

β -sitosterol and lupeol and also for the elemental analysis and spectral data.

Regional Research M. N. S. NAYAR.
Laboratory (Branch), M. K. BHAN.
Sanat Nagar, Srinagar-5, V. GEORGE.
Jammu & Kashmir, (India),
May 24, 1971.

* 60 m c. spectrum.

1. *The Wealth of India* (Ed. B. N. Sastri, 1959, 5, 40).
2. *Phytochemistry* (In press).

A FUNGAL FILTRATE WITH PRONOUNCED INHIBITORY ACTION AGAINST SEMLIKI FOREST VIRUS (SFV) IN MICE*

ABSTRACT

Aspergillus flavus Link, strain 6-MFA, produced antiSemliki Forest Virus substance in stationary culture. 87% of the infected treated mice were protected when 100% of the control mice died due to infection. Major activity was confined to acetone precipitated fraction. Under similar conditions of test acetone fraction (helenine) from *Penicillium funiculosum* NRRL 2075 (standard) could induce only 34% protection. The acetone fraction of 6-MFA filtrate was more effective in protecting mice challenged by subcutaneous route than the intracerebral route. Strains of *Aspergillus flavus* varied in antiviral activity.

We have observed striking virus inhibitory action of a mould filtrate, designated 6-MFA, obtained from a laboratory strain of *Aspergillus flavus* Link, grown in Shope's medium¹ and tested in 35 days old Swiss mice infected with Semliki Forest Virus (original strain, Smithburn and Haddow, ATCC).

It was found that with a dose of virus that produced 57% mortality in the control, the crude filtrate (mycelial mat extracted with culture filtrate) was able to protect treated mice to the extent of 80% (Table I). Similar filtrate obtained from *Penicillium funiculosum* NRRL 2075¹ contained much less antiviral activity and under similar conditions *Penicillium chrysogenum*, strain NRRL 1951, B 25² and NRRL 1951² induced only 70% and 30% protection respectively (Table I).

The acetone precipitated fraction of 6-MFA filtrate prepared according to Shope^{1,3,4} exhibited higher antiviral activity. Mice were injected intraperitoneally with freshly prepared acetone fraction 24 hours in advance of subcutaneous challenge by dilution of 10% SFV infected mouse brain homogenate (Table II). Following such a prophylactic injection

mice were examined daily, morning and evening, for specific symptoms and death due to paralysis for a period of 14 days. In this manner it was observed that about 87% of mice were protected. The control mice succumbed completely to the same virus dilution within an 8-day period. When activity of the acetone fraction of 6-MFA was compared with activity of similar fraction from *Penicillium funiculosum*, NRRL 2075, it was found that the latter could induce only 34% protection (Table II).

We have further observed that the administration of the acetone fraction 24 hours before SFV given directly into mouse brain does not lead to similar resistance in mice, but the onset time of the disease is slightly prolonged (Table III). The two experiments (Tables II and III) show that the virus inhibitory effect of the acetone extract of 6-MFA filtrate is influenced by the route of virus challenge. Isolation, characterization and mode of action of the antiviral agent and method of its fermentation will be the subject-matter of a separate communication.

A finding of some interest is that even the closely related strains of *Aspergillus flavus* vary markedly in the production of anti-Semliki Forest Virus substance (s).

Powell et al.⁵ found that a filtrate designated M5-8450 obtained from fungus *Penicillium stoloniferum* protected 100% of SFV infected and treated mice under conditions when 88% of the untreated mice died due to virus. Shope reported that *Penicillium funiculosum*, NRRL 2075 produced antiviral substance (named helenine) against Col. S. K. and Swine influenza virus¹ during growth in liquid culture that could be extracted with acetone, and observed that the extract protected 77% of the mice challenged with SFV³, and later⁴ achieved nearly 100% protection using a product obtained from culture No. BC. 17-5 by effecting certain improvements in the technique of extraction. Banks et al.² reported the presence of virus-like particles in penicillin producing strains NRRL 1951 and NRRL 1951 B.25 of *Penicillium chrysogenum*, and found that both the virus particles and the ds-RNA derived from these viruses were active inducers of interferon in animals⁶. Our work incidentally confirms the unpublished results of Banks et al.⁶ that virus containing and penicillin producing mould *Penicillium chrysogenum*, strains NRRL 1951 and NRRL 1951 B. 25, both induce production of antiviral substances. We have shown that the antiviral activity of growth

