ACTIVITY OF SOME ACTINOMYCETES AND HIGH PLANTS AGAINST FOOT AND MOUTH DISEASE VIRUS

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Actinomycete cultures extracts and leaves extracts of high plants were tested *in vivo* against foot and mouth disease virus. A virus strain "O" adapted to mice muscle and twenty-one-day-old 10~13 g male mice as laboratory animals were used. From the experimental results the survival index was obtained. Out of 63 assayed Actinomycete strains five were active, as regard plants 3 out of 36 were active.

Various inoculation schemes were assayed. A single dose of extracts administered 24 hours before infection produced a significant survival index from which it is possible to infer the existance of an indirect viral inhibitory mechanism. Also it was proved that there is no inhibitor that acts *in vitro* in treated animals serum. It has not yet been possible to obtain a purified active substance from the extracts and also it was observed that the extracts loose gradually their activity.

Bibliography referring to research on the inhibition of the foot and mouth disease virus by means of antibiotics or synthetic compounds is scarce. Probably, it is the first paper which dealed with the virustatic effect of chlortetracycline in guinea pigs infected with a strain of dermotrope virus.[1]

Lately[2,3], virustatic effects of ribonucleic acid and ribonuclease I on infected mice, guinea pigs and bovine were observed, and it was considered that the activity was caused by the induction of interferon. Successful trials were carried out with guanidine[4], *p*-fluorophenylalanine, thiosemicarbazone, and specially with N-methyl-isatin-β-thio-semicarbazone[5] on cellular systems infected with the virus. Although no activity could have been expected on account of their known mechanism, mitomycin C, actinomycin D and ultraviolet light inhibited the replication of the virus[6].

An inhibitor of the foot and mouth disease virus was produced in cultures of swine leukocytes following treatment with phytohemaglutinin. The biological and physical properties of this inhibitor suggest that it has many properties similar to those of an interferon[7]. The inhibition of the foot and mouth disease virus by normal bovine serum in cellular cultures[8] was also observed.

In our screening for antiviral substances we selected actinomycetes and high plants for their *in vitro* activity against foot and mouth disease virus[9,10,11]. The *in vivo* activity of these extracts and some essays that may lead to study the mechanism of action are summarized in this paper.

Materials and Methods

Virus: Foot and mouth disease virus strain "O" prepared according to previously described methods[10].

Animals: Twenty-one-day old, 10~13 g male mice of a Pirbrigh strain.

Preparation of streptomyces extracts: Cultivated in an EMERSON agar medium (1.5 %, pH 7.5) at 30°C for 8 days. The agar was sliced and macerated at 20°C in phosphate buffer at pH 7 in a proportion of 1 ml per 2.5 g of agar. Four hours later the macerated was filtered through cotton, centrifugated and then sterilized with Millipore 0.8 μ. It was kept at 4°C until use.

Preparation of plant extracts: The leaves of the plants were dried, ground, and refluxed with 50 % alcohol for half an hour. Later its was evaporated *in vacuo*, and the residues dissolved again in the phosphate buffer so that 1 ml of the extract correspond to 4 g of fresh plant material.

Tolerance test: The extracts to be tested were administered daily to lots of 10 animals at different concentrations. The highest non-toxic dosis criterion was employed. It was not necessary to dilute the extracts of streptomyces cultures when they were used immediately after preparation.

Activity test *in vivo*: In the assay, lots of 10~20 mice were used and approximately 100 $I.D._{50}$ of virus per mouse was administered intramuscularly. The extracts were injected intraperitoneally 0.5 ml per mouse: one 24 hours before infection, one at the time of the infection and one daily for 9 days (Tables 1 and 2). This scheme was used to select the active extracts and to adjust time and dose, and to determine the dynamics of the process. Observations lasted for 9 days and the mortality was registered daily.

Survival index: The survival index was obtained from experimental results. Survival index is the quotient of the average life of treated animals over those used as controls. The average life is the antilog of the average of the live logs of each mouse[11]. By means of statistical calculations it was possible to establish, under our experimental conditions, survival indexes for a significant level of $P<0.05$ are: 1.43 for a 9-mice group, 1.41 for a 10-mice group, 1.35 for a 13-mice group, 1.33 for a 15-mice group, 1.27 for a 20-mice group and 1.23 for a 25-mice group. The survival index 1 indicates no activity, while that below 1 indicates toxic action.

In vitro test of antiviral activity of blood serum from mice treated with streptomyces extracts: Lots of mice were inoculated intraperitoneally with 0.5 ml of the extract, and bled at different periods from $5^1/_2$ to 24 hours. Serum was separated, diluted 1:3 or 1:5 with physiological saline solution. Then the serum dilution was mixed with a foot and mouth disease virus suspension to obtain the final virus concentrations of 10~100 $I.D._{50}$. After its incubation at 37°C for one hour, lots of mice were inoculated with the mixture. Normal serum of untreated mice was used as control.

