were the strain 648SG-related sequences, but here the AD values were as low as determined by either procedure ($s_a = 0.56$, $s_b = 0.57$).

Table 1. Protection indices and strain differences in the CPT

Strains (antigens)	Immunized with							
	297BF	301B	472B	482SG	598SG	638SG	648SG	656BF
297BF	3.76	1.75	3.00	4.78	0.58	2.00	2.75	2.41
301B	4.92	1.50	2.26	3.61	−0.25	2.90	2.50	3.26
472B	2.50	1.16	1.09	2.68	0.00	1.70	2.00	1.65
482SG	1.46	0.55	2.79	0.75	−0.50	0.88	0.74	−0.50
598SG	1.75	−0.50	1.25	0.62	−0.37	0.86	0.77	0.26
638SG	3.00	1.24	0.42	0.66	0.56	1.94	2.08	1.04
648SG	2.76	1.50	0.50	2.11	−0.50	0.29	0.50	1.55
656BF	3.74	1.00	1.00	3.26	0.00	1.26	1.00	2.30

Antigenic differences and strain sequences

301B	0	297BF	0	287BF	0	297BF	0	297BF	0	301B	0	297BF	0	301B	0
297BF	1.16	301B	0.25	482SG	0.21	301B	1.17	638SG	0.02	297BF	0.90	301B	0.25	297BF	80.85
656BF	1.18	648SG	0.25	301B	0.74	656BF	1.58	656BF	0.58	638SG	0.96	638SG	0.67	656BF	0.96
638SG	1.92	638SG	0.51	598SG	1.75	472B	2.10	472B	0.58	472B	1.20	472B	0.75	472B	1.61
648SG	2.16	472B	0.59	472B	1.91	648SG	2.67	301B	0.83	656BF	1.64	656BF	1.65	648SG	1.71
471B	2.41	656BF	0.75	656BF	2.00	482SG	4.03	598SG	0.95	482SG	2.02	598SG	1.98	638SG	2.02
598SG	3.17	482SG	1.20	648SG	2.50	638SG	4.12	648SG	1.08	598SG	2.04	582SG	2.01	598SG	3.00
482SG	3.46	598SG	1.25	638SG	2.58	598SG	4.16	482SG	1.08	648SG	2.61	648SG	2.25	482SG	3.75

Table 2. Strain sequences according to mean antigenic differences between protection indexes (PI) and virus-neutralization indexes with brain suspension (VNI_B) and serum (VNI_S)

PI		VNI_B		VNI_S	
SS	AD	SS	AD	SS	AD
301B	0	297BF	0	301B	0
297BF	0.73	598SG	1.89	482SG	0.36
598SG	1.20	482SG	2.06	297BF	0.89
482SG	2.19	301B	2.08	598SG	1.20

In the experiment described, the immunization doses of the individual strains (depending on their extraneural virulence) differed rather widely (from 0.53 to 5.97 and from −0.24 to 5.19 i.c. MLD_{50}/0.03 ml for the total and mean immunization dose, respectively). For this reason some of the experiments were repeated. Roughly the same antigen doses were used for the immunization (2.13−3.76 ± 0.6 and 1.35−2.99 ± 0.6 i.c. MLD_{50}/0.03 ml for the total and mean immunization dose, respectively). The strains were in the same passage, but strains with a low MLD_{50} were used in a higher passage (598SG/M-8; 648SG/M-6). In spite of differences in absolute values of PIs, the SSs according to their AD were essentially the same in both series of experiments. Identity between the challenge and immunization strain influenced its position in SS only vague. No correlation between the PI value and the immunization dose was found either. AD was demonstrated in comparison with other isolates (454B, 460SG, 502BF, 544SG) as well.

To study the relationship between protective and humoral immunity, four strains with the highest AD from previous experiments were used in CPT and VCNT. One of the experiments is illustrated in Table 2. The mean PI ($\bar{x}_5$) equalled 2.64 ± 0.17; $VNI_B = 1.76 \pm 0.24$; $VNI_S = 0.99 \pm 0.59$.

