# PROPERTIES OF THE ALASTRIM VIRUS,

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30 JAN 1968

TRANSLATION NO. 3105

DATE: 30 January 1968

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On page 440 of source (p 2 of translation) reference is made to figure 1, inserted between pp 416-417 of source -- this figure was not provided with document and therefore is lacking in the translation.

UDC: 576.858.13

# PROPERTIES OF THE ALASTRIM VIRUS

Voprosy virusologii
(Problems in Virology)
Vol 10, 1965, pp 439-446

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The alastrim [parasmallpox] virus is the exciter of a variety of smallpox (variola minor). The epidemics which it causes are most frequently of all observed in countries with a hot climate, but in some cases there have also been outbreaks of alastrim in Europe [13, 19, 20]. Hitherto the USSR has recorded no cases of alastrim, but it is obviously just as probable that this infection, as the virus of natural smallpox, may be brought into our country.

The notices of alastrim virus properties in foreign literature are few, fragmentary, and rather contradictory. Just recently it was still believed that the sole difference between the virus of alastrim and that of smallpox was its lesser pathogenicity for man [7, 9, 24]. In recent years reports of the existence of certain other differences have appeared [12, 14]. In the USSR it is only lately that the properties of the alastrim virus have begun to be studied [3, 4, 6].

The present work generalizes the findings on study of the biology of the alastrim virus which have been accumulated in our laboratory in the last four years. The following virus properties were studied: pathogenicity for laboratory animals when administered in different ways, its behavior in chick embryos and tissue culture, the extreme temperature under which it will develop, its hemagglutinating activity, and its antigenic structure. In addition the resistance of alastrim virus to a number of physical factors, chemical substances, and several antibiotics.

## Material and Method

The research material was chorionallantoic cultures of three strains of alastrim virus which the authors obtained from England (Kershaw, Butler, and a strain which the authors call England) whose infectiousness for

developing chick embryos (DCE) is $10^{5.5}$-$10^5$ $ID_{50}$. The Butler strain was acknowledged a commission of the International Congress of Microbiologists in Rome in 1953 to be typical of this type of virus. The comparative experiments used 15 strains of natural smallpox virus and six strains of vaccine virus.

The pathogenicity of the alastrim virus was studied in experiments on rabbits of the Chinchilla breed (weight 2 kg) by inoculation intravenously, intraperitoneally, intracutaneously, on scarified skin, into the brain, and into the anterior chamber of the eye; on guinea pigs (weight 200 gram) by administration into the anterior chamber of the eye; and on white mice (weight 6-7 gram) by inoculation intravenously, intraperitoneally, and into the brain.

Single-layer cultures of monkey-kidney cells (MKC), human embryos (HEC), chick embryos (CEC), and pig kidneys and lungs were used, as well as transplantable lines of HeLa, HEp-2, A-1, SOTs, and KF. In studying the process of plaque formation a CEC culture was employed. The method described by Postlethwaite [23] for vaccine virus was utilized for culturing without agar covering. The agar covering was prepared according to the instructions by Porterfield and Eddison [22].

Gispen's method [17] was employed in setting up the reaction of of double diffusion into gel. The results were taken into consideration on the 3rd, 7th, 14th, and 21st day.

Previously described methods [1, 2, 5] were used to find hemagglutination and hemadsorption in tissue cultures, to detect the cytoplasmic inclusions, and also to study virus heat resistance, effect of pH of the medium, resistance to ultraviolet irradiation, and the action of a number of disinfectants and antibiotics.

## Results

The experimental results showed that the alastrim virus is of low pathogenicity for laboratory animals (Table I)

None of the three strains of alastrim virus caused the death of DCE when injected in the chorionallantoic envelope, where punctate, raised, sharply delimited, dense white lesions were formed. In successive subinoculations the nature of the lesions changed (the elements became somewhat flatter and lost their distinct boundaries) and the virus acquired the capacity to cause death of part of the DECs. Moreover the pathogenicity of the adapted variants of alastrim virus was less for DEC than that of natural smallpox virus.

