SV3T3 supernatants and was not due to glycerol leached from the membrane filters (*13*), which were exhaustively washed prior to use. However, its presence has so far prevented precise quantitation of MIF-like activity in chromatographed SV3T3 supernatants and determination of whether highly concentrated 3T3 supernatants also possess MIF-like activity.

Productive virus infection of fibroblast cultures has previously been shown to stimulate the elaboration of lymphokine-like substances (*14*). The results reported here with 3T3 and SV3T3 fibroblasts provide the first evidence that cells may be induced to elaborate an MIF-like activity as a consequence of transformation with an oncogenic virus and associate such MIF-like activity with the absence of a newly described form of dense-staining cell coat material. Guinea pig macrophages either treated with SV3T3 MIF-like activity or cultured with lymphocytes generating MIF experience a similar loss of stainable cell coat material and undergo significant changes in their migratory properties and intercellular associations. These findings raise the possibility that the MIF-like activity secreted by SV3T3-transformed fibroblasts may also play a role in the striking alterations in cell contact behavior which accompany viral transformation.

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6. The 3T3 cells used in these experiments were well contact-inhibited since their saturation densities in 10 percent FBS were 0.7×10^5 cells per square centimeter (Swiss) and 1.0×10^5 cells per square centimeter (BALB/c), and less than 0.5 percent of confluent BALB/c 3T3 cells incorporated [^{3}H]thymidine by autoradiography after a 4-hour labeling period. All cell lines were checked periodically for contamination with *Mycoplasma* by autoradiographic (*9*) and culture techniques (laboratories of L. Hayflick and L. Dienes) and found to be negative.
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15. We acknowledge the technical assistance of Nancy Gelb, Beverly Ash, Anne Brecia, and Ellen Morgan. This work was supported by PHS contract NCI NO1-CB-44005, PHS grants CA-10126-07 and CA-15136, and by American Cancer Society grant VC-31. M.E.H. is a research scholar of the American Cancer Society (Massachusetts Division); R.O.R. is a faculty research associate of the American Cancer Society (PRA-75); and H.F.D. is research career development awardee 1-KO4-AI-46,352, PHS.

10 May 1974

Barley Stripe Mosaic Virions Associated with Spindle Microtubules

Abstract. *Virions of barley stripe mosaic virus were observed attached to microtubules of the spindle apparatus. This phenomenon was found in barley cells undergoing meiosis and mitosis. The microtubules may be involved in the assembly and cell-to-cell transfer of the virus.*

Examination of ultrathin sections of various cell types of barley (*Hordeum vulgare* L., 'Atlas') which were infected with MI-1 strain of barley stripe mosaic virus (BSMV) revealed an extraordinary relationship between some virions and microtubules of spindles. In microspore mother cells undergoing meiosis (Fig. 1), and in immature cells of the ovule which were undergoing mitosis (Fig. 2), these virions appear to be attached by one end to spindle microtubules. We observed the same phenomenon in rapidly dividing root tip cells and in nondividing cells of other barley tissues (*1*). The number of virions was fewer in root tip cells and cells dividing meiotically than in dividing microspore mother and ovule cells; however, the large number of ribosomes may obscure the actual number of virions that are present. All tissues were prepared for electron microscopy according to the procedure of Mayhew and Carroll (*1*).

The rod-shaped structures in infected tissues were identified as virions of BSMV on the basis of their general morphology and staining characteristics (*2*). The rod-shaped particles were not seen in healthy tissues. The spindle microtubules of dividing barley cells were similar in structure and distribution to those observed in dividing cells of other virus-free plant species (*3*).

The association of viruses with microtubules has been reported for an adenovirus, AV5, infecting HeLa cells

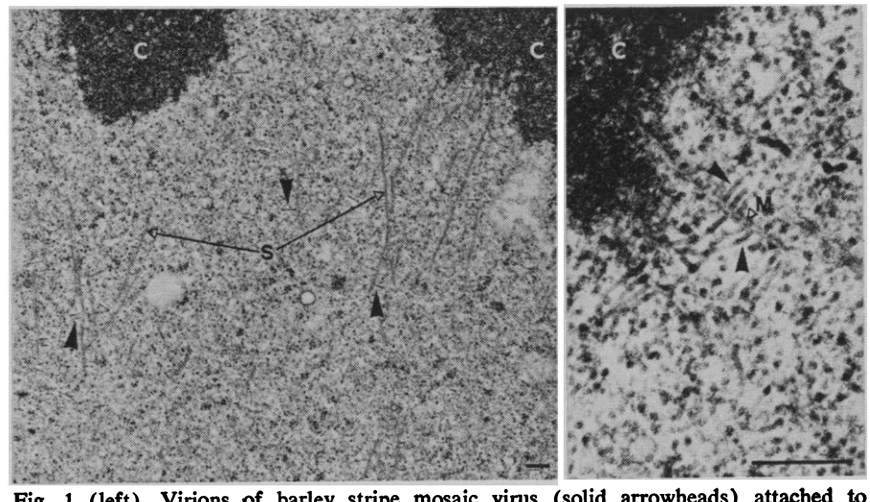

Fig. 1 (left). Virions of barley stripe mosaic virus (solid arrowheads) attached to microtubules of the spindle (*S*). Chromosomes (*C*) are also shown. Scale bar, 0.2 μm. Fig. 2 (right). Virions of barely stripe mosaic virus (solid arrowheads) attached by their ends to a spindle microtubule (*M*) which is connected to a chromosome (*C*). Scale bar, 0.2 μm.