Table 3. Batch sequences in CPT according to mean protection indexes of batches ($\bar{x}_1$) and immunized animals ($\bar{x}_2$) and mean antigenic differences of batches ($\bar{x}_3$) and of immunized animals ($\bar{x}_4$)

Strain	Batch	Code	Batch sequence			
			$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_3$	$\bar{x}_4$
297BF	M-17	A	B 2.45 ± 1.3	D 2.63 ± 0.6	B 1.08 ± 1.1	A 1.29 ± 1.5
301B	M-18	B	C 2.37 ± 1.1	A 2.20 ± 1.5	C 1.16 ± 1.1	c 1.35 ± 0.8
482SG	M-15	C	d 2.30 ± 1.1	B 2.08 ± 1.2	b 1.55 ± 1.2	D 1.36 ± 0.6
598SG	M-11	D	b 2.06 ± 1.2	d 1.55 ± 0.9	d 1.58 ± 1.1	d 1.55 ± 0.9
297BF	M-20	a	A 1.58 ± 1.6	C 1.32 ± 1.4	A 1.95 ± 1.4	b 2.04 ± 1.2
301B	M-21	b	a 1.40 ± 1.0	b 0.88 ± 1.2	a 2.14 ± 1.1	B 2.36 ± 1.2
482SG	M-18	c	c 1.30 ± 1.7	a 0.75 ± 1.2	c 2.23 ± 1.4	a 2.73 ± 1.3
598SG	M-14	e	D 0.91 ± 1.2	c 0.66 ± 0.8	D 2.28 ± 1.2	C 2.74 ± 1.4

Table 4. Sequences of four rabies strains according to antigenic difference of protection indexes (PI). VN indexes (VNI) and VN antibody titres in sera (SNT) at different periods after immunization with CVS strain

Code	Strain	Days after immunization						
		7		14		21		
		PI	SNT	PI	SNT	PI	SNT	VNI
A	CVS	D : 0	A : 0	C : 0	B : 0	A : 0	B : 0	D : 0
B	street-C	C : 1.2	C : 0	A : 0.3	A : 0.4	C : 1.0	D : 0.2	C : 0.2
C	street-F	B : 1.9	B : 0.7	D : 0.6	C : 1.2	D : 1.1	A : 0.6	B : 1.1
D	66B	A : 2.0	D : 0.8	B : 1.2	D : 1.4	B : 2.2	C : 0.6	A : 1.3

The mean ADs in PI = 1.03 ± 0.91; VNI_B = 1.50 ± 1.00; VNI_S = 0.61 ± ± 0.53. The AD, but especially the SS, determined in CPT did not correspond with the AD and SS in VCNT either in the reactions with sera or brain suspensions.

The assumption that the AD value determined in CPT and VCNT is not a permanent and strain-specific characteristics has been confirmed by studying different batches of the same strains (Table 3). The position of the batches in SSs was not influenced by their strain origin, it was identical only in $\bar{x}_1$ and $\bar{x}_3$, but it was different and much more variable than that of strains in the previous CPT. The highest AD was 4.11; mean PI = 1.79 ± 0.57; mean AD = 1.74 ± 0.47. The assumption was also confirmed by a simplified experiment using two batches each of strains 297BF and 598SG.

Antigens prepared from brain tissue were probably not homogeneous enough in many of their biological characteristics. Therefore, two separate lines of 5 mouse passages were run with strain 297BF and strain 598SG,

Table 5. Sequences of four rabies strains according to antigenic differences of protection indexes after passages on immune and non-immune mice

