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Some Effects of High-Intensity Ultrasound on Tobacco Mosaic Virus[1]

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Several investigators have reported the effect of sonic and ultrasonic frequencies on tobacco mosaic virus. Early reports of the exposure of tobacco mosaic virus to sonic frequencies were made by Stanley (*1*) and by Takahashi and Christensen (*2*). Exposure of tobacco mosaic virus to sonic and ultrasonic frequencies was reported by Kausche, Pfankuch, and Ruska (*3*), who showed that the virus could be broken into shorter particles by suitable exposure, with the infectivity reduced. Oster (*4*) exposed tobacco mosaic virus to a frequency of 9,000 c. He found that, as the time of exposure was increased, the basic virus unit, 280 mμ long, was broken into fragments one half and one fourth the original length.

This is a report of some effects of high-intensity ultrasonic waves, oscillating at a frequency of 7 Mc, on the physical structure and infectivity of tobacco mosaic virus. This is a much higher frequency than has previously been used in ultrasonic exposure of viruses.

The juice from Turkish tobacco plants infected with tobacco mosaic virus was purified by the usual technique, involving alternate low- and high-speed centrifugation, and was finally suspended in a phosphate buffer at pH 7. A suspension consisting largely of the basic virus unit 280 mμ long was obtained in this manner.

The ultrasonic vibrations were produced at the face of a quartz crystal ground to vibrate at a frequency of 7 Mc. The crystal controls the oscillator circuit which drives it. Exposure of the virus suspension was made inside a thick-walled lucite cylinder of 5-cc capacity suspended with its lower end about 10 mm above the horizontally mounted crystal. Both ends of the exposure tube were sealed by a 0.003-in.-thick acetate membrane. The entire transducer and exposure tube assembly was mounted under transformer oil having a high dielectric constant. By this arrangement, the ultrasonic waves, originating at the crystal, pass upward through a layer of rapidly circulated ice-cooled oil into the exposure tube, out its upper end, and back into the oil reservoir, where they are deflected and dispersed by screen baffles. Although the outside of the exposure tube was cooled to approximately 10° C, some internal heating did occur, as indicated by a mercury thermometer inserted into the tube following treatment.

To quantitatively evaluate the effect of ultrasonic irradiation upon the infectivity of the virus, Scotia bean plants were infected at the time secondary leaves began to appear. Infection was produced by rubbing one of each pair of primary leaves with an aluminum spatula dipped in ultrasonically treated virus suspension and the other with untreated virus suspension. To aid infection, all leaves were first dusted with No. 600 Carborundum. Infectivity comparisons were made on the basis of 10 replications.

After exposure to ultrasonic energy, microdrop samples of the virus suspensions were deposited upon a thin film formed by evaporating a 0.2% solution of Formvar in ethylene dichloride. The microdrop sample was not allowed to evaporate to dryness, but was removed with a micropipette after standing several minutes. This deposit was then lightly platinum-shadowed *in vacuo* before being photographed in the Universal Model RCA electron microscope.

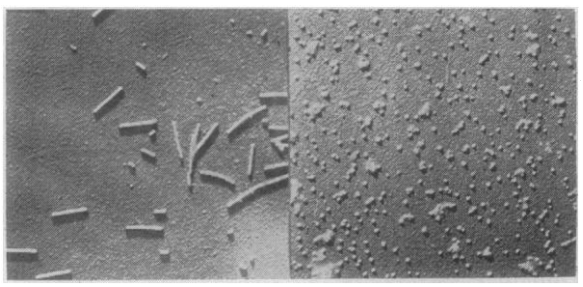

FIG. 1. Tobacco mosaic virus, ×20,000. Electron micrographs show effects of high-intensity ultrasound. Unexposed virus, consisting primarily of units 280 mμ long, is shown at left; highly fragmented virus (fragments 20 mμ–40 mμ long) shown at right.

Fig. 1 shows the results obtained by treatment at the maximum energy output of the oscillator. This high degree of fragmentation of the virus was accompanied by cavitation in the liquid. Experience has shown that the degree of fragmentation revealed by the electron micrographs was directly proportionate to the amount of cavitation as indicated by the number and volume of released gas bubbles. Heating of the virus suspension by ultrasonic energy was not in itself sufficient to inactivate the virus.

Lesion counts made on Scotia bean plants after inoculation of the leaves with unexposed virus and virus exposed to ultrasound for 3.3 min showed a reduction of approximately 95% in the infectivity of the virus exposed to ultrasound.

Using lower intensity levels, between approximately 140 w and 180 w power input, and varying the time of exposure, the following results have been demonstrated:

a) Ultrasonic irradiation of "aged" virus suspension produces an increase in virus infectivity through dispersion of aggregated clusters of virus rods.

[1] This work was jointly supported by the U. S. Public Health Service and Battelle Memorial Institute.

