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DIPYRIDAMOLE IS AN INTERFERON INDUCER*

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Summary. — 2,6-Bis(diethanolamino)-4,8-dipiperidinopyrimido-[5,4-d]-pyrimidine (dipyridamole) induced interferon production in vitro in explanted mouse peritoneal leukocytes and to a lower degree in non-lymphoidal cell cultures of mouse (L cells primary embryo fibroblasts) and human (diploid embryo lung fibroblasts) origin. Dipyridamole induced interferon also in mice after intravenous administration. Peak interferon levels in the blood (128 IU/ml) were attained at 49 hr after injection of 0.1 mg dipyridamole per kg body weight and at 24 and 12 hr after injection of 0.6—1.8 and 16.7 mg/kg respectively. By its pH stability, thermostability and antigenic properties the interferon induced in mice, mouse peritoneal leukocytes and L cells corresponded to IFN-α and IFN-β. This interferon-inducing capacity of dipyridamole may account for its broad-spectrum antiviral effect.

Key words: *dipyridamole*; *interferon inducer*; *interferon neutralization test*

Introduction

Tonew *et al.* (1977, 1978) and Tonew and Dzeguze (1977) described the antiviral effect of dipyridamole [2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-[5,4-d]-pyrimidine], a well known and widely applied coronary vasodilatator and antiaggregant (Persantin, Boehringer Ingelheim, FRG; Curantyl, Germed, GDR) (Simon, 1972). Dipyridamole displays a marked antiviral activity in vitro against a wide range of RNA- and DNA-containing viruses (Tonew *et al.*, 1977; Tonew and Dzeguze, 1977; Oehring and Schmidt, 1978), as well as a therapeutic effect in patients with recurrent herpes simplex (Günther *et al.*, 1977).

We tested the antiviral effect in vitro of dipyridamole against viruses belonging to seven taxonomic groups in agar diffusion plaque inhibition and one-step growth cycle experiments. Our results confirmed those of Tonew *et al.* about an effect against pseudorabies, Newcastle disease, fowl plague

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and vaccinia viruses; an antiviral effect was also demonstrable against Semliki forest virus, vesicular stomatitis virus and poliovirus type 1 (Galabov *et al.*, unpublished).

This unusually broad antiviral spectrum of dipyridamole brought us to the idea of testing the interferon-inducing activity of the substance. Such an investigation was also prompted by the fact that an analysis of the results, concerning the antiviral activity in vitro demonstrated that the effect exerted by the compound is considerably stronger when tests in cell cultures with liquid medium are used instead of plaque inhibition tests. It is known that interferon is adsorbed by the agar layer (Yershov, 1972) and that the agar-diffusion plaque-inhibition test is unsuitable for a direct quantitative assay of interferon.

We tested the interferon-inducing capacity of dipyridamole in vitro on three cellular models derived from mice — explanted mouse peritoneal leukocytes, L cells and primary cultures of mouse embryo fibroblasts, as well as in a cell culture of human origin — diploid embryo lung fibroblasts. Testing in vivo was carried out by intravenous administration of the substance in white mice.

Materials and Methods

Compounds. Dipyridamole was kindly supplied by Dr. E. Tonew, Central Institute of Microbiology and Experimental Therapy, Academy of Sciences of the GDR, Jena. From a stock solution of 100 mmol/l in ethanol, working dilutions were prepared in maintenance medium (for in vitro experiments) and in physiological saline (for in vivo testing). A preparation of double-stranded replicative form of RNA from *Escherichia coli* f2 phage (f2-RNA) (kindly supplied by Dr. J. Doskočil, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague) and poly rI. poly rC (Microbiological Associates, Bethesda, Maryland, U.S.A.) were used as reference interferon inducers.

Cell cultures. Mouse peritoneal leukocytes were prepared and cultures as described (Lackovič *et al.*, 1967; Galabov and Galabov, 1973): 2.5×10^6 cells in 1 ml (per test tube) medium 199 Difco, containing 10% heated calf serum; incubation was at 37 °C. Primary cell cultures of mouse embryo fibroblasts were grown in medium consisting of 0.25% lactalbumin hydrolysate in Hanks' saline with 15% calf serum. Human diploid embryo lung fibroblasts were prepared in the Cell Cultures Laboratory of our institute in growth medium MBE Eutroph plus 10% calf serum.