Code	Strain	No. of passages on immune mice	Immunization with			$\bar{x}_1$
			A	E-5	E-10	
A	CVS	0	E : 0	F : 0	E : 0	2.03 ± 0.32
B	street-C	0	A : 0.17	B : 0.25	G : 0.24	1.92 ± 0.62
C	street-F	0	D : 0.24	D : 0.25	H : 0.25	1.69 ± 0.75
D	66B	0	F : 0.66	E : 0.50	D : 0.50	2.25 ± 0.25
E	CVS	5 (10)	B : 1.24	G : 0.50	A : 1.00	2.58 ± 0.37
F	street-C	5 (10)	C : 1.67	C : 0.52	B : 1.00	2.36 ± 0.47
G	street-F	5 (10)	G : 1.74	H : 0.75	C : 1.00	1.92 ± 1.00
H	66B	5 (10)	H : 2.24	A : 1.02	F : 1.00	1.67 ± 1.27
$\bar{x}_2$			1.50	2.22	2.37	
$\bar{x}_4$			0.99	0.47	0.62	

Table 6. Strain sequence in the VCNT (each serum tested against all strains)

Code	Antigen	Antiserum 297BF	301B	472B	482SG	598SG	648SG	656BF	CVS	$\bar{x}_1$	$\bar{x}_3$
A	297BF	B : 0	A : 0	B : 0	J : 0	E : 0	A : 0	A : 0	A : 0	2.61 ± 1.3	A : 0.39
B	301B	A : 0.74	B : 0.81	A : 0.32	B : 0.24	F : 0.09	C : 1.10	H : 0.52	G : 2.16	2.21 ± 1.3	B : 0.78
C	472B	C : 0.91	E : 0.94	C : 1.26	A : 0.35	B : 0.39	B : 1.31	F : 0.76	E : 2.24	1.37 ± 1.4	E : 1.39
D	482SG	E : 1.66	C : 1.69	E : 1.29	G : 0.40	J : 0.73	F : 1.93	G : 0.76	B : 2.66	1.01 ± 0.7	G : 1.59
E	598SG	G : 1.97	G : 2.25	G : 1.73	C : 0.56	D : 1.33	G : 2.06	I : 0.76	C : 2.66	1.61 ± 1.0	C : 1.62
F	638SG	D : 2.09	D : 2.47	D : 1.93	E : 0.64	H : 1.34	J : 2.09	B : 0.89	D : 2.66	1.20 ± 0.6	F : 1.80
G	648SG	J : 2.39	F : 2.56	F : 2.14	F : 0.82	G : 1.41	D : 2.36	E : 1.10	H : 2.92	1.41 ± 0.5	J : 1.97
H	656BF	I : 2.44	J : 2.77	I : 2.52	I : 1.56	I : 1.67	H : 2.78	J : 1.21	I : 2.97	0.41 ± 0.5	D : 2.05
I	CVS	F : 2.54	I : 3.21	J : 3.23	H : 1.56	A : 1.72	I : 3.12	C : 2.00	J : 3.37	0.72 ± 0.4	I : 2.28
J	street-F	H : 3.17	H : 3.94	H : 3.47	D : 1.62	C : 2.83	E : 3.25	D : 2.00	F : 3.57	1.03 ± 0.6	H : 2.58
	$\bar{x}_2$	2.13	1.78	1.86	1.28	0.92	1.35	0.31	1.13		
	$\bar{x}_4$	1.79	2.06	1.88	0.77	1.15	2.00	1.01	2.52		

Explanation: $\bar{x}_1$ = mean VNI of strain
$\bar{x}_2$ = mean VNI of antiserum
$\bar{x}_3$ = mean antigenic difference of strain
$\bar{x}_4$ = mean antigenic difference of antiserum

one line using undiluted brain suspensions (high multiplicity — HM) and the other the limiting dilution (1—10 i.c. MLD_{50}) (low multiplicity — LM) that still killed experimental animals. This arrangement neither influenced nor elucidated AD. Since the MLD_{50} values of individual passages did not much differ in either line, we also failed to show any mutual interference on titrating combined HM and LM subpassages.

Comparison of three 297BF batches, one of which (M-27) had been passed in mice immunized with batch M-22, and two 598SG batches in CPT, generated an antigen sequence where 297BF was in majority of cases the most virulent virus for immunized mice. The only exception were 598/M-17-immunized animals, where, however, the individual ADs were low ($s = \pm 0.51$) and hence the differentiation was not very pronounced.

