SOME PROPERTIES OF THE AUTOINTERFERING VIRUS RECOVERED FROM THE GASTRIC CONTENT OF PATIENTS WITH EPIDEMIC HEPATITIS

[Following is the translation of an article by D. Kh. Fomin, Uzhgorod Scientific Research Institute of Epidemiology, Microbiology, and Hygiene, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) 11:13-17, 1966. It was submitted on 29 Apr 1964.]

In recent years V. A. Ananyev and A. K. Shubladze [1], Rightsel et al. [6], and others succeeded in isolating a virus from the blood and feces of patients with epidemic hepatitis and the biological properties of these viruses and relation to etiology of hepatitis are being studied. In this report certain properties are discussed of an autointerfering virus (AIF-virus) which was isolated in our laboratory from the gastric content of patients with epidemic hepatitis.

The method for isolation of the virus from the gastric content of the patients was described by us earlier [2,3]. The results of the study of cultural and biological properties of the AIF-virus are presented in the table. Of the number of continuously cultivated tissue cultures the most sensitive to the cytopathic effect of the virus turned out to be the cells of Detroit-6 and HEp-2. On Changa cells the cytopathic action of the virus was manifested up to the 4--6th passage, and on cultures of HeLa and L - in the 1--2nd passage irregularly. The cells of human amnion were not sensitive to the cytopathic effect of the virus.

Degenerative changes in infected cells (cytoplasmic granularity, shriveling of cells) appeared in 24--72 hours after inoculation, and complete degeneration of the cell monolayer set in on the 7--9th day. From these same probes of gastric content the virus was isolated on 9-12 day old chick embryos and individual strains were transplanted from the chick embryos to Detroit-6 cells and from tissue cultures to chick embryos. Results of the investigation of cultural and embryonic lines of the AIF-virus by the method of anaphylaxis on guinea pigs and immunoprecipitation in agar by the method of Ochterlony testify to their antigenic affinity.

In the sera of patients with epidemic hepatitis and reconvalescents antibodies were revealed which neutralized the cytopathic effect of the virus in tissue culture in dilutions of 1:16--1:32. In the study of the embryonic antigens of the virus by the method of

precipitation in agar precipitins were revealed in the sera of hepatitis patients in 86% of the cases, and in the sera of the control group - in 10% (reliable intervals correspondingly of 77--92% and 3.7--18.6%). In the serum of immunized guinea pigs, rabbits, and geese the appearance of precipitating antibodies to the embryonic antigen of the virus was noted.

After the 10th passage on tissue cultures the AIF-virus began to display weak hemagglutinating properties to erythrocytes of roosters (1:2--1:4) and white rats (1:4--1:8). No hemagglutinins were revealed to the erythrocytes of man (O blood group), and also to the erythrocytes of guinea pigs, geese, and sheep. Cultural and embryonic antigens of the AIF-virus are able to sensitize the formalinized erythrocytes of man (O blood group), after which the erythrocytes are agglutinated by the sera of hepatitis patients and reconvalescents in dilutions of 1:80--1:320.

AIF-virus (in the form of virus-containing cultural and embryonic fluid) is highly resistant. It remained active after 18-month storage in a refrigerator at 4--6°. There is a basis to assume that repeated freezing and thawing of the virus-containing fluid has an unfavorable influence on the survival of the virus.

A high resistance of AIF-virus to heating was noted. After a 2-hour exposure at 56° the virus preserved its activity; following heating at 60° for 1 hour or at 65° for 30 minutes the virus also was not inactivated.

The results of preliminary investigations, conducted by us jointly with V. A. Ananyev and V. D. Sobolevaya at the Institute of Virology imeni D. I. Ivanovskiy AMN USSR, characterize the AIF-virus as DNA-containing and ether-resistant. It differs from the known adenoviruses by a high resistance to heating and low resistance to repeated freezing and thawing.

In our first reports /2/ it was noted that the isolated virus displays expressed autointerfering properties. Its cytopathic activity within certain limits changes directly proportional to the degree of dilution of the virus-containing material. The use of native (undiluted) virus-containing fluid in the passages often led to the loss of the line of virus being passaged. This property of the virus made it very difficult to obtain regular results in the passages, especially during titration and setting up of the reaction of neutralization of the cytopathic action of the virus, which dictated the necessity for clearing up the optimum conditions of its cultivation.

