Within the species *Zea mays* L., only a few inbred lines, homozygous for the gene pair *hm/hm,* are known suscepts (*9*). The resistance of most inbred lines of corn to invasion by this fungus may be basically similar to that expressed by the tissues of nonhosts. This paper reports the results of some of the experiments set up to examine this hypothesis (*10*).

The potato tuber was found to be a useful nonhost for this study. Substances inhibitory to the growth of *Helminthosporium carbonum, Ceratostomella ulmi,* and *Fusarium oxysporum* f. *lycopersici* were found in potato peel. The inhibitory substances were not present in potato pulp tissue but were produced in the pulp tissue following inoculation with these fungi. None of the fungi employed for inoculations are known to incite disease in potatoes.

For the experiments, Idaho-grown potatoes (var. Netted Gem) free from visible surface defects were washed with soap and water and surface-sterilized by immersion for 2 minutes in an aqueous 2.5-percent solution of sodium hypochlorite. After the potatoes had been washed with sterile water and dried, peel tissue approximately 1 mm thick was removed. Fresh pulp tissue was obtained from potatoes after removal of the peel. Inoculated pulp tissue was obtained by covering sterile fresh potato slices, approximately 1 cm thick, with a heavy spore suspension of *H. carbonum, C. ulmi* or *F. oxysporum* f. *lycopersici.* Slices were incubated in sterile petri dishes at 22°C for 72 hours in a moist atmosphere. Sections of heavily inoculated slice surface 1 mm thick were removed for extraction. Autoclaved potato slices were also inoculated with each of the fungi and then were incubated under the same conditions. Control pulp tissue was provided by holding sterile potato slices at 22°C for 72 hours.

Extracts were prepared by placing 40 g of tissue in 300 ml of boiling alcohol and boiling for 2 to 5 minutes. After cooling, the tissue was macerated for 5 minutes in a Waring Blendor, and the homogenate was filtered through Whatman No. 2 filter paper on a Büchner funnel. The filtrate was concentrated to dryness under reduced pressure at 40°C; then the residue was redissolved in 50 ml of water and filtered through glass wool. Two grams of dehydrated potato dextrose agar (11) was dissolved in the filtrate, and the solution was autoclaved at 15-lb pressure for 10 minutes. Petri dishes containing 16 ml of the sterile nutrient medium were seeded with dilute spore suspensions of *H. carbonum, C. ulmi,* or *F. oxysporum* f. *lycopersici* and were allowed to incubate at 24°C.

Helminthosporium carbonum made good growth in 5 days, both on the dehydrated potato dextrose agar medium and on the agar medium to which was added the extract of fresh potato pulp or the extract of autoclaved inoculated potato pulp. Moderate to good growth occurred on the medium to which was added an extract of control pulp. The fungus made little or no growth on the medium containing either the extract of peel or the extract of inoculated pulp tissue. *Ceratostomella ulmi* and *F. oxysporum* f. *lycopersici* responded similarly. The results are illustrated in Fig. 1. The fungi showed excellent growth on the autoclaved potato slices but very little growth on the fresh potato slices at the end of the incubation period (72 hours). Potato slices held overnight at −20°C and then inoculated supported excellent growth of these fungi within 72 hours of incubation. Extracts from such frozen, inoculated potato slices, when added to potato dextrose agar, had no inhibitory effect and were in this respect similar to extracts prepared from autoclaved inoculated pulp.

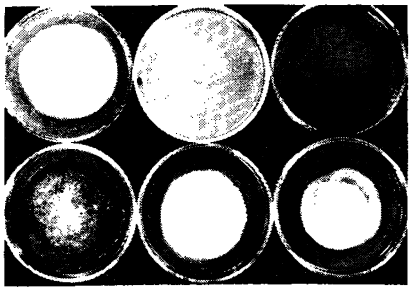

Fig. 1. Growth of *H. carbonum* on potato dextrose agar to which was added extracts of the following potato tissues. (Top, left to right) Fresh pulp; pulp tissue inoculated with *H. carbonum* and incubated for 72 hours at 22°C; peel. (Bottom, left to right) No extract added to the potato dextrose agar; pulp tissue autoclaved, then inoculated with *H. carbonum* and incubated 72 hours at 22°C; control pulp tissue, not inoculated but held 72 hours at 22°C.