Specific anti-mouse-interferon serum globulin obtained from sheep, with a neutralization titre of 256 against 16 interferon units (IU), was a generous gift from Prof. L. Borecký and Dr. N. Fuchsberger (Institute of Virology, Slovak Academy of Sciences, Bratislava).

Interferon induction in vitro. a) In suspension cultures of mouse peritoneal leukocytes: the test compound was added immediately after seeding of the suspension of explanted cells. Sample for interferon assay were taken at the 20th hr of incubation. A control for spontaneous interferon production was run in parallel. *b)* In monolayer tube cultures of L cells, primary mouse embryo fibroblasts and human diploid lung fibroblasts. The scheme used permitted the simultaneous measurement of the interferon-inducing activity and the antiviral effect of the test substance: a 4-hr treatment with the substance tested (contained in the culture medium — medium 199 Difco with 10% heated calf serum); two washings with Hanks' saline; addition of fresh maintenance medium (without the test substance) — medium 199 with 2% heated calf serum and 24 hr incubation at 37 °C. After reading of cytotoxicity, the culture fluid was harvested for interferon assay; the cells were washed with Hanks' saline and inoculated with 100 $TCID_{50}$ of vesicular stomatitis virus (VSV), Indiana strain (multiplicity of infection-MOI = 0.001) and incubated at 37 °C. On the 48th hr after inoculation both the cytopathic effect (CPE, by the 4-cross scale) and the infectious virus yield (by the dilution end-point or the plaque method) were recorded.

Interferon induction in vivo. Dipyridamole was applied intravenously to female white mice of the randombred line H, weighing 20—25 g. Each experimental group usually comprise 40 animals, out of which 8 each were bled on the 6th, 12th, 24th, 48th and 72nd hr. Animals injected with the solvent served as a control (placebo) group. The interferon titre was determined in pooled samples of the blood sera.

Interferon assay. The plaque-inhibition method was used (except for a limited number of experiments in which the CPE inhibition method was employed) on L or human diploid cells, challenged by VSV. The interferon assay of mouse sera was carried out by a micro-variant of the plaque-inhibition method with L-cell monolayers in scintillation vials. The cells were treated with 0.8 ml of the test serum sample (in appropriate dilutions) for 18 hr at 37 °C, then washed twice with Hanks' saline and after inoculation of the challenge virus (adsorption for 60 min at 37 °C) overlaid with 1 ml agar overlay (1% Bactoagar Difco in Eagle MM Difco containing 10% heated calf serum and 1.65 mg/ml $NaHCO_3$). After 48 hr of incubation at 37° C, a second agar overlay (1.5% agar and 0.02% neutral red in physiological saline), 0.7 ml per vial, was added. The interferon titre was expressed in IU/ml, the reciprocal value of the highest dilution of the test sample, reducing twice the PFU number read in the control (untreated cells) or inhibiting the CPE in half of the tube cultures. In the titrations, reference mouse and human interferons (kindly supplied by prof. L. Borecký) were included.

Interferon identification tests. a) Contact test (proving the lack of virucidal effect); *b*) lack of antiviral specificity test; *c*) species specificity test; *d*) stability at pH 2.0 test (18 hr at 4 °C); *e*) heat stability test; *f*) ether treatment (10 min at 4 °C); *g*) trypsin treatment: 100 μg crystalline trypsin Difco (1 : 250) was added to a 1-ml sample and incubated for 1 hr at 37 °C; the enzyme reaction was stopped with 200 μg of purified soybean inhibitor (Fluka); *h*) ribonuclease treatment; and *i*) deoxyribonuclease treatment (Galabov *et al.*, 1973).

Interferon neutralization test. The method proposed by Fauconnier (personal communication, 1969) was applied, using anti-mouse interferon serum globulin, and 4, 2 and 1 IU of both test and reference mouse interferon preparations. The mixtures (0.5 ml) of interferon samples with serial 2-fold dilutions of anti-interferon serum were incubated for 60 min at 37°C and then 24 hr at 4 °C. Interferon neutralization was checked by the plaque micromethod: L cells in scintillation vials were treated with the neutralization mixtures (0.5 ml) for 18 hr at 37° C and challenged by VSV in the plaque test (70-100 PFU per vial).