It has been shown that the alastrim virus exerts a cytopathogenic effect with formation of specific cytoplasmic inclusions (see Fig. 1, an insert between source pages 416-417). The type of tissue culture

determines certain features of cytoplasmic action -- the proliferative nature of the degeneration is observed in cultures of the transplantable lines, and its destructive nature in the primary ones. In the primary tissue cultures the cytopathological action has characteristic features making it possible to differentiate the alastrim virus and other viruses of the smallpox group (vaccine, cowpox, rabbit pox).

TABLE I. Susceptibility of Laboratory Animals to Alastrim Virus

| Laboratory animal | Method of infection | Reaction to injection |
|---|---|---|
| Rabbits | Intravenous | None |
| | Into the brain | None |
| | Intraperitoneally | None |
| | Cutaneously | None |
| | Intracutaneous | Development of infiltrate |
| | Into the testicle | None |
| | Into the anterior eye chamber | Slight keratitis |
| White mice | Intravenously | None |
| | Into the brain | Partial death |
| | Intraperitoneally | None |
| Guinea pigs | Into the anterior eye chamber | Slight keratitis |

In the HEC culture the cytopathological action begins with the appearance of small foci of tissue destruction. In the areas involved isolated rounded cells of different sizes clearly show up, includ- giant cells, distinctly contoured with granular cytoplasm and strongly light-refractive. The alastrim virus is characterized delayed appearance of the cytopathological effect and protracted development of effect from the first focal changes to total degeneration. The first signs of degeneration set in when the culture is infected with a dose of $1 \times 10^3$ to $1 \times 10^4$ TC $ID_{50}$ in 48 hr, and with smaller doses, in 72-96 hr after infection. Total degeneration of the culture is noted 5 to 7 days after appearance of the first degenerative foci.

The duration of the process of degeneration varies with the type of tissue culture. Cytopathological action of alastrim virus in a virus dose of 1000 TC $ID_{50}$ in cultures of pig and monkey livers is noted on the second day and in the HeLa culture, on the fourth day. The difference in sensitivity of tissue cultures to the virus is particularly clearly marked in experiments studying the dynamics of virus accumulation in them (Fig. 2). Maximum and rapid multiplication of the virus is provided by the monkey-kidney culture; the least virus titers in the same periods and their delayed growth are observed in the CEC culture.

We obtained interesting data in experiments culturing the alastrim virus under elevated temperature conditions. It was found that the virus (all three strains) is capable of causing cytopathological changes in CEC only within certain temperature limits -- from 34 to 36°C. No cytopathological action is observed at a temperature of 37.5° (Table II).

In other types of tissue cultures (HEC and MKC) the cytopathological action of the virus is manifested at higher temperatures (to 39°C,

TABLE II. Effect of Various Temperatures on Alastrim Virus in Tissue Culture

| Incubation temperature (in degrees) | Butler strain | | | Kershaw strain | | | England strain | | |
|---|---|---|---|---|---|---|---|---|---|
| | CEC | HEC | MKC | CEC | HEC | MKC | CEC | HEC | MKC |
| 34 | + | + | + | + | + | + | + | + | + |
| 36 | + | + | + | + | + | + | + | + | + |
| 37.5 | - | + | + | - | + | + | - | + | + |
| 38 | - | + | + | - | + | + | - | + | + |
| 39 | - | - | - | - | + | + | - | + | + |
| 40 | - | - | - | - | - | - | - | - | - |

Symbols: + = cytopathological action, - = no such action

except for the Butler strain).

The alastrim virus, exerting no cytopathological effect, continues to remain viable in the tissue culture incubated at high temperature and again acquires the capacity to reproduce, causing distinct changes during transfer of the culture in the usual temperature regime. Moreover the length of time that virus viability is maintained depends on the type of tissue culture and the degree of its adaptation to the given tissue type.

