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REPRODUCTION OF MUMPS VIRUS STRAINS IN VERO CELL CULTURES AT 32, 37 AND 40 °C

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Summary. — Reproduction of various mumps virus strains was studied in Vero cell tube cultures at 32, 37 and 40 °C. Strains of different character could be distinguished based on temperature dependence of their reproduction, but results of several repeated experiments had to be taken into account. The problem of a suitable numerical marker was discussed along with the relationship between temperature dependence of reproduction of a strain and the host response to its action in vivo.

Key words: mumps virus; incubation temperature; strain differences

Introduction

Data on laboratory differentiation of mumps virus strains are not numerous. To our knowledge, reproduction of mumps virus was studied in cultures of chick embryo cells and cells of human origin (Henle and Deinhardt, 1955; Buynak and Hilleman, 1966), in organ cultures of monkey testes and parotic glands (Hosai *et al.*, 1970); virus reproduction was also studied at 32 and 35 °C (Vardanyan *et al.*, 1972) and thermostability of its strains at 50 °C (Vardanyan *et al.*, 1972; Glathe and Nöbel, 1977).

We are reporting the results of experiments on the reproduction of several mumps virus strains in Vero cell cultures at three selected temperatures, aimed at strain differentiation.

Materials and Methods

Virus strains. The following mumps virus strains were used: E, the original Enders' strain (141 chick embryo passages and 11 passages in Vero cells at 37 °C), originating from the World Influenza Centre, London and kindly supplied by Dr. B. Tůmová, Czechoslovak Influenza Centre, Prague; A, the attenuated strain AA/ÚSOL (about 20 passages in chick fibroblast cultures and 6 passages in primary dog kidney cell cultures at 34 °C); B, strain Leningrad 3, isolated from a U.S.S.R. vaccine and adapted by 5 passages to primary dog kidney cell cultures at 34 °C; and five "wild" strains, isolated from cerebrospinal fluids in CV-1 cells, kindly supplied by Dr. P. Červenka, Regional Hygiene Station, Ostrava, and reproduced in Vero cells at 37 °C.

Arrangement of the experiments. Reproduction of the strains was evaluated based on $TCID_{50}$ values determined in Vero cell tube cultures (Izbický and Fröhlichová, 1979) incubated at the given temperature. The test samples were diluted serially 10-fold and 0.2 ml volumes of each dilution were inoculated into 30 tubes each containing 3×10^5 Vero cells (Izbický, 1977) sus-

pended in 1.3 ml of Eagle's minimal essential medium in which glucose was replaced by galactose and sodium pyruvate (Baugh and Tytell, 1967; Slonim, 1974; Izbický, 1977) and which was supplemented with 0.9 g/l $NaHCO_3$ and 7.5% inactivated calf serum. The tubes were stoppered and thoroughly shaken and then divided into three equal groups incubated stationary in inclined position at 32, 37 and 40 °C, respectively, for 10 days. Then the cytopathic effect, haemadsorption and haemagglutination of chick erythrocytes were read directly in the culture tubes and the $TCID_{50}$/ml values calculated according to Reed and Muench for each incubation temperature (log V_{32}, log V_{37} and log V_{40}). The relations between titres at the three temperatures were expressed by the difference between the respective paired values — d_{32} = log V_{32} — log V_{37} and d_{40} = log V_{40} — log V_{37}. The significance of the results was evaluated by Student's t-test.

Table 1. Results of repeated titrations of mumps virus strains E, A and B in Vero cell tube cultures at 32, 37 and 40 °C

Titration No.	log $TCID_{50}$/ml values for strain E 32	E 37	E 40	A 32	A 37	A 40	B 32	B 37	B 40
1	3.3	3.2	1.2	4.2	3.6	3.2	5.2	5.6	5.2
2	3.5	3.3	1.2	4.2	3.8	2.6	4.9	5.3	5.1
3	3.7		1.2	4.1	4.1	3.5	5.5	5.5	5.3
4	3.2	3.5	1.2	4.1	4.6	2.7	5.3	5.4	
5	3.6	3.3	1.2	4.3	4.2	2.9	5.2	5.4	5.3
6	3.3	3.2	1.2	4.1	4.0	3.2	5.2	5.3	5.1
7	3.8	3.3	1.2		3.2	2.4	5.3	5.8	5.4
$\bar{x}$	3.486	3.300	1.2	4.167	3.929	2.929	5.229	5.471	5.233
s	0.210	0.100		0.075	0.416	0.361	0.167	0.167	0.111

Table 2. Differences between $TCID_{50}$ values determined at 32 and 37 °C (d_{32}) and 40 and 37 °C (d_{40}) for mumps virus strains E, A and B (based on data from Table 1)

Titration No.	d_{32} E	d_{32} A	d_{32} B	d_{40} E*	d_{40} A	d_{40} B
1	0.1	0.6	−0.4	−2.0	−0.4	−0.4
2	0.2	0.4	−0.4	−2.1	−1.2	−0.2
3		0	0		−0.6	−0.2
4	−0.3	−0.5	−0.1	−2.3	−1.9	
5	0.3	0.1	−0.2	−2.1	−1.3	−0.1
6	0.1	0.1	−0.1	−2.0	−0.8	−0.2
7	0.5		−0.5	−2.1	−0.8	−0.4
$\bar{x}$	0.150	0.117	−0.243	−2.100	−1.000	−0.250
s	0.243	0.344	0.176	0.100)**	0.469	0.112
Statistical significance***	—	—	+	++	++	++

* Only limiting values at log V_{40} = 1.2 (minimal difference between titres at 40 and 37 °C) are presented.

