THE ANTIVIRAL ACTION OF RIBONUCLEASE

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(With use of a model of the virus of Venezuelan equine encephalomyelitis, the inhibiting effect of a preparation of pancreatic ribonuclease on the accumulation and infectious and hemagglutinating activity of the virus, and interferon production in chicken embryo fibroblast culture were determined. Statistical processing confirmed a high degree of correlation in the suppression of all three types of activity. The mechanism of virus-inhibiting action of ribonuclease is discussed. The article includes two tables, two illustrations and a bibliography of 16 titles).

In the past few years a number of works have been published [8, 10, 12, 13] describing the inhibiting of the reproduction of viruses through the action of nucleases. In some instances, a positive effect from the addition of ribonuclease and desoxyribonuclease has been observed, not only in the laboratory but also in the clinic [4, 5]. The circumstance that ribonuclease, evidently, readily passes the hemato-encephalic barrier has rendered successful the attempt to use this substance in connection with viral affections of the central nervous system [2, 5]. Certain observers are inclined to regard the nucleases as an element of the protective systems of

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the cell and the organism [1, 14]. But in this connection much remains which is unclear as regards the possibilities, prospects and limitations of the use of the nuclease, and also the mechanism involved in the action of these preparations on the reproduction of viruses.

In the present study we attempted to carry through a sequential, statistically reliable comparison of the action of a Soviet-produced preparation of pancreatic ribonuclease on the accumulation of/infectious and hemagglutinating action of a virus, and on the production of interferon, in a modeled system (virus of Venezuelan equine encephalomyelitis) consisting of trypsinized chicken-embryo fibroblasts.

Materials and methods. The Venezuelan equine encephalomyelitis virus, before the study was conducted, underwent 20 passages in chicken embryo fibroblasts. In all of the experiments, we studied trypsinized fibroblasts obtained by the standard method. Titration of the infectious activity of the virus was conducted by the classic method [11]. Interferon was titrated by adding plaques of the test virus. The crystalline pancreatic ribonuclease (official Soviet-produced preparation of the Leningrad Plant of Meat Products) was dissolved in a physiological solution and added to a nutritive medium.

Results. As had been shown earlier [6, 7], the most convenient method for determining the antiviral effect of ribonuclease in the modeled system used by the present writers is the introduction of appropriate quantities of the preparation into an accumulation medium (medium No. 199, with 2% of beef serum) immediately following infection of the cells with Venezuelan equine

encephalomyelitis virus. Multiplicity of the infection amounts to 5-10 BSU per cell.

Samples of the culture liquid were drawn off 24 hours following infection, after which infectious and hemagglutinating activity of the virus were determined, and also (following heating at 60° for 1 hr.) the interferon titers. To establish every control point, 5 independent determinations were made, and for every experimental point 10.

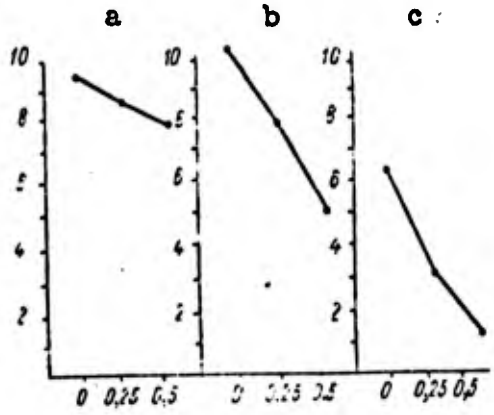

Figure 1. Suppression of the production of infectious virus (a), hemagglutinins (b) and interferon (c) in a culture of chicken embryo fibroblasts in the presence of ribonuclease. On the x-axis are shown, a, infectious activity of the virus (in log BSU/ml; b, activity of hemagglutinins (in $\log_2$ HAU/ml); c, activity of interferon (in $\log_2$ BPE_{50}/ml; on the y-axis is shown the concentration of ribonuclease in the accumulation medium (in mg/ml).

