

These may be used in preparing standard thorium solutions. Directions for use will be furnished with the standards.

(3) *Standard Rock Samples:*

The following rocks, ground to pass 40-mesh screen and be retained on 100-mesh screen, are available in 100-gram samples.

Quartzite (Virginia)
Triassic diabase (Virginia)
Milford granite (Massachusetts)
Chelmsford granite (Massachusetts)
Gabbro-diorite (Massachusetts)
Columbia River Basalt (Idaho)
Berea sandstone (Ohio)
Dunite (North Carolina)
Carthage granite (Missouri)
Carthage limestone (Missouri)
Deccan Trap (India)
Kimberlite (South Africa)

These samples of rock will be analyzed for radium and thorium content and are intended for use as working standards to check methods used in extraction of radon and thoron from rock samples. They may be used for direct fusion in the electric furnace or for carbonate fusion.

All the above samples will be analyzed at a number of laboratories equipped to make such measurements and ultimately certificates will be issued by the National Bureau of Standards. This work is in progress but will require considerable time for its completion so that final figures are available only for a part of the samples at the present time.

Accurate knowledge of the radioactive content of the materials of the earth's crust is of primary importance in many phases of geology, geophysics and cosmology.

Reliable radioactive standards are also essential in studies of radium and thorium poisoning and in biological and medical investigations using the technique of radioactive indicators or internal artificial radioactivity therapy. For the latter purposes calibrated standard sources of β -rays will be made available.

It is hoped that the standards which have been prepared by the committee will provide all workers in these fields with a common basis for comparison of measurements and also improve the accuracy of all measurements of this type. It is likely that they will have other applications, and the committee would appreciate hearing from interested persons who may desire similar standards for their work. The committee is also glad to cooperate as far as possible in aiding investigators to use these standards to the best advantage and welcomes specific inquiries regarding their use. It is urged that any suggestions regarding other desirable radioactive standards, not at present available, be submitted promptly to the committee. In particular, it will facilitate the work of the committee if those laboratories and individuals which can make use of these standards advise the committee of their probable requirements.

Communications may be addressed to the chairman, Professor Robley D. Evans, Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts.

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SPECIAL ARTICLES

REVERSIBLE INHIBITION OF TOBACCO MOSAIC VIRUS IN LIVING CELLS WITH 0.0002 MOLAR SODIUM CYANIDE

Most studies on inactivation of plant viruses have been carried out *in vitro*.^{1,2,3,4} Such data have furnished valuable information as to reactive groups in virus molecules. Little is known, however, of the possibilities of reversible inactivation of virus mechanisms in living cells. Such information should aid in elucidating the substrates and reactions involved in virus multiplication.

Woods⁵ was the first to call attention to the abnormal

¹ W. M. Stanley, *Phytopath.*, 25: 899-921, 1935.

² R. Best, *Annals of Appl. Biol.*, 23: 759-780, 1936.

³ A. F. Ross and W. M. Stanley, *Proc. Soc. Expt. Biol. and Med.* (N. Y.), 38: 260-263, 1938.

⁴ W. B. Allington, *Phytopath.*, 28: 902-918, 1938.

⁵ A. F. Woods, *Centralbl. Bakt. u. Par.*, 5: 745-754, 1899.

oxidase and peroxidase content of virus-infected tissues. A further investigation of the relation between certain oxidation catalysts and the virus mechanism has led in part to the work reported here. Detailed studies have shown that protoplasmic streaming in leaf cells of tobacco is *oxygen sensitive*. In a given leaf the rate of streaming can be reversibly and characteristically inhibited by sodium cyanide. A 0.0002 M solution of sodium cyanide is as effective in inhibiting the rate of protoplasmic streaming as is a 0.02 M concentration. These concentrations of cyanide also inhibit the action of tobacco peroxidase and catalase. The inhibiting effect of cyanide on the hemin-containing respiratory catalysts is well known.⁶

It was found that tobacco leaf tissue could be kept alive over 36 hours in 0.0002 M sodium cyanide by

⁶ C. Oppenheimer and K. G. Stern, "Biological Oxidation," The Hague, 1939.