These results suggest that living potato tuber tissue, when inoculated with *H. carbonum, C. ulmi,* or *F. oxysporum* f. *lycopersici,* produces a substance or substances inhibitory to the growth of these fungi. Potato peel appears to contain a high concentration of inhibitory material, but here inoculation is unnecessary for its elaboration. The inhibitory material seems to be localized at the site of inoculation, since extracts of tissue taken 5 mm from the inoculated surface showed no inhibitory effects on the growth of the fungi. Carrot and turnip tissue responded in a manner similar to that described for potato tuber tissue.

Potatoes, carrots, and turnips appear to have a twofold mechanism for immunity from attack by the fungi studied. Inhibitory substances are present in peel tissue, the first barrier to penetration; however, if the peel tissue is injured or removed, adjacent pulp tissue is capable of producing inhibitory substances immediately around the points of penetration. These inhibitors do not appear to be translocated.

Both the passive resistance of the peel and the active resistance produced by the pulp appear to be nonspecific. The same inhibitors appear to be produced as a response to penetration by the three fungi studied, since the substance produced in response to one fungus was found to inhibit the other fungi.

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19 July 1955

Enteric Cytopathogenic Human Orphan (ECHO) Viruses

The recovery in different laboratories of large numbers of new cytopathogenic viruses from the human intestinal tract led to the cooperative effort described in this report. The work was undertaken as a start in determining the significance of these agents. The viruses were obtained from patients with the aseptic meningitis syndrome (often diagnosed as nonparalytic poliomyelitis) as well as from healthy children in different parts of the world.

Preliminary studies of these viruses indicated that multiple antigenic types exist (*1–4*). Individual prototype strains and serums were exchanged among members of the Committee on the ECHO Viruses for performance of cross-neutralization tests. A uniform technique was adopted for these tests, employing for the inoculum of each tissue culture a

mixture containing 100 TCD_{50} of viruses and an equal amount of antiserum containing 20 units of antibody against its homologous virus. Twenty units represented a 20-fold concentration of that dilution giving 50-percent neutralization of 100 TCD_{50} of virus.

This cooperative study has resulted in the differentiation of the 13 antigenically distinct viruses that are listed in Table 1. These viruses—some of which have been referred to in previous literature as "orphan viruses" (*1*) and others as "human enteric viruses" (*2*)—are now classified as the "enteric cytopathogenic human orphan (ECHO) group" (*5*). They share the following properties. (i) They are cytopathogenic for monkey and human cells in culture (*1–4*). All 13 prototype strains were isolated in cultures of monkey kidney cells, which for the strains tested proved to be more susceptible than HeLa cells. (ii) They are not neutralized by pools of the three types of poliomyelitis antiserum. (iii) They are not neutralized by antiserums for Coxsackie viruses that are known to be cytopathogenic in tissue culture, and they fail to induce disease in infant mice. (Animals less than 24 hours old should be used, for they have greater susceptibility.) (iv) They are not related to other groups of viruses recoverable from the alimentary tract (throat or intestine) by inoculation of primate tissue culture, such as herpes simplex, influenza, mumps, measles, varicella, and the ARD (acute respiratory disease) or APC (adenoidal-pharyngeal-conjunctival) group. (v) They are neutralized by human gamma globulin and by individual human serums; this indicates that they infect human beings.

Other studies of the ECHO viruses (more extensive for some than for others) have provided additional information. Complement-fixing antigens have been detected in the culture fluids of a number of viruses that have been tested (*1, 3*). All the viruses tested were ether-resistant. Ultrafiltration (gradocol membrane) measurements indicated sizes for types 1, 2, and 3 between 11 and 17 mμ (*1*). The size of type 10 is reported to be between 60 and 90 mμ (*2*). Plaque morphology of the ECHO viruses studied (types 1, 3, 4, 5, 6, 7, and 9) is sufficiently distinctive, except for type 7 (Garnett strain), to permit differentiation from polio virus plaques (*6*). The plaques of the ECHO viruses mentioned had irregular diffuse boundaries, and healthy cells could be found within the degenerated areas.