The results of the tests made show that the presence within the accumulation medium of even 0.25 mg/ml of ribonuclease results in a statistically reliable (in all case with P less than 0.01) reduction both

of infection and hemagglutinin titers and of interferon titer. Suppression of virus-induced activity increases still more when 0.5 mg/ml of ribonuclease is used. In addition, it should be remarked that with use of the indicated doses of the preparation, no sign of toxic changes in the cell culture was observed. The data shown in Fig. 1 indicate that there is a directly proportional and approximately linear relationship between the degree of suppression of infectiousness, the hemagglutinins and the interferon, on the one hand, and the preparation dose, on the other. For a more visual comparison of hemagglutinin activity (in hemagglutinating units) and of interferon (in units suppressing the formation of plaques), activities were expressed in decimal logarithms (Table 1). The magnitudes of a particular activity, as expressed in these terms, indicate clearly enough that the process of suppression runs a parallel course in every case. The coefficient of correlation (r) also demonstrates the presence of a quite close bond between variation resulting from the action of ribonuclease on the production of the virus, hemagglutinins and interferon: titer of BSU and HAU = 0.995 ± ± 0.1; titer of BSU and BPE = 0.998 ± 0.08.

Previously, on the basis of data accumulated over the course of a year as a result of tests run under standard conditions (these did not concern the study of the antiviral activity of ribonuclease), we constructed a nomogram (Figure 2) showing the interconnection between infectious activity (in lg BSU/ml) and hemagglutinating activity (in $\log_2$ GAE/ml) of the virus. In the construction of this nomogram we made use of the standard procedure for the determination of regression. The zones set off by solid lines reflect the distribution of results for a given number of observations (n) as indicated on the graph.

TABLE 1

Comparative Data Showing the Degree of Suppression of Infectivity, Hemagglutinins and Interferon by Ribonuclease

(1)	(2)	(3)	(4)	(5)	(6)	(7)
	(9)		(10)		(11)	
(8) Контроль	9,4	—	3,07	—	1,93	—
0,25	8,5	0,9	2,35	0,72	0,92	1,01
0,5	7,8	1,6	1,54	1,53	0,36	1,57

Key: 1 - Dose of ribonuclease in accumulation medium (in mg/ml); 2 - Virus activity; 3 - Degree of suppression; 4 - Hemagglutinin activity; 5 - Degree of suppression; 6 - Interferon activity; 7 - Degree of suppression; 8 - Control; 9 - in lg BSU/ml; 10 - in HAU/ml; 11 - lg BPE_{50}/ml

Using a nomogram, we determined the titers of infectious activity of the Venezuelan equine encephalomyelitis, proceeding from the hemagglutinin titers as determined in the control and in the presence of 0.25 and 0.5 mg/ml of ribonuclease.

The data shown in Table 2 below show that the difference between the experimentally determined and the computed (by hemagglutinin activity infection titers does not extend beyond the limits of ordinary statistical straggling.

Thus, the presence of an accumulation of effective concentrations of ribonuclease within the accumulation medium leads in equal measure to a

a statistically reliable suppression of the production of both hemagglutinin and infectious virus, as well as to the formation of interferon ; here the suppression is directly proportional to the preparation dose. In other words, the determination of the degree of inhibition of any of these three indexes can serve as an objective criterion of the suppression of the other two.

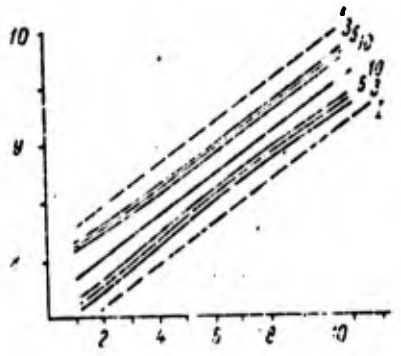

Figure 2. Nomogram showing the distribution of the infectious activity of the virus of Venezuelan equine encephalomyelitis virus (hemagglutinin titer)

On the y-axis is shown the infectious activity of the virus (in lg BOE/ml); on the x-axis, the hemagglutinin activity (in $\log_2$HAU /ml). The numbers indicate the zones of distribution of the results of a given number of observations (n).