Results

Interferon-inducing activity of dipyridamole in vitro

In explanted mouse peritoneal leukocytes. Under the effect of the compound added to the cell culture immediately after seeding, marked interferon production was observed after 20 hr of incubation at 37 °C (Table 1). The minimal interferon-inducing concentration (MinIFN-IC) was 3 μmol/l and the maximal interferon titre (256 IU/ml) was attained at a concentration of 100 μmol/l (optimal interferon-inducing concentration — OptIFN-IC), an effect equal to or stronger than that of f2-RNA (5 μg/ml).

In L cells. A clear-cut interferon-inducing activity of dipyridamole was observed in L-cell monolayer cultures (Table 2). The interferon production in "aging ' cultures (96 hr after seeding) was higher as compared to "young" 48-hr-cultures. In both cases MinIFN-IC was 10 μmol/l, but along with the higher interferon titres in "aging" cultures, the OptIFN-IC values were lower 30-300 μmol/l (the OptIFN-IC for the 48-hr cultures was 1000 μmol/l).

The development of virus-resistant state in these cells, tested against VSV (MOI = 0.001), as a rule corresponded to the level of interferon production. The virus growth was markedly inhibited (under the effect of $\geqq$ 10 μmol/l of

Table 1. Interferon-inducing activity of dipyridamole in mouse peritoneal leukocytes in vitro

Dipyridamole concentration μmol/l	Interferon titre (IU/ml)*	
	Exp. 1	Exp. 2
1000	ND	128
300	ND	128
100	64	≧ 256
30	16	8
10	8	8
3	< 8	8
1	< 8	< 8
0	< 8	< 8
f2-RNA (5 μg/ml)	64	128

* Determined after 20 hr of incubation at 37 °C.
ND — not done.

dipyridamole in "aging" cells), as expressed by a sharp reduction of the degree of the CPE.

A cytotoxic effect of dipyridamole (for L cells) was only established at a concentration of 1000 μmol/l after treatment lasting as long as 24 hr, the degree of cytotoxic alterations having been very mild. The data obtained, showing a reduction of both interferon production and antiviral effect at this concentration (in "aging" cells). suggest the presence of a cytotoxic effect,

Table 2. Interferon-inducing activity of dipyridamole in L cells

Dipyridamole conc. μmol/l	"Young" (48-hr) cell cultures				"Aging" (96-h) cell cultures			
	Interferon titre IU/ml	Virus-resistant state assay*			Interferon titre IU/ml	Virus-resistant state assay		
		CPE**	Infectious virus yield log $TCID_{50}$ per ml	Δ log***		CPE	Infectious virus yield log $TCID_{50}$ per ml	Δ log
1000	64	0.9	2.0	4.3	4	0.5	6.3	0.7
300	8	2.7	5.5	1.0	≧ 128	0.2	5.7	1.3
100	8	2.7	5.5	1.0	≧ 128	1.2	5.7	1.3
30	8	3.1	5.5	1.0	128	0.2	5.5	1.5
10	8	3.2	5.5	1.0	4	0.8	5.7	1.3
3	< 2	2.1	5.5	1.0	< 4	1.4	6.7	0.3
1	< 2	3.2	5.5	1.0	< 4	1.6	6.7	0.3
0	< 2	3.9	6.5	—	< 4	3.6	7.0	—
Poly rI. poly rC 10 μg/ml	16	0.1	3.5	3.0	32	0.7	ND	—

* In multicycle growth set-up (VSV MOI = 0.001): CPE and infectious virus yield were determined 48 hr after inoculation (for details see "Materials and Methods").
** Mean values in the 4-cross scale assessment of the CPE.
*** Δ log = difference between the infectious virus yields (log $TCID_{50}$/ml) in cells treated with dipyridamole or poly rI. poly rC and the control untreated cells.
ND = not done.