Strains CVS, street-C, street-F and 66B were passaged in a similar way in CVS-immunized mice. The points of interest were the dynamics of protective and VN activites (Table 4) and changes of AD after 5 and 10 passages in immune animals (Table 5). The first part of the experiment showed a continual rise of PI values of the homologous CVS strain. The sera displayed different VN activity, which might have been due to differing avidity or doses of the reacting strain. The SS-AD in CPT was again different from SS-AD in VCNT. The second part of the experiment failed to demonstrate any regularity in the AD variation. The mutual PI differences were minimal and did not allow clear differentiation into SS.

The AD could not be elucidated in term of virion disintegration following UV irradiation or storage at —70 °C.

The simultaneous testing of greater numbers of antigens and antisera in the VCNT and SCNT was technically impossible. Therefore, the testing was carried out in two steps: 1) simultaneous tests of all antigens against individual antisera, and 2) an opposite test. In simultaneous testing of all strains against the individual hyperimmune sera in the reaction (Table 6), where $\bar{x}_5$ was 1.35 ± 0.59, the strain 656BF was neutralized at worst ($\bar{x}_1 = 0.41 \pm \pm 0.53$) and the strain 297BF at best ($\bar{x}_1 = 2.62 \pm 1.31$). The highest AD value (4.47) was obtained with strain 656BF tested against 472B serum. The SS turned out the same whether arranged according to $\bar{x}_1$, $\bar{x}_2$ or $\bar{x}_3$.

Since in the previous experiment the serum antibody level after immunization with some strains (598SG, 656BF) was low (probably because of low antigenic doses) or, in contrast, the animals had died (638SG) during the immunization, a similar experiment was set up with sera having a mean VNI of 2.95 ± 0.98 (Table 7). All the strains were neutralized to a roughly equal degree by all sera (the maximum VNI difference $\bar{x}_1$ was 0.73). The highest AD (2.72) for strain 656BF was shown in anti-CVS serum, which also possessed the lowest antibody level ($\bar{x}_2 = 0.74$) of all the sera tested. The SS based on the mean neutralizability-avidity ($\bar{x}_1$) again did not differ from SS determined according to the mean AD values ($\bar{x}_3$). The same position in the SS by both experiments was found only for strains 301B, 472B ans 648SG.

The SS in the rests where each strain was simultaneously tested against all hyperimmune sera differed from the SS described in Tables 6 and 7. The SS arranged according to the mean values from all eight experiments

Table 7. Strain sequence in the VCNT (each serum tested against all strains)

Code	Antigen	Antiserum 297BF	301B	472B	482SG	598SG	638SG	648SG	656BF	CVS	street	$\bar{x}_1$	$\bar{x}_2$
A	297BF	J : 0	F : 0	A : 0	A : 0	E : 0	C : 0	E : 0	D : 0	F : 0	B : 0	2.94 ± 1.2	E : 0.82
B	301B	F : 0.85	G : 0.19	E : 0.24	G : 0	B : 0.10	H : 0.09	C : 0.74	I : 0.16	I : 1.46	G : 1.00	3.26 ± 1.1	B : 0.84
C	472B	B : 1.02	H : 0.53	D : 0.50	F : 0.13	A : 0.36	B : 0.12	I : 0.82	B : 0.24	C : 1.72	F : 1.12	2.99 ± 1.2	F : 0.89
D	482SG	H : 1.05	C : 0.69	G : 0.50	H : 0.58	F : 0.50	A : 0.75	H : 1.50	E : 0.50	E : 1.86	C : 1.33	2.55 ± 0.9	G : 1.11
E	598SG	A : 1.22	E : 0.95	B : 0.59	E : 0.66	I : 0.50	I : 0.92	B : 1.60	G : 0.50	B : 1.96	J : 1.33	3.28 ± 1.2	C : 1.11
F	638SG	E : 1.42	J : 1.19	F : 0.80	D : 1.00	G : 0.74	F : 1.02	A : 1.91	J : 0.89	D : 2.22	D : 1.38	3.21 ± 0.8	A : 1.17
G	648SG	I : 1.54	I : 1.20	I : 1.26	B : 1.10	H : 0.74	D : 1.24	G : 2.09	D : 1.24	G : 2.48	H : 1.42	3.00 ± 1.4	I : 1.22
H	656BF	C : 1.62	A : 1.29	J : 1.58	C : 1.58	D : 1.00	J : 1.26	D : 2.19	A : 1.75	A : 2.66	A : 1.78	2.87 ± 1.2	H : 1.23
I	CVS	D : 1.96	B : 1.71	H : 1.74	I : 1.85	C : 1.50	E : 1.26	J : 2.26	F : 1.98	J : 2.67	D : 2.00	2.89 ± 1.0	J : 1.46
J	street-F	G : 2.38	D : 2.24	C : 2.00	J : 1.92	J : 1.58	G : 1.27	F : 5.29	H : 2.00	H : 2.72	I : 2.50	2.70 ± 1.2	D : 1.55
	$\bar{x}_2$	2.30	3.44	2.57	3.99	2.79	2.46	3.93	3.57	0.74	3.61		
	$\bar{x}_3$	1.30	0.99	0.92	0.88	0.70	0.79	1.57	0.92	1.97	1.38		

