10th day of hepatoma development. At all times of the investigation its level in the experimental animals was significantly lower than in the control ($P < 0.001$), especially after 12 days of growth of the tumor (Fig. 2).

The results are evidence that, first, CP influences the time of appearance of AFP and its blood level in animals with hepatoma 22a and, second, that the character of the changes in AFP concentration depends on the stage of tumor development in which the cytostatic was injected. On the 4th day of the growth cycle of the hepatoma CP had the most effective action on AFP; this suggests that it is at this stage of tumor development that the population of AFP-producing cells arises. CP injected into animals at this stage of tumor development delays the formation of the AFP-synthesizing cell population.

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INHIBITORY ACTION OF A COMBINATION OF NEURAMINIDASE AND INTERFERON IN MICE WITH RAUSCHER LEUKEMIA

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There are many reports in the literature of the inhibitory effect of the enzyme neuraminidase on growth of various types of neoplasms [2, 5, 9]. The effectiveness of action of the enzyme is due to its ability to enhance the specific immune response of the host organism to tumor-bound antigens. The successful use of interferon and, in particular, highly purified and concentrated preparations of interferon, in experimental and clinical oncology has been the subject of recent reports [3, 4].

In 1976 the present writers published the results of a study of the effect of neuraminidase from *Vibrio cholerae* on development of Rauscher leukemia in mice [1]. In particular, it was shown that preliminary treatment of virus-containing spleen cells from affected mice with this enzyme had an inhibitory action on the onset of the disaese following intraperitoneal injection of these cells into healthy animals.

The object of the present investigation was to study the combined action of neuraminidase and interferon on the development of Rauscher leukemia.

EXPERIMENTAL METHOD

BALB/c mice weighing 16-20 g were used. Rauscher's leukemia virus was first passed through mice of this line by intraperitoneal inoculation of 0.1 ml of a suspension of spleen

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TABLE 1. Effect of Combined Action of Neuraminidase and Interferon on Development of Rauscher's Mouse Leukemia

Experimental condition	Number of surviving animals	Mean length of survival after infection, days
Administration of interferon before infection	0/30	26.2*
Infection with virus-containing material treated with neuraminidase	11/30	26.5*
Injection of interferon _ infection with virus-containing material treated with neuraminidase	27/30	19.5
Injection of interferon before and after infection	0/30	24.2*
Infection with virus-containing material not treated with neuraminidase (control)	0/30	17.4

Legend. Numerator — number of surviving animals; denominator — number of animals in group; *) differences significant compared with control.

cells from mice from the previous passage, affected with this type of leukemia. Development of the disease was assessed on the basis of the splenomegaly which began to appear in the infected mice on the 17th-19th day after infection. A sample of spleen from animals with leukemia also was subjected to pathomorphological investigation. The results of infection were finally assessed on the 40th day after infection. The animals were infected intraperitoneally with Rauscher's leukemia virus in a dose of 100 LD_{50} per mouse.

Neuraminidase was obtained from filtrates of cultures of unagglutinated vibrios at the Gor'kii Institute of Experimental Microbiology. The preparation contained 500 units of enzyme in 1 ml. It was diluted with calcium acetate buffer (pH 5.6) so that 1 ml contained 100 units of enzyme. The diluted enzyme was mixed with a suspension of spleen cells from mice with Rauscher's leukemia, containing $2.5 \cdot 10^7$ cells/ml. After incubation of the infected cells with neuraminidase preparations at 37°C for 45 min, the enzyme was washed off by centrifugation, and the cells treated with it were injected in a volume of 0.1 ml intraperitoneally into mice.

The interferon preparations were obtained by infecting mice weighing 18-20 g with Newcastle disease virus. The virus was injected into the caudal vein. Blood was taken from the animals 4 h later and the interferon content in the serum from it was determined. A culture of 1/929 mouse cells and 100 TCD_{50} of vesicular stomatitis virus were used for this purpose.

All the animals were divided into groups with 30 mice in each group. The mice of group 1 received a single injection of interferon in a dose of 1500 units per animal 4 h before infection with Rauscher's leukemia virus. The mice of group 2 were infected intraperitoneally with mouse spleen cells containing Rauscher's leukemia virus and treated beforehand with neuraminidase. The animals of group 3 were given a single injection of 1500 units interferon per mouse 4 h before infection with virus-containing spleen cells treated with neuraminidase. The animals of group 4 were infected with virus-containing spleen cells not treated with neuraminidase. Interferon was injected in the same doses into each animal 4 h before infection and 5 days thereafter. Animals of group 5, which were the control, were infected with only spleen cells containing Rasucher's leukemia virus, untreated by the enzyme.

The experimental results were summed up after 40 days and subjected to statistical analysis.

EXPERIMENTAL RESULTS

As Table 1 shows, administration of interferon before infection did not prevent death of the animals but lengthened their survival period compared with the control. Injection of interferon had the same effect whether given before or after infection.

Preliminary treatment of the cells with neuraminidase before infection protected 11 of the 30 mice against the disease, and in the 19 animals which died the duration of the disease after injection was lengthened. As a result of combined administration of the two preparations 27 of the 30 mice survived.

As was stated above, these investigations were a continuation of those described in [1]. In neither case did neuraminidase inhibit the development of Rauscher's leukemia in the mice when the animals were infected with virus-containing spleen cells treated beforehand with neuraminidase.

In the last series of experiments injection of interferon into the mice before or after infection did not prevent death of the animals. However, just as after preliminary treatment of the virus-containing material with neuraminidase, injection of interferon prolonged the survival of the infected animals. In this case the action of neuraminidase must be regarded as more effective, for some of the animals (11 of 30) survived.

The mechanism of the antitumor action of neuraminidase and interferon is different and has not been fully explained. According to some workers [4], it can be attributed to the ability of neuraminidase to unmask antigens on the cell surface, whereas according to others [5] it is due to the ability of the enzyme to bind with the cell surface, so that the action of neuraminidase can be regarded as that of a hapten or adjuvant.

So far as interferon is concerned, its effectiveness is most probably connected with its antiviral action [3].

The combined use of the two preparations was thus far more effective than administration of each one separately. It would seem worthwhile to undertake similar tests on models of other neoplasms. The effect of doses of these preparations and also of the periods and methods of their administration must be studied so as to determine the optimal conditions for their use. Depending on the results thus obtained, the combined use of neuraminidase and interferon under clinical conditions will perhaps be recommended.

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