Table 3. Interferon-inducing activity of dipyridamole in primary cultures of mouse embryo fibroblasts

Dipyridamole concentration	Interferon titre	Virus-resistant state assay (with VSV)		
		CPE	Infectious virus yield	
μmol/l	IU/ml		log $TCID_{50}$/ml	Δ log
1000	< 4	1.1	3.3	≧ 4.2
300	16	2.9	6.0	≧ 1.5
100	4	3.0	6.0	≧ 1.5
30	< 4	2.7	≧ 6.5	≧ 1.0
10	< 4	2.4	≧ 7.5	0
3	< 4	4.0	≧ 7.5	0
1	< 4	4.0	7.0	≧ 0.5
0	< 4	3.7	≧ 7.5	—

Explanations as in Table 2.

although "hidden", even after a 4-hr treatment. No such phenomenon was observed in the 48-hr cultures. These results offer additional evidence for the major role of the interferon mechanism in the antiviral effect observed. The definitely stronger interferon-inducing effect in "aging" L-cell cultures is a well known phenomenon, observed in a number of cell cultures under the effect of various inducers (see Galabov *et al.*, 1972; Stewart, 1979).

In mouse embryo fibroblasts. Dipyridamole stimulated interferon production also in this cell culture of mouse origin although to a much lesser extent (Table 3). MinIFN-IC was 100 μmol/l and the maximal effect was only noted at 300 μmol/l (only 16 IU/ml). A virus-resistant state was also demonstrated, expressed by the inhibition of the infectious VSV yield at concentrations of the substance ≧ 100 μmol/l with no marked reduction in the viral CPE observed.

Table 4. Interferon-inducing activity of dipyridamole in human diploid embryo fibroblasts

Dipyridamole concentration μmol/l	Interferon titre IU/ml	Virus-resistant state assay (with VSV)		
		CPE	Infectious virus yield	
			PFU/ml	% inhibition
600	< 4	CT	6.9×10^3	99.90
300	32	1.7	2.8×10^6	59.42
100	16	2.4	3.5×10^6	49.28
30	4	2.9	4×10^6	42.00
10	4	2.8	4×10^6	42.00
3	< 4	3.2	4×10^6	42.00
1	< 4	3.2	4.3×10^6	37.68
0	< 4	3.2	6.9×10^6	—

Explanations as in Table 2.
CT = cytotoxic effect.

Table 5. Mouse serum interferon levolsin response to intravenous injection of dipyridamole

Dipyridamole i.v. dose mg/kg weight	Interferon titre (IU/ml)* at various intervals after administration of the drug				
	6 hr	12 hr	24 hr	48 hr	72 hr
50.0	ND	8	8 (4)	ND	ND
16.7	ND	32	16	ND	ND
5.5	ND	16	16	ND	ND
1.8	ND	16	16	ND	8
0.6	< 4	8	32	16	16
0.2	ND	ND	≦ 4	32	32
0.1	≦ 4	≦ 4	≦ 4	128	32
0.05	ND	ND	< 4	64	32
0.01	ND	ND	ND	ND	16
0 (Placebo)	< 4	< 4	< 4	< 4	4

* Interferon titres in pooled sera from groups of 8 female white mice each were determined by a micro-variant of the plaque-inhibition method.
ND = not done.

In human diploid embryo fibroblasts. "Aging" (96-hr) cultures were used for testing the dipyridamole effect. Interferon was produced but its titres did not exceed 16—32 IU/ml (Table 4). MinIFN-IC was 10μ mol/l and OptIFN-IC — 100—300 μmol/l. A twofold higher concentration (600 μmol/l) exerted a cytotoxic effect. The virus-resistant state assessed against VSV was not clearly expressed in this cell culture.

Interferon-inducing activity of dipyridamole in vivo

The compound was administered intravenously to adult white mice in single doses ranging from 0.01 to 50 mg/kg body weights, the maximal dose corresponding to 0.33 LD_{50} (150 mg/kg) as determined by Tonew *et al.*

Table 6. Some characteristics of dipyridamole-induced interferon

Treatment		Titre of interferon induced by dipyridamole in		
		L cells	mouse peritoneal leukocytes	mouse sera
Control		16	32 (16)	≧ 128
pH 2.0	18 hr	16	32	≧≧ 128
56° C	30 min	8 (4)	16	≧ 128
	60 min	4	8	8
65° C	30 min	4	8 (16)	4
75° C	30 min	4	< 4	ND
Trypsin		< 4	< 4	< 4
Ether		16 (8)	32	ND
Ribonuclease		16	32	≧≧ 128
Deoxyribonuclease		16	32	≧≧ 128

ND = not done.