Explanation:

$\bar{x}_1$ = mean VNI of virus strain
$\bar{x}_2$ = mean VNI of antiserum
$\bar{x}_3$ = mean antigenic difference of virus strain
$\bar{x}_4$ = mean antigenic difference of antiserum

Table 8. Mean antigenic difference values of VNI from all (eight) experiments

Code	Strain	Antiserum 297BF	301B	472B	482SG	598SG	638SG	648SG	656BF	CVS	street	$\bar{x}_1$	$\bar{x}_3$
A	297BF	B : 0	B : 0	B : 0	G : 0	C : 0	C : 0	C : 0	I : 0	B : 0	B : 0	2.37 ± 0.3	B : 0.39
B	301B	A : 0.08	D : 0.20	B : 0.29	A : 0.01	E : 0.23	H : 0.09	F : 0.64	D : 0.23	F : 0	G : 1.00	2.51 ± 1.0	C : 0.55
C	472B	D : 0.28	A : 0.28	A : 0.34	D : 0.19	F : 0.53	B : 0.12	E : 0.65	E : 0.28	D : 0.25	F : 1.12	2.38 ± 0.7	D : 0.61
D	482SG	E : 0.56	G : 0.46	D : 0.40	C : 0.35	D : 0.60	A : 0.75	D : 0.77	A : 0.36	C : 0.52	C : 1.33	2.29 ± 0.3	A : 0.62
E	598SG	C : 0.61	E : 0.52	G : 0.79	F : 0.61	A : 0.62	I : 0.92	A : 0.96	C : 0.36	G : 0.65	J : 1.36	2.28 ± 0.5	E : 0.65
F	638SG	G : 0.88	F : 0.82	F : 0.98	H : 0.67	G : 0.64	F : 1.02	I : 1.18	B : 0.42	A : 0.68	E : 1.38	2.13 ± 0.7	G : 0.74
G	648SG	I : 1.11	C : 0.84	I : 1.52	E : 0.78	B : 0.77	D : 1.24	G : 1.26	G : 0.51	E : 0.85	H : 1.42	2.19 ± 0.6	F : 0.78
H	656BF	H : 1.33	I : 1.30	H : 1.52	B : 1.02	H : 1.17	E : 1.26	H : 1.34	F : 0.56	I : 1.14	A : 1.79	1.78 ± 0.8	H : 1.15
I	CVS	J : 1.34	H : 1.59	C : 1.55	I : 1.06	I : 1.40	J : 1.26	B : 1.37	H : 1.13	H : 1.28	D : 2.00	1.74 ± 0.5	I : 1.21
J	street-F	F : 1.51	J : 1.86	J : 2.17	J : 1.41	J : 1.57	G : 1.27	J : 2.02	J : 2.21	J : 2.05	I : 2.50	1.31 ± 0.9	J : 1.62
	$\bar{x}_2$	1.70	1.89	1.82	1.86	1.64	2.38	2.05	1.97	1.89	3.61		
	$\bar{x}_4$	0.77	0.68	0.95	0.61	0.75	0.79	1.01	0.50	0.74	1.38		