** Statistical significance $p_{0.01}$ at the given mean d_{40} value was still preserved at s = 1.16.

*** Values statistically insignificant (—) or significant at $p_{0.05}$ (+) or $p_{0.01}$ (++).

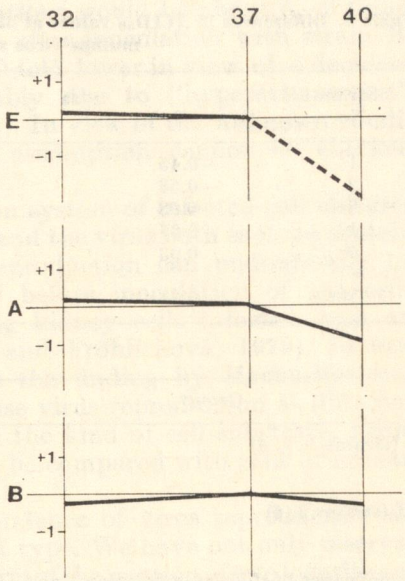

Fig. 1.

Titres (log $TCID_{50}$) of strains E, A and B in Vero cell tube cultures at 32 and 40 °C in comparison with those at 37 °C

Results

Table 1 summarizes the results of seven independent experiments on strains E, A and B at 32, 37 and 40 °C. The calculated indices d_{32} and d_{40} for the individual strains are presented in Table 2.

The d_{32} values were statistically significant ($p_{0,05}$) only in strain B, the reproduction of which decreased at 32 °C to 57% ($d_{32} = -0.243$ log) of that reached at 37 °C.

All d_{40} values were statistically significant at $p_{0,01}$. As compared with 37 °C, the reproduction at 40 °C was lowered in strain E on the average to 0.8% or less, in strain A to 10% and in strain B to 56%. The relationship between the titres of the strains tested is illustrated in Fig. 1.

The difference in d values between the strains at 32 °C was statistically significant ($p_{0,05}$) only between strains A and E on the one hand and strain B on the other. At 40 °C the difference in d values was significant ($p_{0,01}$) between all strains. The virus strains tested in the given system could thus be comparatively reliably differentiated by comparing the paired $TCID_{50}$ value s determined at 32, 37 and 40 °C.

Examination of the five "wild" strains isolated from clinical cases yielded mean values $d_{32} = -0.31$ and $d = -0.18$ log (Table 3). But based on temperature dependence of their reproduction, these strains could be separated into two groups with $d_{32} = -0.54$ and $d_{40} = -0.35$ log (group I) and $d_{32} = 0.03$ and $d_{40} = 0.04$ log (group II).

Table 3. Differences in $TCID_{50}$ values at 32 and 37 °C (d_{32}) and 40 and 37 °C (d_{40}) of five "wild" mumps virus strains in Vero cell cultures

Strain No.	d_{32}		d_{40}	
	$\bar{x}$	s	$\bar{x}$	s
1	−0.45	0.25	−0.33	0.25
2	−0.53	0.25	−0.33	0.11
3	0.03	0.05	0.27	0.39
4	−0.67	0.41	−0.33	0.37
5	0.03	0.08	−0.13	0.24
Mean	−0.31	0.37	−0.18	0.35

Group		d_{32}	d_{40}
I (strains 1, 2, 4)	$\bar{x}$	−0.540 (++)	−0.345 (++)
	s	0.320	0.227
	n	10	11
II (strains 3, 5)	$\bar{x}$	0.029 (−)	0.043 (−)
	s	0.070	0.366
	n	7	7
Significance of difference between I and II		++	+

The values for individual strains represent means from 3—4 tests; n = number of tests in the given group.
−, + and ++: differences statistically insignificant and significant at $p_{0.05}$ and $p_{0.01}$, respectively

Discussion

Our experiments showed that, like with other viruses, mumps virus strains of different character can be distinguished based on the temperature dependence of their reproduction, especially in the region of temperatures about 40 °C. We found differences both between "wild" and between attenuated mumps virus strains. In view of the variability of the results, mean values from repeated experiments had to be taken into account. Variability of the results depended among others on the so far ill defined quality of cell cultures. We therefore included in each experiment a control examination of the Enders' laboratory strain; another evaluation proved to be unreliable.