TABLE 1
INFLUENCE OF 0.0002 M SODIUM CYANIDE ON MULTIPLICATION OF TOBACCO MOSAIC VIRUS IN DETACHED HALF LEAVES OF
Nicotiana tabacum

| Experiment | Hours between which cyanide treatments were made (Time of inoculation = 0) | Hours of virus test | Amount ^a of virus CN-treated: control | Per cent. total time in NaCN | Per cent. reduction in virus concentration due to NaCN treatment |
|------------|---|---------------------|--|------------------------------|--|
| 1 | $\frac{1}{2}$ to 15 $\frac{1}{2}$; 24 to 40; 51 to 64 | 66 | 25/93 | 66.6 | 73.2 |
| 2 | 10 $\frac{1}{2}$ to 21 $\frac{1}{2}$; 36 to 46; 59 $\frac{1}{2}$ to 70 $\frac{1}{2}$ | 76 $\frac{1}{2}$ | 762/1263 | 41.8 | 39.7 |
| 3 | 48 to 72 (sample A) | 74 $\frac{1}{2}$ | 535/856 | 32.2 | 37.5 |
| | (sample B) | 97 | 817/885 | 24.7 | 7.7 |

^a Concentration expressed as number of necrotic lesions developed on opposite halves of *N. glutinosa* (or hybrid) leaves.

maintaining a constant flow of solution. When the cyanide-treated tissue was washed with running tap water for several hours complete recovery in the rate of protoplasmic streaming usually resulted. By alternately treating with cyanide and dialyzing with water, tobacco leaves were kept alive for periods of several days. With this information as a background whole leaves were inoculated with a single-lesion strain of severe mottling tobacco mosaic virus, rinsed with tap water and split lengthwise into two halves through the mid-rib. One half of each leaf was immersed in a 0.0002 M sodium cyanide solution and the other half, the control, was immersed at the same time in tap water. A slow flow of solutions was maintained from reservoirs at room temperature, average approximately 25° C. Freedom from death of the cells due to cyanide was determined by the macroscopic and microscopic appearance of the treated tissues. In all the experiments reported here protoplasmic streaming was still active in the leaf cells at the end of the tests. Retardation of virus multiplication can not therefore be attributed to death of cells during treatment with cyanide. All samples were dialyzed with water for several hours to free them of traces of cyanide before inoculation tests. Some of the results obtained are summarized in Table 1.

The data of Table 1 show that 0.0002 M sodium cyanide retards the multiplication of tobacco mosaic

ments showed that virus multiplication had been resumed. The virus had almost reached the level of concentration in the controls which were apparently nearing the maximum.

Experiments with detached leaves of an F₂ necrotizing hybrid (*Nicotiana tabacum* × *N. glutinosa*) illustrated the reversible inhibition of virus in a very striking manner. Actual virus concentrations were not measured, but the time of appearance of the local necrotic lesions was recorded. Necrotic spots usually appear in detached leaves of this hybrid held in air for 60 to 75 hours after inoculation. Lesions develop in leaves immersed in oxygenated water in about the same time. The data in Table 2 show that 53 hours' treatment with 0.0002 M sodium cyanide delayed appearance of lesions 45 hours.

Seventy and one-half hours after inoculation, when the control halves were heavily spotted with lesions 2 to 3 millimeters in diameter, the cyanide-treated tissues showed no symptoms of virus infection. Lesions finally appeared after cyanide treatment had been stopped for 50 hours.

With respect to ability to multiply in living cells, tobacco mosaic virus responds to 0.0002 M sodium cyanide in much the same way that certain hemin-containing catalysts do. This indicates that the virus mechanism either depends on the activity of hemin-containing respiratory catalysts of the cell or the

TABLE 2
EFFECT OF 0.0002 M NaCN ON TIME OF APPEARANCE OF NECROTIC LESIONS OF TOBACCO MOSAIC VIRUS IN DETACHED LEAF TISSUE

| Hours between which cyanide treatments were made (Time of inoculation = 0) | Hours after inoculation | Number necrotic lesions in control tissue | Number necrotic lesions in cyanide-treated tissue |
|---|-------------------------|---|---|
| 2 to 18 $\frac{1}{2}$; 25 to 40 $\frac{1}{2}$; 44 $\frac{1}{2}$ to 65 $\frac{1}{2}$ | 70 $\frac{1}{2}$ | approx. 250 | none |
| Treatment with water after 65 $\frac{1}{2}$ hours | 97 $\frac{1}{2}$ | approx. 250 | none |
| | 115 $\frac{1}{2}$ | approx. 250 | approx. 250 |

protein. In the third experiment measurements of virus concentration made two and one-half hours from the last cyanide treatment indicated a strong inhibition in virus multiplication. A second series of measurements made 25 hours after cessation of cyanide treat-

ment showed that virus multiplication had been resumed. The virus had almost reached the level of concentration in the controls which were apparently nearing the maximum.

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