alimentary route. Active synthesis of their own immunoglobulin G_2 does not take place in young rats under the age of 42-45 days. It must be noted that the microflora plays a much greater role in determining the serum IgG_2 level of both adult rats and their young than genetic predetermination.

The "standard" data obtained for the IgG_2 levels are essential as a basis for comparison in many types of immunological experiments on rats.

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INCREASED RESISTANCE OF CELLS TO VIRUS CAUSED BY mRNA FOR ANTIVIRAL PROTEIN

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The resistance of L-929 mouse cells to virus after administration of a single dose of homologous and heterologous preparations of messenger RNA for antiviral protein (AVP-mRNA) was studied. If homologous AVP-mRNA was used, inhibition of virus production reached 90-93% and remained steady after passage of the cells for 1.5 months (period of observation). After contact between the cells and heterologous AVP-mRNA inhibition of virus production in the first six passages was about 90%, increasing by the 16th passage to 99.9%. The results indicate a steady increase in the resistance of cells to virus by means of AVP-mRNA, and this could prove to be a new and effective method of nonspecific protection of cells against viruses.

KEY WORDS: **interferon; antiviral protein; mRNA; resistance to viruses.**

The writers showed previously that mouse cells can produce heterogenic chick interferon for long periods after administration of a single dose of chick interferon mRNA, which has template activity [1].

In the chain of formation and action of interferon, antiviral protein (AVP) is the final product which determines the resistance of the cells to virus [2].

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TABLE 1. Antiviral Effect of AVP-mRNA in Homologous and Heterologous Cells

Source of AVP-mRNA	Inhibition of VEE virus, log PFU/ml	
	L-929	CEF
L-929 cells	0,8—2,9[1]*	0,7—3
CEF cells	1—3,1	0,9—3,3

* Degree of inhibition of reproduction of VEE virus compared with control cells (not treated with AVP-mRNA).

TABLE 2. Inhibition of Production of VEE and VS Viruses in L-929 Cells after a Single Dose of Homologous AVP-mRNA

Type of RNA used	Test virus	Inhibition of virus products (in log. PFU/ml at undermentioned passages)			
		0*	2*	4*	6*
AVP-mRNA	VEE	1,2	1	1,2	2,2
"	VS	1	0,9	0,7	0,8
Control RNA†	VEE	—	0,1	—	0,1
Control RNA	VS	—	0,3	—	0,2

Legend. *) No. of passages, †) RNA preparation isolated from intact L-929 cells.

It was suggested that if additional genetic information for AVP can be introduced into homologous cells, the resistance of the cells to virus infection may be increased. It was found that in a series of passages reproduction of viruses can be inhibited steadily and significantly by this simple method [3].

The object of the present investigation was to determine how long the resistance of homologous and heterologous cells into which a single dose of AVP-mRNA was introduced retain their resistance to virus.

EXPERIMENTAL METHOD

Primarily trypsinized chick embryonic fibroblasts (CEF) and a primary line of mouse cells (L-929), obtained and subcultured by the usual methods, were used.

To induce and isolate AVP-mRNA, after monolayer formation the cells were treated with homologous interferon (100-200 units/ml). The culture fluid was poured off after 18 h and the cells were washed and removed from the walls of the vessel by means of a rubber spatula into STE buffer (Tris 0.01 M, NaCl 0.1 M, EDTA 0.001 M, pH 7.4). The cell residue was treated with 1% sodium dodecyl sulfate, shaken, and treated with an equal volume of a mixture of phenol and chloroform (1:1), saturated with STE and heated to 37°C. The mixture was shaken for 15 min at room temperature, then centrifuged at 2500 rpm for 20 min. The aqueous phase was treated twice more with the phenol-chloroform mixture saturated with STE. DNA was removed from the aqueous phase by the addition of 1 volume of alcohol and by winding it on to a glass rod. A second volume of alcohol with 0.1 M NaCl was then added. The sample was stored at −20°C for two days. RNA was sedimented at 20,000 rpm for 30 min and dissolved in 0.1 M NaCl.

To test the functional activity of the AVP-mRNA, cells were grown in penicillin flasks and treated with actinomycin D (2 μg/ml) 4 h before treatment with RNA. The RNA was diluted in 0.02 M phosphate buffer, pH 7.2, containing 1 M NaCl and 200 μg/ml DEAE-dextran. RNA was added to each flask in a dose of 50 μg. After adsorption for 20-60 min the RNA was removed, the cells were infected with Venezuelan equine encephalomyelitis (VEE) or vesicular stomatitis (VS) viruses with a multiplicity of 5-10 PFU/cell. After 60 min the virus was removed and the cells were washed and covered with medium 199 with 2% bovine serum. Samples were collected after incubation for 18-20 h at 37°C.

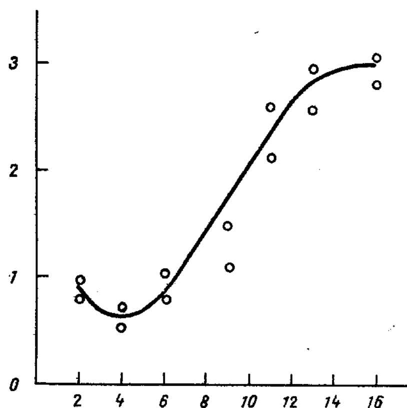

Fig. 1. Resistance of L-929 cells to VEE virus after a single dose (passage 0) of AVP-mRNA obtained from CEF cells. Abscissa, number of passages; ordinate, inhibition (in log PFU/ml) compared with control intact L-929 cells.