Table 1. *In vivo* anti-foot and mouth disease activity of actinomycete cultures.

Actinomycete strains	Number of mice	Survival index
No. 25,554	13	1.97*
No. 28,573	20	1.74*
No. 21,160	10	1.49*
No. 13,804	13	1.42*
No. 19,749	10	1.60*

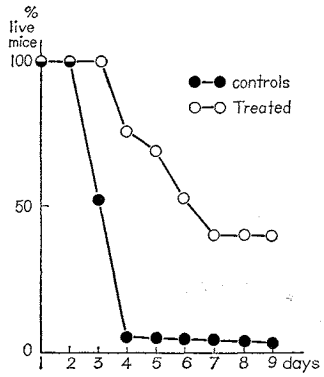
The injection scheme: One dose 24 hours before infection, one at the time of infection and one daily for 9 days.
* Indicates a significant survival index ($P<0.05$).

Table 2. *In vivo* anti-foot and mouth disease activity of plant extracts.

Plants	Number of mice	Dosis (mg/mouse)	Survival index
Eringium eburneun	10	2.50	1.51*
Aechema disticantha	10	0.75	1.43*
Dunalia breviflora	20	5.00	1.62*

The injection scheme: One dose 24 hours before infection, one at time of infection and one daily for 9 days.
* Indicates a significant survival index ($P<0.05$).

Fig. 1. Response of mice to the treatment with an extract of *Streptomyces* No. 25,554. Administered 24 hours before the virus, once at the time of inoculation and daily thereafter.



The mortality was registered daily for 9 days to determine the survival index.

Results

From 63 actinomycetes strains tested, five were active *in vivo*; as regards plants, 3 of 36 were active. Some of the experimental data are indicated in Tables 1 and 2. The difference between the survival curve of treated mice and control are shown in Fig. 1.

The number of dosis and times of injection of the extracts were modified through different schemes and are presented in Table 3. Table 3 proves that single dose of extract administered 24 hours before infection produced a significant survival index, thus allowing to expect that an indirect viral inhibitory mechanism exist and suggesting it might be an inhibitor induction process as the case of helenine and statolon[12,13,14,15].

Table 3. *In vivo* anti-foot and mouth disease activity of actinomycetes and plants using different injection schemes.

Actinomycete strains and plants	Number of mice	Injection scheme	Survival index
No. 25, 554	15	d	1.51*
No. 25, 554	20	c	1.30*
No. 25, 554	20	c	1.32*
No. 21, 160	10	b	1.54*
No. 13, 804	20	c	1.49*
No. 28, 573	15	g	1.10
No. 25, 554	20	h	1.19
Eryngium eburneun	10	f	1.67*
Eryngium eburneun	10	e	1.66*

b) 5 hours before infection and daily thereafter
c) 24 hours before infection
d) 48 hours and 24 hours before infection
e) 24 hours before infection and at the moment of infection
f) 48 hours and 24 hours before infection and at the moment of infection
g) At the time of infection and subsequent days
h) 48 hours before infection
* Indicates a significant survival index ($P<0.05$).

Table 4. Connection between activity and age of an extract from Actinomycete strain No. 25554.

Days after extract preparation	Number of mice	Survival index
4	13	1.97*
20	12	1.49*
30	10	1.08

* Indicates a significant survival index ($P<0.05$).

Table 5. *In vitro* neutralization test of foot and mouth disease virus of mice treated with the extract of streptomyces No. 25, 554.

	Hours after treatment with the extract			Control
	6.30	17	24	
Average life	2.61	2.45	2.71	2.29
Survival index	1.13 P=0.4	1.07 P=0.4	1.18 P=0.2	

Serum is diluted with physiological saline solution (1 : 3).

When the extracts are administered at the time of infection and each subsequent day, or a single dose is administered 48 hours before infection, no significant survival index is obtained.

We have observed that samples gradually loose their activity even if they are kept at 4°C (Table 4), and in some cases their toxicity increases.

To determine if the serum of treated animals contains an inhibitor of the virus, the serum was collected at different times after the treatment and mixed *in vitro*

with foot and mouth disease virus suspension. Table 5 shows that there is no difference between the serum of treated animals and the controls, in other words, there is no inhibitor that would act *in vitro* which on the other hand agrees with the observations of RYTEL *et al.*[13] with respect to inhibitor induced by helenine.

Up to this moment all endeavours to isolate one or several active substances have not been successful. When attempts to fraction the active extracts were made, they lost their activity. However, the active extracts do not lost their activity by heating at 100°C for 1 hour. Possibly it is not a single active substance but a more complex phenomenon which its necessary to clarify.

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