Explanation: $\bar{x}_1$ = mean VNI of virus strain
$\bar{x}_2$ = mean VNI of antiserum
$\bar{x}_3$ = mean antigenic difference of virus strain
$\bar{x}_4$ = mean antigenic difference of antiserum

was also different (Table 8). In this case, however, AD diminished markedly (max. 1.20—1.30) and would not be demonstrable in terms of the usual criteria.

Discussion

The identification of rabies virus isolates is merely done by comparison of VNI of the new isolate with that of reference strain using positive and negative reference sera (Schneider and Schoop, 1972). The identification of the "mouse" isolates was performed in the same way (Sodja *et al.*, 1982). Among other criteria, decisive for referring to the lyssavirus family is VNI 2.00 $\log_{10}$ in the VCNT. All of the small-rodent isolates fulfilled this criterion. The AD seen in CPT and VCNT described above, were probably not due to technical error or test variability, because the results were reproducible providing that identical conditions were maintained, and especially if the same strain batches were used.

Strain sequence was significantly influenced neither by identity of immunizing and challenge strain nor by the PI and VNI level, even in case of early death. However, if a strain situated in the middle of SS had been used in the comparison, the antigenic difference would not be unequivocal. These findings confirmed that there is no correlation between protection and humoral immunity, at least not in mice (WHO, 1980). In contrast, it seems that virions initiating protection might be better inducers of VN-antibody formation. Neither was found any correlation between protective activity and the MLD_{50} values of immunizing doses, i.e. strains displayed a variable immunogenicity.

The rabies virion contains several antigenic components, a decisive role in virulence is attributed to the glycoprotein (Wiktor, 1972). Antigen prepared from disintegrated brain tissue may contain particles of different maturity including "incomplete" particles. However, we failed to influence the SS in CPT using antigens prepared from the brains of animals infected with variable virus doses. AD could neither be explained by virion denaturation after UV irradiation nor by deep-freeze storage.

Yuvchenko (personal communication) assumes that wildstrain isolates include different biological variants. This assumption has been confirmed by the isolation of different variants of rabies virus from the same host (Gribencha *et al.*, 1982). Clark and Wiktor (1972) have demonstrated spontaneous genetic variations in Lagos bat and Mokola viruses propagated in cell culture, with individual strains being variable neutralized by antirabies serum. Different clones and variants have also been demonstrated with rabies viruses (Wiktor, 1980; Charlton *et al.*, 1982; Sureau *et al.*, 1983, etc.). Therefore, one may logically assume the existence of diverse antigenic variants in so nonhomogeneous material as the brain suspension.

By passaging in immune mice we obtained a rabies virus variant highly virulent for animals immunized by each virus strain or batch under study. Probably, not even this can be unequivocally explained by an antigenic change. The test performed suggested that the "antigenic difference" shown in CPT was not strain- but batch-specific: a characteristic probably emerging

in the assembly of virions or their glycoproteins when new antigenic determinants are formed. On the other hand, there was no direct dependence between virulence (an active biological property), antigenity and immunogenicity of rabies viruses.

Variability is greater in VCNT than in CPT, especially if the experimental arrangements differ, and VCNT is also more liable to be influenced by sera with different antibody levels. AD is most often demonstrable only when comparing many strains in one test, and especially between strains in extreme SS positions. If mean values from different VCNT are used, the AD cannot be detected.

By our tests it was impossible to show whether "AD" was a property specific for "mouse isolates", but, for several reasons given above, this seems to be a more general phenomenon. Although AD remains hidden in most cases, it could negatively affect vaccination and assert the challenge by strains occupying extreme SS positions, in particular if the challenging dose was high. AD might perhaps account for some of the postexposure vaccination failures described.

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