Kidney cells of different monkey species vary in their susceptibility to the ECHO viruses. Rhesus (*Macaca mulatta*) and cynomolgus (*M. irus*) cells are susceptible to all 13 types studied. Cells from the South American capuchin (*Cebus capucina*) were found to be resistant to types 1, 2, 3, 7, 8, 9, and 11 (*2, 6*). However, they were susceptible to type 10 (*2*). Cells from the African red grass military monkey (*Erythrocebus patas*), which were resistant to types 1, 2, 3, 4, 5, 6, and 9, were as susceptible as those from the rhesus monkey to the type 7 Garnett strain (*6*).

It is emphasized that this committee is not an authoritative body but rather a group of investigators who, together with others present at the Conference on Orphan Viruses, felt the need for a working approach to a classification of this heterogeneous assembly of viruses that were encountered while poliomyelitis studies were being made. Agreement within the committee was obtained by verification of the specificity of each prototype strain in at least two laboratories.

If requested by other investigators, the committee is prepared to assign numbers to new prototype strains that satisfy the criteria employed for differentiation of the strains listed in Table 1. To avoid unnecessary confusion in the literature, the committee is willing to function as a clearinghouse for characterization of new strains by comparison with established prototypes. In this way the distinction of new prototypes may be hastened.

The antigenic classification presented here is only a preliminary step toward understanding the role that these viruses of the human enteric tract play in disease. If and when any one of the established types is identified as the etiologic agent of a clinically distinct disease, it will be removed from the ECHO group of viruses.

COMMITTEE ON THE ECHO VIRUSES*
National Foundation for Infantile Paralysis

Table 1. List of antigenically distinct ECHO viruses.

Type	Prototype strain	Geographic origin*	Illness in person yielding virus	Reference
1	Farouk	Egypt	None	(*1*)
2	Cornelis	Connecticut	Aseptic meningitis	(*1*)
3	Morrisey	Connecticut	Aseptic meningitis	(*1*)
4	Pesascek	Connecticut	Aseptic meningitis	(*1*)
5	Noyce	Maine	Aseptic meningitis	(*1*)
6	D'Amori	Rhode Island	Aseptic meningitis	(*1*)
7	Wallace	Ohio	None†	(*2*)
8	Bryson	Ohio	None	(*2*)
9	Hill	Ohio	None†	(*2*)
10	Lang	Ohio	None	(*2*)
11	Gregory	Ohio	None	(*2*)
12	Travis 2-85	Philippine Islands	None	(*3*)
13	Hamphill 2-188	Philippine Islands	None	(*3*)

* Strains belonging to type 1 have also been recovered from the Philippine Islands (*3*) and from India (*1*). Strains belonging to types 8, 9, 10, 11, and 13 have also been isolated from healthy children in Mexico (*2*).

† Strains belonging to types 7 and 9 have been recovered from patients having the aseptic meningitis syndrome in West Virginia (*1*).

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* Members of the committee are G. Dalldorf, J. F. Enders, W. McD. Hammon, A. B. Sabin, J. T. Syverton, and J. L. Melnick (chairman). The committee was called together as a result of a recommendation made at the Conference on Orphan Viruses that was held by the National Foundation for Infantile Paralysis in New York, 19–20 May 1955.

22 November 1955

Calcium Uptake by a Coral

In the course of a series of experiments designed to test the usefulness of radioisotope tracers for the study of calcium deposition by corals, we have obtained some interesting data on the common Atlantic coral *Astrangea danae* (*1*). Pieces of living coral were placed in beakers one-third full of glass beads and containing 40 to 60 ml of sea water that had previously been filtered through No. 4 Whatman filter paper. Stirring and aeration were accomplished by means of jets of water-saturated air impinging on the surface. After the coral polyps became reextended, radioactive calcium-45 in neutralized sea water was added by pipette in amounts sufficient to give about 1000 counts per minute from 0.1-ml aliquots. All samples were spread to uniform area on copper planchets and counted at the same geometry with a 1.8-mg/cm² mica end-window Geiger-Müller tube. The high specific activity of the calcium-45 permitted very small additions of total calcium, never exceeding