Table 7. Neutralization of dipyridamole-induced mouse interferon by anti-interferon serum globulin

Interferon preparation	IU per neutralization test mixture	Dilution end-point of anti-interferon serum globulin neutralizing the interferon activity
Reference mouse interferon*	4	1 : 8192
	2	1 : 16384
	1	1 : > 65536
Dipyridamole-induced mouse interferon**	4	1 : ≧ 32
	2	1 : > 1024
	1	1 : > 1024

* NDV-induced in L cells.
** Serum samples from mice injected i.v. with dipyridamole.

(personal communication, 1978). The dose-response dependence and the kinetics of interferon production were studied in parallel.

Dipyridamole showed a marked interferon-inducing effect in mice (Table 5). This effect was strongly dose-dependent. Maximal levels of interferon in the blood (128 IU/ml) were reached at a dose of 0.1 mg/kg, but this was attained at the 48th hr, followed by a 4-fold decrease by the 72nd hr. When dipyridamole was administered in a twice lower dose (0.05 mg/kg), peak interferon levels were induced at the 48th — 72nd hr but the values observed were 4-fold lower. A dose 10-fold lower (0.01 mg/kg) induced "late" interferon production as well, but the titre was very low (8 IU/ml). With 0.2 mg/kg, a dose twice higher than the optimal one, the effect was comparable to that obtained with 0.05 mg/kg.

When dipyridamole was applied in doses ranging from 0.6-1.8 mg/kg maximal interferon levels in the blood were attained earlier — as early as at the 24th hr, with a peak value of 32 IU/ml (0.6 mg/kg). Serum samples taken earlier or later contained less interferon. Under the action of relatively high dipyridamole doses (5.5—50 mg/kg) maximal interferon titres were reached as early as at the 12th hr (32 IU/ml with 16.7 mg/kg).

Some characteristics of dipyridamole-induced interferon

By a series of interferon identification tests we established (Table 6) that interferon induced by dipyridamole in explanted mouse peritoneal

Table 8. The interferon-inducing capacity of dipyridamole in various cell cultures

Cell culture	MinIFN-IC μmol/l	OptIFN-IC μmol/l	Highest interferon titre IU/ml
Mouse peritoneal leukocytes	3	100—1000	≧ 256
L cells	10	30— 300	≧ 128
Mouse embryo fibroblasts	100	300	16
Human diploid embryo fibroblasts	10	100— 300	32

leukocytes and the found in the sera of mice (after intravenous administration of the substance) were identical, in the first place regarding their heat stability (resistant for 30 min at 56 °C, with a partial loss of activity after 60 min at 56 °C and 30 min at 65 °C).

Interferon induced in L cells, like the one in mouse peritoneal leukocytes and the mouse serum interferon was stable at pH 2.0, but differed from them by its heat lability (inactivated within 30 min at 56 °C).

In all three cases we are dealing with the "classical" type I interferon (IFN-α and IFN-β). This was confirmed by an interferon neutralization test with anti-interferon serum globulin (against mouse interferon). One and 2 IU/ml of the mouse serum interferon samples (taken 12 or 24 hr after dipyridamole administration) were neutralized by the anti-interferon serum diluted 1 : 1024, and 4 IU/ml were neutralized by dilutions of 1: $\geqq$ 32 (Table 7).

Discussion

Our results indicate that dipyridamole, the antiviral activity of which was recently discovered, is actually an interferon inducer. This representative of pyrimido [5,4-d] pyrimidines may be included into the group of low-molecular-weight interferon inducers (Mayer and Krueger, 1970; Diederick *et al.*, 1972; Gláz *et al.*, 1973; Hoffman *et al.*, 1973; Siminoff *et al.*, 1973; Soehner *et al.*, 1974; Khaitovich and Lvovsky, 1975; Kern *et al.*, 1976; Nichols *et al.*, 1976; Suzuki *et al.*, 1977; Wierenga *et al.*, 1980). Most of them induce interferon production only in vivo and in lymphoid cell cultures. E. g., acranyl dihydrochloride and mepacrine dihydrochloride applied in maximal subtoxic concentration do not stimulate interferon production in cultures of human lung fibroblasts, but in lymphoblastoid cells they induce 35 and 90 IU/ml, respectively (Dennis *et al.*, 1972). The first low-molecular-weight interferon inducer in non-lymphoid cells reported is S,2-aminoethylisothiouronium, inducing 32 IU/ml in human HSV-1 cells and minimal titres in L cells (Khaitovich and Lvovsky, 1975; Lvovsky *et al.*, 1977). Degré and Gláz (1977) found that 2,7-bis-(2-diethylaminoethoxy)-fluorene-9-one dihydrochloride (tilorone hydrochloride) can induce interferon production in cultures of human embryo lung fibroblasts (60 IU/ml at a concentration of 25 μg/ml). This compound, applied in the same concentration, induces in human lymphoblastoid cells and in normal human leukocytes 90 and 20 IU/ml, respectively.