The titers of virus were determined by the plaque method under agar. Functional activity of the AVP-mRNA was determined from the difference between virus production in the experimental and control samples [4]. The biological activity of the AVP-mRNA preparations was tested on L-929 and CEF cells.

EXPERIMENTAL RESULTS

After addition of AVP-mRNA the level of resistance of the homologous and heterologous cells to virus varied in different experiments from 0.7 to 3.3 log units (Table 1). As these results show, no preferential inhibition by the samples was observed in homologous cells, evidence of the nonspecificity of action of the AVP-mRNA.

In a series of experiments mouse AVP-mRNA was added to homologous cells. For this purpose L-929 cells grown in 250-ml flasks were washed, after monolayer formation, with Hanks' solution. AVP-mRNA in a dose of 200-300 μg was then added to each flask in the presence of 1000-1500 μg DEAE-dextran. After contact for 30 min at room temperature the RNA was removed and the cells washed twice with Hanks' solution and covered with medium 199 and 2% bovine serum and antibiotics. Cells treated with RNA from cells untreated with interferon, and also intact L-929 cells, were used as the control. The first passage of the culture treated with AVP-mRNA and of the control cells was carried out after 24 h. Subsequent passages were carried out weekly, and some of the cells were poured into penicillin flasks in order to determine their antiviral resistance.

The results are given in Table 2, which shows that the resistance of the cells to VEE virus after addition of AVP-mRNA was 1.2 log unit and it showed little change during the next 1.5 h of culture (period of observation). The resistance of the same cells VEE virus was a little lower (0.8-1 log unit), but also remained stable in the course of passage. Control RNA preparations had virtually no inhibitory action.

In the next experiments a heterologous system was used: AVP-mRNA obtained from CEF cells induced by interferon was added to L-929 cells. Otherwise the experimental method was the same as that described above. The period of observation was increased to 4 months (16 passages). Prolonged passage of the cells in these experiments led to a gradual increase in the resistance of the cells, probably as a result of the more rapid growth of cells containing additional information from the AVP,and of the gradual displacement of the remainder of the cell population by these cells.

The results of determination of the reproduction of VEE virus in cells after different numbers of passages are shown in Fig. 1. The resistance of the cells increased gradually over the control level (intact L-929 cells) from 1 to 3 log units. In other words, inhibition of VEE virus production in these cells during the first six passages was about 90%, and it increased to 99.9% by the 16th passage. Compared with interferon, the isolation

of AVP in preparative quantities and the study of its properties are extremely difficult tasks [6, 5], and this restricts the investigation of this most important factor in antiviral immunity. Technically it seems a more promising approach to use messenger RNAs for AVP, as was done in the present investigation. The methods of induction, isolation, and testing of AVP-mRNA developed by the writers [1] enable samples with marked biological activity to be obtained comparatively simply. During translation of AVP-mRNA, the specificity of action characteristic of interferon was not observed [3].

The long-term resistance developed by the cells in response to administration of homologous and, what is particularly important, of heterologous AVP-mRNA may be a new and effective method of nonspecific protection of cells against viruses.

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PROTECTIVE ACTION OF SOME DIPHOSPHONATES AGAINST INJURY TO LYMPHOCYTES BY ANTILYMPHOCYTIC SERUM

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Rabbit antilymphocytic serum and complement were used in quantities causing death of 50% of human lymphocytes isolated in a Ficoll–Verografin (amidotrizoate) density gradient. The experimental samples (0.2 ml of a lymphocyte suspension containing $2.4 \times 10^4 - 6 \times 10^4$ cells) were treated with 10 mM solutions of diphosphonates [disodium salt of hydroxyethylenediphosphonic (HEDP) acid, alkylated HEDP-acid, aminomethyl-HEDP-acid, aminobenzyldiphosphonic and aminoisopropyldiphosphonic acids] in doses of 0.001 to 0.2 ml. A decrease in the number of dead cells was observed after staining with 0.1% trypan blue. The disodium salt of HEDP-acid and alkylated HEDP-acid proved to be most effective and exhibited protective properties in doses as low as 0.01 ml. In a dose of 0.1 ml, all the tested compounds had a marked protective action.

KEY WORDS: lymphocyte; antilymphocytic serum; diphosphonates.

Certain diseases are based on immunologic injury to the outer cell membranes, frequently accompanied by the liberation of biologically active substances of pathogenetic significance. As a rule these reactions depend on complement and require the participation of calcium and magnesium ions. Marcelli and Renoux [5], for instance, showed that the intensity of liberation of histamine from mast cells is reduced in the absence of calcium ions, which are bound by EDTA. More recently work has been published on various substances which have protective properties in relation to cell membranes. In particular, synthetic diphosphonates have been shown to possess this property. These compounds are complex ones which can interfere with calcium and magmetabolism [1-3].

The object of the present investigation was to study the protective action of a series of disphosphonates against immunologic injury to the outer cell membrane of human lymphocytes by antilymphocytic serum in the presence of complement in vitro.

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