[2] The author wishes to acknowledge the capable technical assistance of John Kissel.

b) Highly end-to-end aggregated virus is disaggregated primarily into basic virus units 280 mμ long with a subsequent increase in the infectivity of the ultrasonically exposed virus.

c) At somewhat higher energy levels, statistically significant measurements show a tendency for the basic infective unit, 280 mμ long, to fracture, first at a constant distance from the end of the virus rod, with subsequent random fragmentation as the power is increased. This indicates a structural weakness at one definite point in the virus rod.

It is felt that, with further study and refinements of techniques, there is a definite possibility of preparing viral and bacterial vaccines by exposure to ultrasound which may be superior in the treatment of disease to those produced by the usual procedures, since the method of inactivation is physical and therefore probably less likely to alter antigenic properties than in the case of chemical inactivation. Ultrasonic inactivation of microbiological materials may also furnish a means of "uncovering" desirable antigens.

By the use of comparatively low-energy ultrasonic treatment, "aged" vaccines may be reactivated and their useful period possibly extended.

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Report on Fossil Vertebrates from the Upper Magdalena Valley, Colombia

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The field-work program for collection of fossil vertebrates in the Upper Magdalena Valley (Department of Huila, Colombia) during the summer of 1950 was sponsored jointly by the Associates in Tropical Bio-Geography at the University of California and by the Servicio Geológico Nacional de Colombia. The writer was paleontologist for the party. Diego Henao Londoño, geologist for the Servicio, managed the field group. Oliver Pearson, of the Museum of Vertebrate Zoology at the University of California, joined us in Huila to facilitate his work on the zoological studies in the upper Magdalena. Stanley G. Smith, of the Botany Department, was also attached to our field party for a month and was able to carry on the program of plant studies.

This was the second year in which one or more representatives from the Museum of Paleontology had participated in the jointly sponsored program. In the summer of 1949, R. A. Stirton and Robert W. Fields joined with Sr. Henao for the initiating of detailed stratigraphic studies; but prior to that time, in 1944 and 1945, Stirton and Royo y Gomez had collected a large assemblage of fossil vertebrates from the region. The most important paleontological discoveries made by the 1950 field party were: a complete cranium of a toxodont, skeletal parts of a leontiniid,

TABLE 1

THE LA VENTA FAUNA—LATE MIOCENE

- Class, Dipnoi
  - Order, Lepidosireniformes
    - Family, Lepidosirenidae
- Class, Teleostomi
  - Order, Cypriniformes
    - Suborder, Siluri (catfish)
      - Unidentified teleost
- Class, Amphibia
  - Order, Anura
    - Family, Leptodactylidae
- Class, Reptilia
  - Order, Chelonia
    - Suborder, Pleurodira
      - Family, Pelomedusidae
    - ?Suborder, probably two genera
  - Order, Sauria
    - Family, ?Iguanidae
    - Family, ?Teiidae
  - Order, Serpentes
    - Family, ?Boidae
  - Order, Sebecosuchia
    - Family, Sebecidae (*Sebecus*)
  - Order, Eosuchia
    - Family, Alligatoridae
    - Family, Crocodylidae
    - Family, Stomatosuchidae
    - Family, ?Gavialidae
- Class, Aves
  - Order, unidentified
- Class, Mammalia
  - Order, Marsupialia
    - Family, Didelphidae
    - Family, Borhyaenidae (cf. *Borhyaena, Lycopsis,* and *Cladosictis*)
  - Order, Chiroptera
    - Family, Phyllostomatidae (n. gen. and n. sp. Savage [*8*])
  - Order, Primates
    - Family, Cebidae
      - Subfamily, Pitheciinae (*Cebupithecia sarmientoi* Stirton and Savage [*6*])
      - Subfamily, Alouattinae (*Homunculus tatacoensis* Stirton [*9*]; *Homunculus* sp. Stirton [*9*])
      - Subfamily, Cebinae (*Neosaimira fieldsi* Stirton [*9*])
  - Order, Edentata
    - Family, Megalonychidae
    - Family, Megatheriidae
    - Family, Mylodontidae (at least three genera)
    - Family, Myrmecophagidae
    - Family, Dasypodidae (at least two genera)
    - Family, Glyptodontidae (at least two genera)
  - Order, Rodentia
    - At least two families and four genera
  - Order, Condylarthra
    - Family, ?Didolodontidae
  - Order, Litopterna
    - Family, Macraucheniidae (two genera)
    - Family, Proterotheriidae (two genera)
  - Order, Notungulata
    - Family, Leontiniidae
    - Family, Toxodontidae
    - Family, Interatheriidae (n. gen. and n. sp. Stirton and Savage [*7*])
    - Family, ?Hegetotheriidae
  - Order, Astrapotheria
    - Family, Astrapotheriidae (two genera)
  - Order, Sirenia
    - Family, Trichechidae (*Potamosiren magdalenensis* Reinhart [*10*])