We do not dispose of sufficient clinical data to evaluate the relationship between the temperature dependence of the reproduction of a virus strain and its action in vivo, i.e. the degree of attenuation in the usual sense. It appears, however, that in accordance with usual criteria the attenuated strains belong to strains the reproduction of which is limited at higher temperatures. This would correspond to the idea of the mechanism of feedback by which virus reproduction in the inoculated organism is limited by a temperature increase before major pathological sequelae may develop. For example if (based on data in Table 2) change in temperature from 37 to 40 °C would result in a reproduction decrease to 10% in strain A and 56% in strain B, at such temperature reaction of the organism to inoculation

viraemia and thus also the risk of complications would be about 5—6 times lower after inoculation with strain A than after inoculation with strain B. With strain E it would be still at least 10-fold lower in view of a decrease in reproduction to less than 1% (probably due to "hyperattenuation" in the course of 141 chick embryo passages). In view of the unknown conditions of previous passages, however,this assumption cannot be checked clinically.

The effect of temperature on the titration system of infected cell cultures concerns its basic components, i.e. the cells and the virus both each separately and in their mutual interaction. Virus reproduction can undoubtedly be affected also by the temperature history before inoculation of the cells employed, as shown, e.g., in primary dog kidney cells infected with an attenuated mumps virus strain (Izbický and Fröhlichová, 1979). In this connection attention should be called to the finding by Markushin and Ghendon (1967) that foot-and-mouth disease virus reproduction at different temperatures may considerably depend on the kind of cell substrate. From this point of view Vero cell cultures should be compared with cells of human origin.

In characterizing the temperature dependence of virus reproduction we intentionally did not use markers of the rct type. We have not only reservations as to the mode of mathematic formulation of the relation of two logarithmically expressed values ($10^a : 10^b = 10^{a-b}$ and not $10^{a/b}$), but we did so especially in view of the fact that rct belongs to the relative markers, the value of which depends on the right selection of the reference strain. The validity of, e.g., the rct_{32} values reported by Vardanyan *et al.* (1972) as characteristic of a virulent mumps virus strain based on the use of the reference strain Leningrad 3 therefore probably is only limited. In our opinion the course of the curve of the temperature dependence of reproduction and the value of the optimal reproduction temperature are more suitable characteristics of a virus strain. The reference strain should only serve as a control of good functioning of the test system. In accordance with the idea of the temperature feedback mentioned above a comparison of the optimal reproduction temperature of a virus strain with the normal physiological temperature of the presumed host could serve as a certain measure of virus attenuation. The shape of the curve of temperature dependence would be an expression of the homogeneity or heterogeneity of the virus population. In any case, for sake of comparability of the results, it is desirable to introduce generally standardized procedures on defined cell cultures with uniform reference strains. Taking into account the possible variability of the results, each sample should be subjected to an appropriate number of examinations with their eventual statistical evaluation.

References

Baugh, C. L., and Tytell, A. A. (1967): Propagation of the human diploid cell WI-38 in galactose medium. *Life Sci.* **6**, 371—380.

Buynak, E. B., and Hilleman, M. R. (1966): Live attenuated mumps virus vaccine. 1. Vaccine development. *Proc. Soc. exp. Biol. Med.* **123**, 768—775.

Glathe, H., and Nöbel, B. (1977): Biologische Eigenschaften des Mumpsvirus. — Das Verhalten im T_{50}-Marker. *J. Hyg. Epidem.* (*Praha*) **21**, 162—168.

Henle, G., and Deinhardt, F. (1955): Propagation and primary isolation of mumps virus in tissue culture. *Proc. Soc. exp. Biol. Med.* **89**, 556—560.

Hosai, H., Yamanishi, K., Ueda, S., Minekawa, Y., Ogino, T., Suzuki, N., Takahashi, M., and Okuno, Y. (1970): Studies on live attenuated mumps virus vaccine. 1. Attenuation of mumps virus by serial passage in the chorioallantoic cavity of developing chick embryos and field trials by the inhalation method. *Biken J.* **13**, 121—126.

Izbický, A. (1977): Kultivace buněk Vero v Eagleově minimálním esenciálním médiu a jeho modifikaci s galaktózou a pyruvátem. *Čs. Epidem.* **26**, 9—14.

Izbický, A., and Fröhlichová, S. (1979): Influence of preincubation of primary dog kidney cell cultures on the multiplication of attenuated mumps virus. *Acta virol.* **23**, 473—480.

Markushin, S. G., and Ghendon, Yu. Z. (1967): Mechanism of the variability of viruses in the course of passaging in tissue culture at lowered temperature. III. Variability of foot-and-mouth disease virus. *Acta virol.* **11**, 100—107.

Slonim, D. (1974): Study of the population dynamics of the human diploid cell strain WI-38. II. The composition of the medium. *J. biol. Standardization* **2**, 51—58.

Vardanyan, N. V., Dzagurov, S. G., and Popov, V. F. (1972): On features of reproduction of mumps virus strains (in Russian). *Vop. Virus.* **17**, 472—476.