Discussion. The results of the experimental study made show that ribonuclease in a chicken-embryo culture, without any question, suppresses the multiplication of Venezuelan equine encephalomyelitis virus in nontoxic amounts, and that such suppression is directly dependent upon the preparation dose. Prospects for the use of ribonuclease as an antiviral prepara-

tion are in fact promising, mainly because the substance is physiologically antiviral in its action. Ribonuclease in normal circumstances is present in the tissues and fluids of the organism. This, evidently, is due to its ability to readily surmount the hemato-encephalic barrier. Increase in the content of ribonuclease in the cerebrospinal fluid of ill persons through the action of encephalitis is by some observers associated with a mild course of that disease [1, 2].

TABLE 2

Computed and Experimentally Determined Suppression of Infectiousness of the Virus of Venezuelan Equine Encephalomyelitis in Chicken-Embryo Culture, in the Presence of Ribonuclease

(1) Доза рибонуклеазы в среде накопления (в мг/мл)	(2) Экспериментально определенная активность вируса ВЭЛ	(3) Расчетная исходя из активности гемагглютининов, активность вируса ВЭЛ
	lg БОЕ/мл (4)	
Контр(5)	9,4±0,03	9,5±0,3
0,25	8,5±0,05	9,0±0,3
0,5	7,8±0,16	8,6±0,9

Key: 1 - Ribonuclease dose in accumulation medium (mg/ml); 2 - Experimentally determined activity of virus; 3 - Activity of virus as computed from hemagglutinin activity; 4 - lg BSU/ml; 5 - Control

Of interest was the considerable suppression of the production of interferon within cells affected by ribonuclease which we observed. To the degree to which ribonuclease suppresses the multiplication of the virus within the cells, it blocks the production of the defensive factor, namely interferon. This fact leads directly to the conclusion of the ex-

pedience of combining the action of two physiological antiviral agents—ribonuclease and interferon. Actually, as a result of the tests which we ran, it was possible to observe that under comparable conditions the combined use of ribonuclease and interferon leads to effective suppression of the multiplication of model viruses [6, 7].

As regards the mechanism involved in the antiviral activity of ribonuclease, there are two points of view. The first of these associates this antiviral effect with the direct inactivating action of this enzyme on the infection viral nucleic acids [9, 16]. The second, by contrast, is based on the effect of ribonuclease on the biosynthetic processes of the cell. As is well known, there is a reverse suppression of protein synthesis as a result of the penetration of ribonuclease into various cells [15]. The data which we obtained may perhaps serve as indirect confirmation of this second point of view, demonstrating that in the infected chicken-embryo culture and the ribonuclease-treated cells, the production of infection virus and of virus-induced proteins (hemaglutin/in and interferon) take place in equal degree. As has already been established [3], the levels of production of the infection virus and of hemagglutin/ins and interferon in a virus-infected chicken-embryo culture to a great degree depend on the total level of viability and productivity of the cell culture.

In ribonuclease-treated cultures there is a suppression of the synthesis of cell protein [15], a reduction in the content of cell RNA, and a suppression of cell reproduction. Directly associated with this, evidently, is the reduction in the ability of cells to produce both infection virus and hemagglutin/in and interferon. It is especially important that this process

bears a reversible character: 24-48 hours following removal of the ribonuclease, the initial productivity of the cell culture is almost totally restored, which fact, the authors presume, may indicate restoral of normal or nearly normal levels of viability of the cells. However, the question of the mechanism of antiviral action of ribonuclease, has not until now been fully resolved, and requires additional experimental treatment to that end.

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ANTIVIRAL EFFECT OF RIBONUCLEASE

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A preparation of pancreatic ribonuclease was shown to inhibit accumulation and the infectious and hemagglutinating activity of Venezuelan equine encephalomyelitis virus and interferon production in the chick embryo fibroblast culture. Statistical treatment showed a high extent of correlation in the level of inhibition of all three kinds of activity. The mechanism of the virus-inhibiting effect of ribonuclease is discussed.