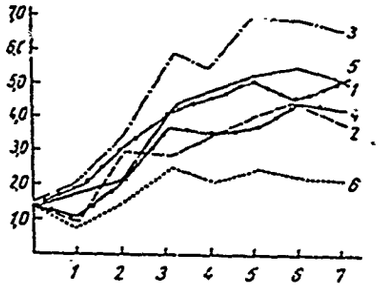


Fig. 2. Dynamics of Accumulation of Alastrim Virus (Butler Strain) in Various Tissue Cultures. 1 -- HEC, 2 -- HeLa, 3 -- MKC, 4 -- HEp-2, 5 -- A-1, 6 -- KF. Ordinate axis -- log TC $ID_{50}$/0.1 ml, abscissa axis -- incubation period, days

The alastrim virus forms plaques in tissue culture without an agar covering from 96 hr after infection. Initially they may be distinguished only by means of a loupe or microscope. Subsequently their size increased slightly (diameter to 0.5-0.8 -- 1 mm). On magnification the interior of such a plaque shows a clear reticular structure of a degeneratively changed tissue focus. Under an agar covering the alatrim virus plaque did not put in an appearance before the fifth day. Their size was likewise very small -- less than 1 mm (during ten days of observation), if neutral red was added to the composition of the agar covering before application to the monolayer, and up to 2.5 mm when the dye was added on the fourth day of incubation. Raising the incubation temperature leads to disappearance of the plaques, while the temperature conditions exert a greater effect on the plaques beneath the agar covering and a less effect without it. Thus, at 37.5°C in the case of culturing without agar covering, the plaques were found in both strains studied (Butler and England), but in only one of them (England) under an agar covering; they did not form at 38°C.

Indication of small alastrim virus doses is facilitated by using the hemadsorption reaction, which is usually positive until the appearance

TABLE III. Comparative Characteristics of Properties of Alastrim, Natural Smallpox, and Vaccine Viruses

| Tests | Viruses | | |
|---|---|---|---|
| | Alastrim | Natural smallpox | Vaccine |
| Chick embryos (DCE): inoculation onto chorionallantoic envelope of DCE | Embryos do not die; chorionallantoic envelope has dense white punctate elevated sharply delineated lesions | Embryos do not die; lesions on chorion-allantoic envelope indistinguishable from those with alastrim virus | Embryos die on 3rd-5th day; round whitish flat lesions on chorionallantois |
| Successive subinoculations | Elements flatten, lose distinctness of outline; some embryos die | Same as with alastrim virus. Pathogenicity for embryos somewhat higher | No essential change in nature of lesions; embryos die earlier |
| Injection of sub-inoculated material into allantoic cavity | Embryos do not die; nature of lesions seemingly returns to original | With few exceptions embryos do not die; nature of lesions typical for virus | Embryos die; lesions typical of vaccine virus |
| Hemagglutinating activity with respect to chick erythrocytes | Lacking or very weakly marked | Lacking or very weakly marked | Pronounced |
| Rabbit reaction to injection: | | | Generalized infection with rash on skin |
| intravenous | None | None | and mucous membrane |
| into brain | none | | encephalitis, death |
| into testicle | none | | orchitis |
| onto scarified skin | none | | hyperemia, infiltration, pustulous rash |
| intracutaneous | infiltrate | infiltrate | infiltrate |
| into anterior eye chamber | keratitis | keratitis | keratitis |
| White mouse reaction to injection: | | Sporadic death in injection of high | |
| intravenous | None | virus suspensions | Death of animals |
| into brain | death of young animals | death of young animals | death of animals |
| Tissue culture: cytopathological action | Focal type, cells rounded, sharp borders, often enlarged | Same as with alastrim virus | Marked, spreading to whole monolayer, cell borders eroded |
| intracellular inclusions | regularly in cytoplasm | regularly in cytoplasm | in cytoplasm |
| extreme temperature of development of cytopathological effect in CEC | 37°C | 38°C | above 40°C |