As Spies noted /7/, autointerference is also inherent to the Motol virus, which was isolated by Kubelka and associates in 1958 /5/.

Table 1

Certain properties of the AIF-virus, isolated from the gastric content of patients with epidemic hepatitis

Property	Characteristic
Spectrum of sensitivity of transplanted tissue culture	
Detroit-6 cells	++++
HEp-2 "	+++
KEM "	+++
Changa "	++ (up to 5--6th passage)
HeLa "	+
L "	±
Sensitivity of 9--12 day chick embryos	Up to 8--12th passage
Serological characteristics	
a) in sera of reconvalescents: neutralizing antibodies	In titers of 1:8--1:32
precipitins	" " " 1:8--1:16
b) in sera of immunized animals: precipitins	In titers of 1:8--1:16
neutralizing antibodies	Not investigated
Hemagglutinins: to erythrocytes of man (0 blood group)	Absent
" " " guinea pigs, sheep, geese	Absent
" " of roosters, rats	In dilutions of 1:2--1:4
Phenomenon of passive hemagglutination with erythrocytes of man (formalinized)	Positive
" " of rats	Irregular
" " of geese, roosters, guinea pigs, sheep	Absent
Resistance to temperature influences at 4°	Activity preserved; 18 mos. (period of observation)
56°	2 hours
60°	1 hour
Alcohol (97°) for 24 hours	Did not perish
Pepsin (0.1% solution) for 24 hrs	" " "
Trypsin (0.025% solution) for 24 hours	" " "

Assuming that during the interaction of virus with sensitive cells in suspension the multiplicity of virus particles, absorbed by a cell (multiplicity of infection), is characterized by Poisson distribution, Spies on the basis of a statistical analysis of his experimental data came to the conclusion that autointerference developed at a multiplicity greater than 2. In other words, suppression of the cytopathic effect of the virus was noted when the susceptible cell adsorbed 3 or more virus particles.

It was difficult for us to judge to what extent the noted mathematical regularities depleted the essence of autointerference which was inherent to the Motol virus. However, it is apparent that the statistical analysis of multiplicity of infection with the application of the Poisson theory does not explain completely the peculiarities of reproduction in tissue culture of the AIF-virus studied by us.

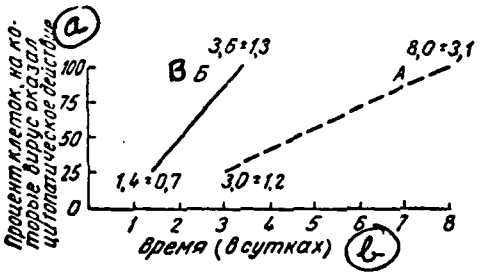
In studying the resistance of AIF-virus to heating we established a fact which was paradoxical at first glance; virus which was heated at 56-60° for 30 minutes manifested its cytopathic activity in a tissue culture more intensively than warmed virus. Cytopathic action was revealed 24 hours after inoculation and complete degeneration of the monolayer set in on the 3--4th day.

The results of further investigations showed that the "effect of heating" noted above was conditioned by the influence of temperature not on the virus, but on some substance which was found in the culture medium and suppressed the cytopathic action of the virus. In its nature this substance may be a substance of the interferon or inhibitor type. In connection with this there is interest in the investigations of Ya. Ya. Tsilinskiy [4], who established that cultural fluid and cell extract of transplanted cultures of HeLa, Detroit-6, HEp-2, and certain others contain inhibitors of virus activity.

Data from the investigations conducted testify that the substance detected by us, which suppresses the cytopathic activity of virus, differs from interferon and the inhibitors described by Ya. Ya. Tsilinskiy by its thermolability. It is destroyed by heating at 56° for 30 minutes. The cytopathic activity of the AIF-virus is increased after its treatment in cultural fluid with an 0.1% solution of pepsin or 0.025% solution of trypsin. This creates a basis for the assumption that the stated proteolytic enzymes destroy the substance which suppresses the cytopathic action of the virus. Naturally the stated antivirus substance does not manifest its activity in the event of a frequent "change of soil" on which the virus is cultivated, for example, with the alternation of passages on cells of HEp-2 and Detroit-6 or HeLa, Changa, KEM, and on culture embryos.

