FORMALDEHYDE INDUCED MUTATIONS OF THE VIRUS OF WESTERN EQUINE ENCEPHALOMYELITIS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56,
No. 9, pp. 73-76, September, 1963
Original article submitted December 21, 1962

Investigations on induced mutagenesis in experiments with viruses makes it possible to resolve not only theoretical questions on the molecular level, but also purely practical problems in the area of obtaining "useful" viral strains (live vaccines).

Spontaneous mutants of the virus of western equine encephalomyelitis (WEEML), forming fine plaques in chicken fibroblast tissues cultures, have been described in the literature [3,6,7]. Formaldehyde was used for the study of induced mutations, since its intensity and selectiveness of action give it an advantage over many other mutagenic factors.

EXPERIMENTAL METHOD

In the experiments, we used a $10^{-2.5}$M solution of formaldehyde, prepared in Henk's solution, with the addition of 0.5% hydrolysate of lactalbumin. The intracellular virus of WEEML in the tissue cultures of chicken embryo fibroblasts was treated with formaldehyde for a period of 4 h at 37°. The control was represented by the intracullular virus with the same nutrient medium but without formaldehyde. After 4 hours, the culture fluid in the experimental and control tissue cultures was removed, and the cells were washed and resuspended in nutrient medium, after which the experimental and control viruses were titrated in cultures of chicken embryo tissue by the method of plaques [5].

EXPERIMENTAL RESULTS

Out of 143 plaques obtained with the control virus, 140 were large, measuring 4-6 mm in diameter, with rough edges, and 3 were small–1-2 mm in diameter–with an even border (Fig. 1a). Out of 105 plaques with the experimental virus, 104 were small, and 1 was large (Fig. 1b). For a detailed study of pure lines of the virus, we isolated

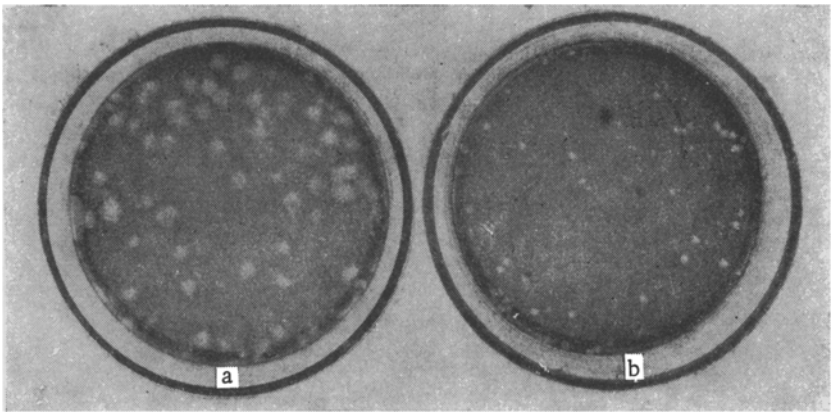

Fig. 1. Plaques of the virus of WEEML. a) Control; b) experiment.

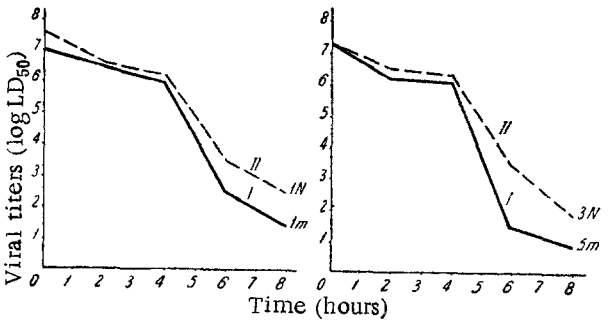

Fig. 2. Sensitivity to formaldehyde of the experimental (I) and control (II) clones of the WEEML virus.

10 of the small plaques from the tissue cultures with the experimental virus, and 5 of the large plaques from the control tissue cultures. All viral clones were separated according to their cytopathogenic activity in chicken embryo tissue cultures. At the same time that we inoculated the tissue cultures of chicken fibroblasts, we inoculated cultures of rabbit kidney cells. Beginning with the first passage, and in the process of the subsequent 3 passages, the behavior of the clones isolated from the large plaques differed from that of the clones isolated from the small ones. After 48 h, in the cells inoculated from the control cultures (clones 1-5 N), we noted clear cytopathogenic activity, which was not manifested in the presence of the specific immune serum. In 7 out of the 10 experimental clones (1 m, 2 m, 4 m, 5 m, 6 m, 8 m, 9 m), there were no morphological changes in the cells that could be recorded under low magnification of the microscope. No cytopathogenic activity was detected with these cells even with prolonging the interval of incubation to 72-96 h, although the virus multiplied, which was proven by titration of the culture fluids from these chicken embryo cell tissue cultures (see table).

Studying the pathogenicity of the isolated viral clones for white mice weighing 7-8 g, inoculated intracerebrally, we found that the titer of the viral clones 1-5 N ranged from 7.0-8.0 log LD_{50}. The viral clones 1 m, 2 m, 4 m, 5 m, 6 m, 8 m, and 9 m, were apathogenic when injected into white mice intracerebrally. As indicated by the table, these viral clones also did not cause cytopathogenic effects on the rabbit kidney cells. Clones 3 m, 7 m, and 10 m, exerted a partial cytopathogenic action in the cultures of rabbit kidney cells, and were pathogenic when inoculated into the brain of the white mice, although the viral titers were lower than in the clones 1-5 N. In order to determine the stability of the apathogenicity characteristic of the clones for white mice, we carried out 3 consecutive passages by means of intracerebral inoculation of the mice at intervals of 6 and 3 days. Of the 60 mice inoculated for each clone, not one died during the course of a 3 week observation period.

