

came apparent that reoviruses and wound-tumor virus might belong to the same group, provided they are serologically related.

Serologic tests were facilitated by the availability of wound-tumor antiserum (9) prepared against highly purified wound-tumor virus extracts of plant tumors. The reovirus titers were $10^{-5.5}$ tissue culture infective dose (50/ml) for each strain (10). Because there is a common complement-fixing antigen in the three reoviruses (1), it was decided to use complement fixation for the tests. The reovirus antigens were the respective three strains of virus, grown in monkey kidney cells with Earle's basic salt solution, 10 percent lactalbumin hydrolyzate, and fetal calf serum, diluted 1 to 4 in veronal buffer at pH 7.2.

For preliminary experiments, a constant concentration of antigen and antiserum was used with complement at various dilutions. The results indicated that more complement was used with reovirus I, II, and III and wound-tumor antiserum than in the controls with either normal antigen plus wound-tumor antiserum, or reoviruses I, II, and III and normal serum. The normal serum was obtained from untreated healthy rabbits. The normal antigen consisted of monkey kidney cells in Earle's basic salt solution, 10 percent lactalbumin hydrolyzate, and fetal calf serum, diluted 1 to 4 in veronal buffer at pH 7.2.

The positive results of preliminary tests were followed by a quantitative assay with the technique described for wound-tumor complement fixation by Windsor (11) and by Ellen M. Ball (12). Twofold serial dilutions of antigen and antiserum were made up to a dilution of 1:128 and tested against each other in a grid titration. Complement fixation took place up to and including a dilution of wound-tumor antiserum of 1:128, and of reovirus antigen dilution of 1:64, while the controls remained negative at 1:64 and 1:128.

There seems little likelihood that any normal plant antigens may have contributed to the reaction since the control tests were negative at the higher dilutions. As pointed out by Sabin (1), the presence of small amounts of reovirus antibodies can always be expected in normal sera. The results indicate the presence in the normal serum of a component that reacted at a dilution of 1:32 with reovirus antigen.

Serological relationship between a

human (animal)-pathogenic virus and a plant-pathogenic virus has thus been indicated. It is recognized that this evidence is only strongly suggestive but not conclusive. Before these results can be accepted as final, further tests will be required, including antiserum end-point titration, reciprocal reactions between reovirus antiserum and wound-tumor antigen, and controls in which serum from animals injected with healthy plant material are employed. Attempts will also be made to obtain normal serum from animals that lack reovirus antibodies.

Ordinarily, relationships would not be evident between seemingly widely separated viruses. The significance of such observations has been discussed recently by Macleod and Markham (13). Certainly, similarities in host range can hardly continue to be considered as criteria for relationships among viruses (14).

This reported serological relationship between reoviruses and wound-tumor virus is, to our knowledge, the first known instance of a common complement-fixing antigen between a human-pathogenic and a plant-pathogenic virus. The wound-tumor virus causes tumors in several plant species and it has been shown to multiply not only in plants but also in insect vectors (15). It can be transmitted easily to its insect host by needle inoculation (16), but only in a low percentage of cases can it be introduced mechanically into susceptible plants (2). The similarity in morphological structure and serological relationship indicates that wound-tumor and reoviruses should be classified as belonging to the same group. An effort is being made to establish whether agallian leafhoppers can be rendered "plant infective" by

the three reovirus strains. If so, will the reoviruses cause a plant disease distinguishable from the wound-tumor disease?

The finding of a complement-fixing antigen common to both reoviruses and wound-tumor virus would have far-reaching implications in the study of virus reservoirs, virus survival in nature, fundamental aspects of virus-host interactions, and in public-health problems. It might also influence ideas concerning the origin of viruses (17).

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8. The impetus for beginning this investigation was provided by Dr. Albert B. Sabin, who first suggested this problem to us.
9. Kindly supplied by Dr. L. M. Black, University of Illinois, Urbana.
10. Made available through the courtesy of Dr. Maurice R. Hilleman, Merck Research Institute, West Point, Pa. Additional reovirus was purchased from the American Type Culture Collection.
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17. Supported in part by U.S. Public Health Service grant AI-04290.

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18 April 1963

Base Composition of the RNA of a Reovirus Variant

Abstract. A variant of reovirus 3, Dearing strain, isolated after repeated passage of the original Dearing virus in L cells, is similar to the parent virus in many ways. It is, however, less sensitive to specific antibodies and metabolic inhibitors, and is released from L cells to a lesser extent than the original virus. Calculated as moles per 100 moles of total base in RNA, the percentage of guanine is 20.2, adenine, 29.8, cytosine, 21.0, and uracil 29.1. These values closely approximate those reported previously for the parent virus.

Reovirus type 3 contains a minimum complement of 10.2×10^6 molecular weight units of RNA per particle (1), an amount larger than that present in any other RNA-containing virus thus far

examined. Furthermore, reovirus RNA appears to have a secondary structure similar to that of DNA (1). The ratios of its bases are complementary. Reovirus RNA melts sharply in a narrow

Table 1. Base composition of the RNA of a variant of reovirus type 3. The percentages given are calculated as moles of base per 100 moles of total base in the RNA.