TABLE I

Mice treated with filtrates of fungi given i/p and challenged s/c by SFV

Treatments (crude filtrate)	Medication	Challenge virus	Proportion of mice survived	Survival (%)
<i>Aspergillus flavus</i> , strain 6-MFA	.. 0.5 ml × 5 2 doses given 12 hourly one day ahead and 3 after virus 24 hourly	0.5 ml of 10 ⁻²	12/15	80
<i>Penicillium funiculosum</i> , NRRL 2075	8/14	57
<i>Penicillium chrysogenum</i> , NRRL 1951. B 25	14/20	70
<i>Penicillium chrysogenum</i> , NRRL 1951	6/20	30
Control	.. No treatment	..	6/14	43

TABLE II

Mice treated i/p with acetone fraction of *A. flavus* compared with standard
P. funiculosum, NRRL 2075 challenged with SFV given s/c

Fungi	Medication	Challenge virus	Surviving mice	Survival (%)
<i>A. flavus</i> 6-MFA	.. 2.00 ml × 1 i/p, 24 hrs before virus	0.5 ml of 10 ⁻¹	14/16	87
<i>P. funiculosum</i> NRRL 2075	6/18	34
Control (buffer saline)	0/18	0

TABLE III

Mice treated i/p with acetone fraction of *A. flavus* compared with standard
P. funiculosum, NRRL 2075 challenged with SFV given i/c

Fungi	Medication	Challenge virus	Mice surviving	Survival (%)
<i>A. flavus</i> , 6-MFA	.. 2.00 ml × 1 i/p	0.03 ml of 10 ⁻¹	0/10*	0
<i>P. funiculosum</i> NRRL 2075	0/10	0
Control (buffer saline)	0/10	0

* Symptom onset time prolonged in the case of mice treated with acetone fraction of *A. flavus*.

products of these two moulds extends to SFV infection in Swiss mice which are protected prophylactically to a fairly large degree.

We thank Dr. M. L. Dhar and Dr. A. B. Kar, Director and Deputy Director of the Central Drug Research Institute, for their interest in this project, and Dr. K. G. Mukherjee and Dr. B. M. Johri of Delhi University, for the supply of original cultures of fungi from their collection.

Central Drug Research Institute,
Chattar Manzil Palace,
Lucknow, July 8, 1971.

K. CHANDRA:

B. M. GUPTA.

R. K. MAHESHWARI.*

3. Shope, R. E., *J. Exp. Med.*, 1953, 97, 627.4. —, *Ibid.*, 1966, 123, 213.5. Powell, H. M., Culbertson, C. G., McGuire, J. M., Hoehn, M. M. and Baker, L. A., *Antibiot. Chemother.*, 1952, 2, 432.6. Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, Joan, E., Himmelweit, F., Ratti, G., Sharpe, T. J. and Planterose, D. N., *Nature*, 1970, 227, 505.

PALAEOMAGNETISM AND THE CONTINENTAL DRIFT

RESULTS of palaeomagnetic studies on Indian rocks by Deutsch *et al.* (1959), Athavale *et al.* (1963), Prasad (1966), etc., have given significant support to the hypothesis of Continental drift.

During the course of investigation of the pre-Cambrian dolerites of the Tirupati area, oriented samples were collected from 25 dykes for palaeomagnetic work. The natural remanent magnetic directions and the intensities of the dykes were measured with suitably sensitive astatic magnetometers. Stability

* Communication No* 1659

** Junior Research Fellow, C.S.I.R., India.

1. Shope, R. E., *J. Exp. Med.*, 1953, 97, 609.2. Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E. and Himmelweit, F., *Nature*, 1969, 222, 89.