The stability of the feature of forming small plaques in clones 1-10 m, and large plaques in clones 1-5 N, was determined by titrating the viruses by the method of plaques. In this experiment, 500-700 plaques were investigated with each viral clone. All the tested viral clones caused formation of plaques that were strictly monotypic in their diameter and contours.

Cytopathogenic Activity of the Clones of WEEML Virus in Certain Tissue Systems, and Their Pathogenicity for White Mice

Material	Intensity of the cytopathogenic action in rabbit kidney cells	log LD_{50}/ /0.2 ml in the culture of chicken embryo fibroblasts	log LD_{50}/ /0.2 ml in the cultures of human embryo fibroblasts	log LD_{50}/ /0.03 ml with intracerebral inoculation into white mice
1 m	0	>6,5	4,2	<1,0
2 m	0	5,5	4,4	<1,0
3 m	2—3+	4,7	—*	5,4
4 m	0	5,5	>5,5	<1,0
5 m	0	5,0	4,5	<1,0
6 m	0	5,5	>5,5	<1,0
7 m	2+	6,0	—	3,4
8 m	0	6,5	>5,5	<1,0
9 m	0	6,3	—	<1,0
10 m	3—4+	7,0	—	5,5
1 N	4+	>6,5	>5,5	7,5
2 N	4+	>6,5	—	7,3
3 N	4+	>6,5	—	7,4
4 N	4+	>6,5	—	7,6
5 N	4+	>6,5	—	—

* Experiment not done.

To resolve the question of whether, under the conditions of the experiment, selection of the mutants from the virus population took place, or whether there was a high percent of mutants caused by the mutagenic action of formaldehyde, we studied the sensitivity of clones from the large (1 N and 3 N) and small (1 m and 5 m) plaques to formaldehyde. It was postulated that, in the case of selective action, the experimental clones would be more resistant to formaldehyde.

As can be seen from the graph in Fig. 2, the inactivation curves in the experimental and control viruses are close to one another; in every case the experimental viral clones are somewhat less sensitive to formaldehyde than the controls, but these data range within the limits of possible statistical error.

On the basis of the obtained results, all the studied viral clones can be divided into 3 groups, according to 3 genetic characteristics.

Clones of the first group form small plaques with clear borders, do not cause cytopathogenic effects in the cultures of rabbit kidney cells, and are nonpathogenic when inoculated intracerebrally into white mice.

Clones of the second group form small plaques with clear borders, exert a partial cytopathogenic action in the cultures of rabbit kidney cells, and possess intracerebral activity for white mice (3.0-6.0 $\log LD_{50}$).

Clones of the third group form large plaques with rough borders, give rise to a manifest cytopathogenic action in the cultures of rabbit kidney tissue, and possess high intracerebral activity for white mice (7.3-7.5 $\log LD_{50}$).

The frequency of experimental mutations that arose in the presented experiment, close to 100%, represents the result of a very high intensity of action on the part of formaldehyde. The formaldehyde mutation output in experiments with Drosophila [1], plants, and bacteria [4], often exceeds the potential of all the other mutagens, even with relatively weak selection of the primary treated material. Thus, the thousands or tens of thousands of times more severe action – if we judge from the selection of viruses in contact with the formaldehyde – must lead to the ideally "pure" outcome of mutations. On the basis of a number of characteristics, it may be postulated that in the total mass of mutations that are produced there are several types which differ from one another both morphologically (size of the plaques) and biologically (pathogenicity for mice, cytopathogenic activity). In its biochemical behavior, formaldehyde is distinguished, on the one hand, by its bonds with certain nucleotides, particularly with adenine [2], and on the other, by its affinity for amino groups [1], i.e., it is allotted with a general, high affinity for nucleoprotein substances. Thus, although the affinity of formaldehyde for gene material is general, and most often the total number of elementary mutone blocks that react with it is numbered in the hundreds, the character of distribution indicates a definite selectivity, specifically: the frequency of bonding with certain mutones is many times greater than the induced reorganization of other mutones.

Apparently, with adenine in the structure of the nucleotides, formaldehyde bonds especially easily, but it is possible, in turn, that some adenine manomeres are considerably more susceptible to its action than others, depending on the neighboring nucleotide blocks, the amino acids with which they are linked in the composition of the triplet, and the general steric properties.

SUMMARY

As demonstrated, a high percentage of mutants is produced by the action of formaldehyde on the virus of western equine encephalitis. These mutants form small plaques when cultured on chick embryo cells. The viral clones isolated from the small plaques may be subdivided into 2 groups: 1) viral clones that are nonpathogenic for 7-8 g albino mice when injected intracerebrally, and causing no cytopathogenic effects in cultures of rabbit kidney cells; 2) viral clones that are pathogenic for albino mice when injected intracerebrally, and cause a partial cytopathogenic effect in cultures of rabbit kidney cells. The viral clones isolated from the "wild" type of virus were highly pathogenic for albino mice, and possessed a marked cytopathogenic activity against the rabbit kidney cells.

LITERATURE CITED

1. I. A. Rapoport, Zh. obshchei biol., 8, 5, 359 (1947).
2. T. Alderson, Nature, 187, 485 (1960).
3. M. Dunayevich, H. Johnson, and W. Burleson, Virology, 15, 295 (1961).
4. D. Pennington, J. Bact., 59, 617 (1950).
5. J. Porterfield, Bull. Wld. Hlth. Org., 22, 373 (1960).
6. L. Quersin-Thiry, Brit. J. exp. Path., 42, 511 (1961).
7. R. Ushijima, D. Kill, G. Dolana, et al., Virology, 17, 356 (1962).