Percentage			
Guanine	Adenine	Cytosine	Uracil
<i>Dearing strain variant</i>			
20.2	29.8	21.0	29.1
<i>Dearing prototype strain (1)</i>			
19.3	29.7	20.5	30.5

temperature range, reacts only slightly with formaldehyde, and is resistant to hydrolysis by pancreatic ribonuclease. Thus, it is likely that reovirus RNA is a double-stranded helix (1). Reovirus and wound-tumor virus RNA's (1) are the only RNA's in nature known to possess such a secondary structure.

A variant of the original Dearing strain of reovirus type 3 has been isolated in this laboratory (2). Dearing strain variant is not significantly different from the original Dearing virus in antigenic constitution, size, fine structure, rate of adsorption to L cells, latent period, or cytopathic effects. However, it is less sensitive to specific antibodies and is released to a lesser extent from L cells. Our results indicate that the base composition of the Dearing strain variant is similar to that of the original prototype strain.

Cultures of L cells were grown for 48 hours in monolayers in phosphate-free reinforced Eagle's medium containing 5 percent fetal bovine serum and 30 $\mu\text{C}/\text{l}$ of carrier-free P^{32} orthophosphate per milliliter. They were then inoculated with the Dearing strain variant, and incubation at 37°C in the radioactive growth medium was continued. When the cells had degenerated, the cultures were frozen and thawed three times and the suspension was clarified at 8000g for 10 minutes. The supernatant was then centrifuged at 42,000g at 10°C for 2 hours, and the sediment was resuspended in a medium containing deoxyribonuclease, 16 $\mu\text{g}/\text{ml}$, ribonuclease, 20 $\mu\text{g}/\text{ml}$, and 0.003M MgCl_2 . The suspension was incubated for 12 hours at 4°C and for 30 minutes at 37°C. Fetal bovine serum was then added to a final concentration of 5 percent; and the suspension was centrifuged at 80,000g for 2 hours. The pellet was resuspended in 0.02M phosphate buffer, pH 7.2, and fractionated on a column of diethylaminoethyl cellulose by increases in the NaCl concentration. The radioactivity, hemagglutinating activity, and infectivity of the var-

ious fractions were determined. Virus was first eluted from the column at a salt concentration of 0.3M. The elution pattern showed a sharp simultaneous rise of the radioactivity and virus which reached a maximum and then gradually declined. To the eluate showing maximum infectivity and radioactivity were added the eluates from neighboring tubes and the combined eluates were centrifuged at 80,000g for 2 hours. The pellet was resuspended in a cesium chloride solution of an average density of 1.34 and centrifuged at 100,000g for 24 hours. No bands were visible because relatively small amounts of virus had been used. Successive drops were collected from below, and assayed for radioactivity and infectivity. The peak concentrations of radioactivity and infectivity were coincident, and were located below the center of the centrifugation tube. The contents of the tubes containing virus were pooled and centrifuged at 80,000g for 2 hours.

The pellet was resuspended in deionized water. The nucleic acid was extracted from the virus with phenol at 50°C. The RNA was hydrolyzed in 1N KOH at 22°C for 22 hours, and then K^+ ions were removed (3). Carrier ribonucleotides were added to the hydrolysate and the nucleotides were separated by electrophoresis on Whatman No. 3 paper (4, 5). The ultraviolet absorbing areas were located and eluted; the radioactivity of these eluates was measured. The base composition

of the RNA was estimated from the amount of P^{32} incorporated in the nucleotides.

The base composition of the RNA of the Dearing strain variant is similar to that of the original Dearing prototype strain of reovirus 3 (Table 1). The close similarity is specially noteworthy since the analytical procedures used were different; with the original Dearing virus, the bases were measured by direct chemical determination (1). In both virus strains, calculated as moles per 100 moles of total base in RNA, the percentage of guanine closely approximates that of cytosine, and that of adenine approximates that of uracil. The combined percentage of guanine plus cytosine is 41.2 percent of the total in the variant, whereas in the original Dearing virus it was 39.8 percent. No unusual bases have been detected in either strain (6).

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22 April 1963

Plant Morphology: Its Control in *Proserpinaca* by Photoperiod, Temperature, and Gibberellic Acid

Abstract. *Growth forms controlled by the photoperiod are modified by temperature and gibberellic acid. Gibberellic acid changes leaf orientation and prostrate growth habit of the short-day plant to that typical of the long-day plant and modifies leaf shape in both types of plants. Low temperature resembles short days in its effect on leaf shape and stem orientation of long-day plants. Geotropic responses of the stem appear to be modified by photoperiod, temperature, and gibberellic acid.*

Heterophylly, or change of leaf shape, of the aquatic angiosperm *Proserpinaca palustris* L., is controlled by photoperiod and temperature (1). However, little attention has been given to several other morphological characters that are also modified by these stimuli. We wish to draw attention to the many reversible responses of a single plant species to changes in photoperiod, temperature, and the growth regulator, gibberellic acid.

Figure 1 shows that aerial *Proserpinaca* plants grown under short days scarcely resemble those grown under long days. Short-day plants are prostrate and have short internodes and brown or green stems which bear dark-green dissected leaves (Fig. 2F). The leaves are inserted helically but are not oriented helically. They are appressed to the stem and oriented in the plane parallel to the stem, which gives the shoot a dorsiventral symmetry. Long-