Obviously, dipyridamole should be added to this type of low-molecular-weight interferon inducers. It induces interferon both in vivo (in mice) and in vitro in cultured mouse peritoneal leukocytes, as well as in non-lymphoid cell cultures, derived from mice (L cells and primary embryo fibroblasts) and man (diploid embryo lung fibroblasts). Out of the four types of cell cultures tested, the interferon-inducing effect of dipyridamole was most clearly expressed in the explanted mouse peritoneal leukocytes (Table 8): the MinIFN-IC in them was 3 μmol/l; in L cells and in human diploid fibroblast it was more than threefold higher (10 μmol/l); the lowest, almost insignificant activity was demonstrated in primary mouse embryo fibroblasts —

— more than 33-fold higher MinIFN-IC (100 μmol/l). A similar conclusion was reached on the basis of OptIFN-IC values and the interferon titres induced with the OptIFN-IC.

The kinetics of interferon production in mice under the effect of dipyridamole, applied intravenously, deserves particular interest in view of the somewhat unusual curve of blood interferon levels, strongly dependent on the inducer dose: an "early" peak at the 12th hr with 16.7 mg/kg, a peak at the 24th hr with 0.6-1.8 mg/kg, and at the 48th hr when 0.1 mg/kg were applied, the highest titres having been observed at this concentration (128 IU/ml).

The data on the interferon induction in mice treated with tilorone, the pyrazolo[3,4-b] quinolines BL-20803 and BL-3849A, the arylpyrimidine derivative U-25, 166 or other low-molecular-weight interferon inducers (Krueger and Mayer, 1970; Rohovsky *et al.*, 1970; Krueger *et al.*, 1971; DeClercq and Merigan, 1971; Siminoff *et al.*, 1973; Soehner *et al.*, 1974:; Nichols *et al.*, 1976; Kern *et al.*, 1976; Stringfellow, 1977) stress the strong dependence of both the size of the inducer effect and the kinetics of interferon production on the type of inducer and the way of its administration. Our results demonstrate that dipyridamole (applied intravenously) was inferior in its in vivo activity to the other inducers mentioned, but one must bear in mind that the intravenous way of administration is totally inefficient if tilorone is given to mice (Krueger *et al.*, 1971). Moreover, the effective dose of dipyridamole is considerably lower than that of other low-molecular-weight inducers — only 0.1 mg/kg, and special mention deserves the extremely high selectivity index of this substance ($\geqq 1500$).

As to the type of interferon induced by dipyridamole, based on their characteristics, including pH stability, heat stability and antigenic properties, the serum interferon in mice and the interferon produced in mouse peritoneal cells and in L cells correspond to IFN-α and IFN-β.

In conclusion, the present results suggest that the antiviral action of dipyridamole is due to its interferon-S-inducing capacity.

In connection with our study on the interferon-inducing activity of dipyridamole, a substance so far considered a virus inhibitor, we would like to stress some methodological considerations about the scheme of testing a given substance for antiviral activity. In fact, there are very few examples of low-molecular-weight interferon inducers with an in vitro effect in non-lymphoid cell cultures. Nevertheless, if in the course of a wide-spectrum screening programme for antiviral activity in vitro, a substance applied in concentrations non-toxic for the cells, demonstrates an inhibitory effect towards viruses, representatives of several viral taxonomic groups (families), it may be suggested that the substance is an interferon inducer. To avoid leaving an interferon inducer undecected it is desirable that a test for interferon-inducing capacity on a lymphoid cell culture sensitive to different types of interferon inducers, be included in the wide-spectrum antiviral screening test-system. A cell culture of this type was used in the present work, namely the explanted mouse peritoneal leukocytes in vitro.

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