polare, which is presumed to have grown on the land (*9*). If it had been washed or blown onto T-3 in 1935, it would have lived 19 years to the time when a piece of it was revived in the laboratory (early in 1954; the next year the remainder could not be revived). This is precisely the longest period of which a record could be found of a moss tussock remaining viable in a herbarium (*10*).

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Electrophoretic Separation of Hemoglobins from the Chicken

Investigations of the dissociation of oxyhemoglobin in birds (*1, 2*) have led to the suggestion that there are two hemoglobins, one embryonic and one adult. Such differences exist in various mammalian species; these have been summarized by Lecks and Wolman (*3*). Recently, electrophoretic methods have been successfully used in separating mammalian hemoglobins (*4*). It appeared that the nature of avian hemoglobins could profitably be examined by a similar technique (*5*).

Hemoglobin was obtained from embryos and adults of White-Olympian-New Hampshire cross chickens. Blood samples were drawn from the vitelline artery, the heart, or from the radial veins, depending on the age of the chicken. Embryonic bloods from several individuals in a single age group were pooled to form a single sample for analysis. Heparin was employed as the anticoagulant. The cells were separated from the plasma by centrifugation and washed three times with 0.85-percent sodium chloride. The hemoglobin solution for analysis was prepared by adding 2 vol of distilled water to 1 vol of packed erythrocytes. The supernatant fluid, after centrifuging, was stored at −10°C, then thawed at room temperature for use. Electrophoretic patterns were obtained by applying 5 μl of this hemoglobin solution to the filter paper strips (S. and S. No. 204-313, 20 mm wide) in a thin line. A controlled voltage (300 v) was applied to the strips for 6 hours; the current increased from 6 to 12 ma. The strips were moistened with Veronal buffer (*p*H 8.8, ionic strength 0.05) prior to application of the sample. Boundary diagrams were prepared by direct readings of paper strips in a spectrophotometer (*6*). The results are plotted in Fig. 1.

The samples tested were from embryos (13, 15, 18, and 20 days), hatched chicks (up to 6 hours and 1, 2, 4, 6, 8, 11, and 18 days), and 6-month-old and 2-year-old chickens.

Two hemoglobins were observed in each age group tested. No marked differences were apparent among specimens. The faster moving component, designated as α-hemoglobin, appeared to be in lower concentration. The slower moving component was β-hemoglobin. Migration was toward the anode; the rate of migration was uniform in all samples (Fig. 1). Percentage composition of the hemoglobins, as determined by planimetry of boundary diagrams, appeared to vary with age. There was an approximate 30-percent reduction of the α component in the 2-year-old chicken as compared with the 18-day embryo—that is, 30 percent α and 70 percent β in the embryo, and 20 percent α and 80 percent β in the adult. Determinations on a limited number of samples indicated that the major portion of the change occurred within a few days after hatching.

As early as the 13th day of incubation, two hemoglobins are present. If one were essentially an embryonic hemoglobin and the other an adult hemoglobin (*1*), the replacement of the former by the latter would be expected. It appears that no such major replacement occurs. The average life-span of an avian erythrocyte has been reported to be approximately 32 days (*7*); yet the hemoglobin of both 6-month and 2-year chickens exhibits α and β components in relatively the same proportions as is found in young (18-day) chickens. A logical explanation of hemoglobin types is readily available in mammals; however, in birds, where no placental transfer of oxygen occurs, this explanation does not directly apply.

Fig. 1. Electrophoretic analysis of chicken hemoglobin.

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Production of Fungistatic Substances by Plant Tissue after Inoculation

Among the various attempts to learn how plants are able to resist invasion by pathogens, the most successful is the work of Link *et al.* (*1–3*), Angell, Walker, and Link (*4*), and Walker and Link (*5*), who showed that protocatechuic acid and catechol in the outer scales of colored onions were responsible for resistance to smudge and neck rot. Other instances of specific resistance owing to the presence of biochemical entities have been suggested but not proved. Johnson and Schaal (*6*) suggest that chlorogenic acid in the potato peel participates in resistance to scab. Müller and Behr (*7*) point out that tanninlike substances in the potato may be associated with resistance to late blight. The possibility that a pathogen-inhibiting substance might be produced by plant tissue in response to the presence of a pathogen has been conceived by some workers in the field. However, experimental proof has been lacking heretofore.

Helminthosporium carbonum race I, the incitant of a leaf-spot disease of corn, has an exceedingly narrow host range (*8*).

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.

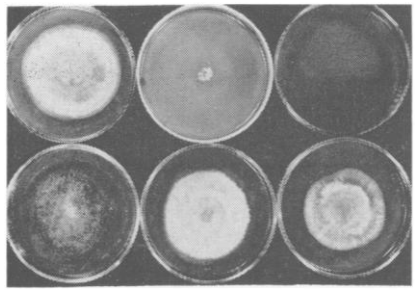

Fig. 1. Growth of *H. carbonum* on potato dextrose agar to which was added extracts of the following potato tissues. (Top, left to right) Fresh pulp; pulp tissue inoculated with *H. carbonum* and incubated for 72 hours at 22°C; peel. (Bottom, left to right) No extract added to the potato dextrose agar; 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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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