(*4*). Dales and Chardonnet (*4*) suggest that the virions are moved vectorially from the plasma membrane to the nuclear envelope along pathways furnished by the microtubules. The virus subsequently begins replicating in the nucleus. Esau and Gill (*5*), studying dividing cells of tobacco leaves infected with tobacco mosaic virus (TMV), did not observe close association of TMV with spindle microtubules, even though the virus particles were found distributed among the chromosomes and spindle. To the best of our knowledge, there have been no other reports in the literature describing the close relationship of plant viruses and spindle microtubules. Because there is no convincing evidence that the MI-1 strain of BSMV replicates in the nucleus, and since the virions appear to be attached to the spindle microtubules, we suggest that, in the case of this strain of BSMV, the microtubules may be involved in virus assembly or transport of virions from mother to daughter cells during cell division, or both.

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3 May 1974

Mitogenic Activity of Sterculic Acid, a Cyclopropenoid Fatty Acid

Abstract. *Hepatocytes in rainbow trout and rat are stimulated to augmented DNA synthesis and cell division by low concentrations of cyclopropenoid fatty acids in the diet. Sterculic acid isolated as the methyl ester from* Sterculia foetida *oil has been identified as one of the mitogenic principles.*

Cyclopropenoid fatty acids (CPFA's), constituents of plants of the order Malvales, which includes several important sources of food for man and animals, have long been known to exert toxic (*1*) and other adverse biological effects (*2*, *3*) in a variety of animals. Two such fatty acids have been identified and have the following structures

Sterculic acid: $CH_3(CH_2)_7C{=}C(CH_2)_7COOH$ (cyclopropene ring)

Malvalic acid: $CH_3(CH_2)_7C{=}C(CH_2)_6COOH$ (cyclopropene ring)

In farm animals, especially poultry, these effects have been largely obviated by decreasing the content of the residual oil in cottonseed meal, which is used as a major protein supplement in the diet. Although it has been established that CPFA's are incorporated into animal fat and thus carried in the food chain, it is generally considered that the concentrations of these compounds currently present in foodstuffs are well below the threshold dose for deleterious effects (*1*). Several years ago, interest in these compounds was renewed when it was shown that at very low doses CPFA's markedly potentiate the carcinogenic effect of aflatoxin B_1 in liver of rainbow trout *Salmo gairdnerii* (*4*, *5*). The study reported here establishes a hitherto unknown effect of CPFA's; their ability to induce hepatocytes of trout and rat to undergo cell division in vivo.

Cyclopropenoid fatty acids in *Sterculia foetida* oil were incorporated into a synthetic diet (*6*) at a dose level of 200 parts per million (ppm) and fed freely to fingerling rainbow trout; control fish were fed the synthetic diet alone. Fish were killed after 4 weeks of feeding. Male Sprague-Dawley rats (50 g) were fed Purina rat chow containing CPFA's (500 ppm); control animals were pair fed and received Purina rat chow alone. One hour before the rats were killed they were injected intraperitoneally with [^{3}H]thymidine (Schwarz/Mann, specific activity 10.0 c/mmole) at a dose of 1 μc per gram of body weight. Rats were killed after 2 and 4 weeks of feeding. Liver weights and liver DNA were determined for all animals. Approximately 4 g of liver was homogenized in cold, normal saline with a Potter-Elvehjem homogenizer to make a 20 percent homogenate (weight to volume). The DNA was extracted by a modification of the Schmidt-Thannhauser-Schneider method (*7*) and separated from RNA by differential acid solubility (*8*). The DNA content was determined colorimetrically (*9*). Specific activity of [^{3}H]thymidine incorporated into DNA was measured with a Packard Tri-Carb liquid scintillation spectrometer by use of a mixture of 2,5-bis(5′-*tert*-butylbenzoxazoyl)-2′-thiophene (BBOT, Packard Instrument Company, La Grange, Illinois) and toluene. The internal standard, [^{3}H]-toluene (Packard Instrument Company), had a counting rate of 2.26×10^6 disintegrations per minute (dpm) per milliliter. After percentage efficiency was determined, the counting rates of the samples and values of the specific activity of DNA were calculated. Thin slices of liver were fixed in calcium formalin, embedded in paraffin, and prepared for light microscopy, and mitoses in hepatocytes were counted.

The livers of all animals fed CPFA's were large and moderately fatty; liver weight, content of hepatic DNA, mitotic index, and incorporation of [^{3}H]thymidine into nuclear DNA of hepatocytes were increased significantly above those of control animals, as shown in Table 1. These findings indicate that the hepatomegaly was accompanied by augmented DNA synthesis and number of hepatocytes. Histologic and cytologic alterations were evident in hepatocytes after 2 weeks of CPFA feeding, and were strikingly similar in both species studied. The cytoplasm had a foamy appearance and contained numerous fat droplets. The nuclei were large and hyperchromatic and contained enlarged nucleoli, some of which were multiple and occupied much of the nuclear volume. Mitoses were frequent (Fig. 1) and all stages were present; although most of these appeared to be normal, there were occasional abnormal figures characterized by fragmented and lagging chromosomes. Examples of isolated hepatic cell necrosis, which were rare (0.4 percent) at 2 weeks, were more numerous (0.7 percent) at 4 weeks.

In an attempt to establish whether sterculic acid, the CPFA compound present in highest concentration in *S. foetida* oil, might be capable of stimu-