TABLE III (continued)

| Reaction of double diffusion into gel with antivaccine serum | Two main precipitation zones | Two main precipitation zones | Two main and 1 – 4 additional zones |
|---|---|---|---|
| Resistance to: | | | |
| temperature of 50, 60, and 70°C | More labile than smallpox virus | More resistant than alastrim virus | Same as in alastrim virus |
| ultraviolet irradiation | Intermediate position between smallpox and vaccine | same | less resistant than alastrim virus |
| 3% solution of chloramine | inactivated in 1 hr | not inactivated in 3 hr | inactivated in 1 hr |
| 5% solution of phenol | inactivated in 2 hr | same | inactivated in 2 hr |

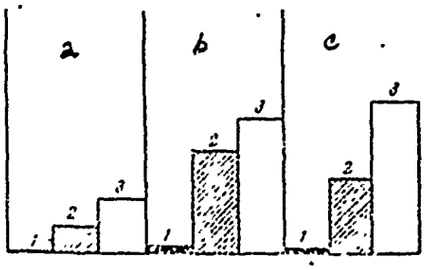


Fig. 3. Thermal Resistance of Viruses of (a) Smallpox, (b) Alastrim, and (c) Vaccine. 1 -- 50°C, 2 -- 60°C, 3 -- 70°C. Ordinate -- inactivation rate in log/min.

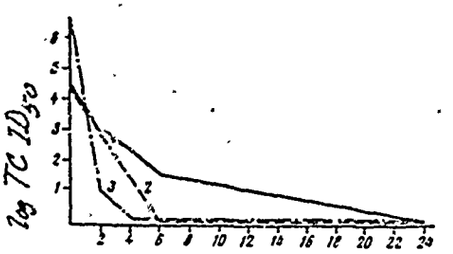


Fig. 4. Effect of Ultraviolet Rays on Infectiousness of Viruses of (1) Smallpox, (2) Alastrim, and (3) Vaccine in Clarified Suspensions

of distinct cytopathological action. Hemagglutinatinins during multiplication of the alastrim virus in the susceptible tissues are lacking or their titer is very low (1:2 – 1:4 in the tissue culture; up to 1:20 in suspensions of DCE chorionallantoic envelope).

The antigenic structure of the alastrim virus in the study in the reaction of double diffusion into gel with antivaccine serum is characterized by presence of only the main precipitation zones (I and III).

The following was brought to light in studying the thermal resistance of the alastrim virus. Protracted storage of the virus at 4-6°C was not perceptibly reflected in its infectiousness: after storage for eight months at -25°C the titer had not changed; in storage for the same period at 4-6°C the titer fell 1 log. At room temperature (20-28°C) the virus kept its viability for three months; at 34°C, for one month.

Figure 3 gives the results of determining the thermal resistance of this virus in heating for a single time at 50, 60, and 70°C. The rate

of inactivation expressed in the drop in infectiousness in log/min at 50°C was 0.01 log/min, at 60°C -- 1.4 log/min, and at 70°C -- 1.7 log/min. At 100°C the alastrim virus was inactivated in 1 min.

Optimum pH for the alastrim virus is 7.4; at a pH lower and higher than 9 its activity fell drastically; a medium of pH 3.0 led to inactivation of the virus in 1 hr.

Figure 4 represents the results of experiments studying the effect of ultraviolet irradiation on the virus in clarified suspensions. Clarification was brought about by freezing, thawing, and then centrifuging. A 30-watt bacterial lamp with rays of wavelength 2537 Å was used for irradiation, which took place for 2 to 24 hr at a distance of 30 cm from the object.

As is apparent from Fig. 4, the alastrim virus was inactivated between the 4th and 6th hour of irradiation.