On the drawing the dynamics are shown for the increase of cytopathic action of virus (strain Uzh-76) during continuous passages on

Detroit-6 cells (A) and during transplanting of virus from cells of HEp-2 cells to Detroit-6 (B). Consideration was given to the time of appearance of the cytopathic effect for 1 plus (25%) and the time of onset of total degeneration of the cell monolayer (100%). Data given on the drawing show that with a "change of soil" the cytopathic effect of the virus was manifested considerably more actively than during passages of it on the same tissue culture. The noted phenomenon is explained easily if it is assumed that the cytopathic effect of virus, cultivated without a "change of soil", is suppressed by interferon. A characteristic for the latter, as noted by Vil'chek [8], is the significant (though not absolute) tissue specificity; it is manifested only in homologous tissue.



Dynamics of the increase of the cytopathic effect of AIF-virus. A - during passages on Detroit-6 cells; B - during transplanting of virus from HEp-2 cells to Detroit-6 cells (explanation in text). Key: (a) Percentage of cells on which the virus exerted a cytopathic effect; (b) Time (in days).

These investigations contributed to the clearing up of the optimum conditions of cultivation of AIF-virus. It was established that suppression of reproduction of virus as a result of autointerference can be overcome by various methods of treatment of the virus-containing culture fluid: dilution, heating at 56-60° for 30 minutes, treatment with solutions of pepsin (0.1%) and trypsin (0.025%), and also by alternation of passages on cells of a different type ("change of soil"). The investigations conducted contributed to clearing up the mechanisms of interaction of virus with the cell, in connection with which there is also a certain theoretical interest.

Conclusions

1. From the gastric content of patients with epidemic hepatitis an autointerfering virus was isolated which was capable of reproducing in passages on transplanted cultures of Detroit-6, HEp-2, KEM, and in 9-12 day old chick embryos.

2. The virus isolated possesses expressed autointerfering properties; its cytopathic activity was manifested more intensively during dilution of virus-containing culture fluid to 10^{-7}--10^{-10}.

3. In cultures of transplanted cells of Detroit-6, HEp-2, HeLa, KEM, Changa, and L, infected with the AIF-virus, a substance of the interferon or inhibitor type was revealed which suppressed the cytopathic effect of the virus. It is destroyed by heating up to 56-60° for 30 minutes, and under the influence of 0.1% solution of pepsin and 0.025% solution of trypsin. The antivirus activity of the substance is manifested in homologous tissue culture and is absent in heterologous. Based on these properties the substance revealed is close to interferon.

4. The capacity of AIF-virus to be cultivated in transplanted tissue cultures and chick embryos, its autointerfering properties, high resistance to heating, absence of hemagglutinating activity, and other properties pointed out in the work distinguish it from the known enteric and respiratory viruses.

5. In the sera of patients with epidemic hepatitis the presence of antibodies which precipitate and neutralize the cytopathic effect of the virus creates the basis for the assumption of a possible significance in the etiology of Botkin's disease.

6. Materials cited in the work make it possible to recommend, for the isolation of viruses from patients with epidemic hepatitis and their study in passages, the dilution of native virus-containing material, heating it at 56-60° for 30 minutes, and also frequent "change of soil" (alternation of tissue cultures of different types).

Literature

1. Anan'yev, V. A., Shubladze, A. K., Vopr. virusol., 1961, No 5, p 538. - 2. Fomin, D. Kh., Zheltvay, A. A., In the book: Epidemic Hepatitis, Thesis of Reports of the 15th Scientific Session of the Institute of Virology, AMN USSR imeni Ivanovskiy, Moscow, 1962, p 6. - 3. Fomin, D. Kh., In the book: Materials for the Conference on the Problem of Botkin's Epidemic Hepatitis, Gor'kiy, 1963, p 9; In the book: Epidemic Hepatitis, Thesis of Reports of the 15th Scientific Session of the Institute of Virology, AMN USSR imeni Ivanovskiy, Moscow, 1962, p 16. - Tsilinskiy, Ya. Ya., Inhibitors of viral activity from noninfected cultures of transplanted cells, Authors abstract, Moscow, 1964. - 5. Kubelka, V., Slavik, K., Sousek, O., Zbl. Bakt., 1 Abt. Orig., 1958, Bd. 171, S. 401. - 6. Rightsel, W. A., et al., J. A. M. A., 1961, v 177, p 671. - 7. Spies, K., Z. Hyg. Infekt.-Kr., 1961, Bd. 147, S. 277. - 8. Vil'chek, Ya., Uspekhi sovr. biol., 1963, v 55, No 3, p 391.