Penicillin, streptomycin, gramicidin, and biomycin were included in the experiments on antibiotic action. The results of the experiments demonstrated that penicillin, streptomycin, and gramicidin in vitro during contact for 24 hr at room temperature exert no perceptible effect on alastrim virus, while biomycin in a dosage of more than 1000 ED/ml sharply suppresses the action of this virus.

Our investigations also studied the effect on the alastrim virus of a number of chemical disinfectant agents for the purpose of selecting the optimum conditions for inactivation. The experiments were conducted with the most active and practically convenient disinfectants preselected in experiments with vaccine virus. Figure 5 displays the findings.

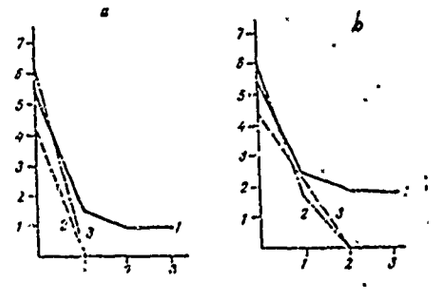


Fig. 5. Resistance of Viruses of (1) Smallpox, (2) Alastrim, and (3) Vaccine to (a) 3% Solution of Chloramine and (b) 5% Solution of Phenol. Ordinate axis -- log TC $ID_{50}$, abscissa axis -- time in hr.

As may be seen, the alastrim virus was successfully inactivated by a 3% solution of chloramine in 2 hr.

When comparing the findings with the results of a parallel study of the properties of viruses of natural smallpox and vaccine it may be seen that both in experiments on laboratory animals, chick embryos, and tissue culture and in study of the resistance of the virus to effects of a physical and chemical nature a number of substantial differences are disclosed which make it possible to distinguish the alastrim virus and the viruses of natural smallpox and vaccine (Table III).

## Discussion

From the findings cited it is evident that the alastrim virus is weakly pathogenic to all the types of laboratory animals used. In this respect it was scarcely distinguished from the virus of natural smallpox and was sharply distinguished from the vaccine virus. It is to be pointed out that Cleland and Ferguson, Green, Blacksol (quoted by Sadov [8]) and Helbert [18] noted the weak pathogenicity of smallpox and alastrim viruses for laboratory animals in comparison with the pathogenicity of vaccine virus.

In experiments on DCEs we discovered the lesser pathogenicity of alastrim virus in comparison with the virus of natural smallpox. The nature of the lesions on the chorionallantoic envelope of the DCE was identical with those of smallpox. Dinger [14] and Helbert [18] have obtained similar findings.

In the character of its cytopathological action the virus of alastrim is not distinguished from that of the virus of natural smallpox. At the same time the type of the cytopathological changes permits clear differentiation between these viruses and the vaccine virus. Baltazard et al. [11] also pointed this out.

Our investigations established the identity of the intracellular inclusions in infection by viruses of natural smallpox, alastrim, and vaccine. It must be noted that the difference in the nature of the inclusions detected by Torres and Teixeira [25], which in their opinion allowed these infections to be distinguished with sufficient accuracy, have not been corroborated either by our research or that of others [10, 16, 20].

Investigators in Downie's laboratory in experiments on neutralizing complement-binding and hemagglutination-inhibition reactions have detected no antigens distinguishing these two viruses.

At the same time while using the method of virus neutralization by adsorbed serums they found that antigenically the viruses of smallpox and alastrim are closer to each other than to the vaccine virus [15, 21]. Our results from studying the virus antigen structure by the method of double diffusion into gel do not conflict with these data.

In the accessible literature we have found no allusions to the resistance of alastrim virus to physical and chemical effects. Our findings permitted the establishment of several distinctions between the viruses of alastrim, natural smallpox, and vaccine. The alastrim virus proved to be more labile with respect to high-temperature, ultraviolet-radiation, chloramine, and phenol action than is smallpox virus. In its resistance to disinfectants the alastrim virus is no different from that of vaccine, but is more resistant than the latter to ultraviolet light.

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Received 14 August 1964.