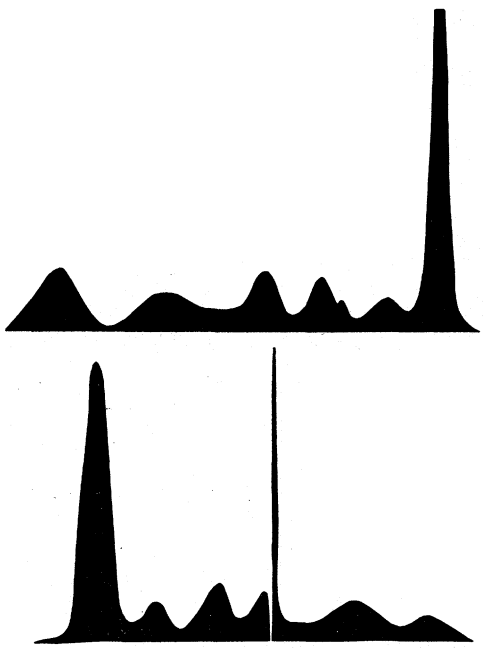

FIG. 2. Standard electrophoretic pattern of the 7-day serum. (Veronal buffer pH 8.6, ionic strength 0.1, 15 ma, 180 min.)

This peak is roughly in the area where the radioactivity localizes.

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Relation of Chlorogenic Acid to Scab Resistance in Potatoes[1]

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The nature of the resistance of potatoes to common scab caused by *Streptomyces scabies* is not fully understood. Lutman and Cunningham (*1*) and Longree (*2*) attributed resistance to structural differences, whereas Kiessling (*3*) and Wingerberg (*4*) considered resistance to be based on physiological factors. Müller and Behr (*5*) suggested that substances giving typical tannin reactions are associated with resistance of potatoes to late blight caused by *Phytophthora infestans*. Walker *et al.* (*6*) found high concentrations of protocatechuic acid in skins of onion varieties resistant to the attacks of onion smudge, *Collitotrichum circinans*.

The presence of chlorogenic acid in potato tubers was first demonstrated by the use of $FeCl_3$. Phenolic compounds having the *ortho*-dihydroxy grouping give a green color with $FeCl_3$. In this test the epidermis of a tuber was carefully removed from a test spot, a drop of 5% $FeCl_3$ solution added, and the exposed tissue macerated with a sharp knife. The green color developed immediately, and its intensity varied with the resistance of the variety to scab. Nine named and 36 seedling varieties of potatoes of known scab resistance were given the $FeCl_3$ test. All varieties highly resistant to scab showed a strong color reaction when treated with $FeCl_3$. The intensity of the green color varied with the degree of scab resistance.

The identification of chlorogenic acid in the potato (*Solanum tuberosum*) was established by the use of paper chromatography and ultraviolet absorption techniques as described by Johnson *et al.* (*7*) in work on peach tannins. Only two phenolic compounds, chlorogenic acid and tryosine, were found to be present in significant quantities in potatoes. The $FeCl_3$ test indicated that chlorogenic acid was concentrated in a very thin layer, in the periderm perhaps not over 2 cells thick.

To demonstrate the presence of chlorogenic acid in the periderm of potato tubers, the following technique was used. One hundred grams of potato peelings, removed with a vegetable paring knife and having an average thickness of 1 mm, were extracted with 300 ml 95% ethanol in a Waring blendor for 5 min. The extract was filtered and concentrated to 25 ml under reduced pressure. This concentrate was then reduced to dryness in a vacuum oven at 35° C. The same procedure was used for the flesh of the potato. A 50-mg sample of the extract powder from the skin and flesh from two scab-resistant varieties, Russet Burbank and Yampa, was extracted three times with 5 ml petroleum ether in a 15-ml centrifuge tube to remove any fatty or waxy materials. The petroleum ether was decanted after centrifugation. The dry powder was dissolved in 0.5 ml water. Two µl was chromatographed on Whatman No. 1 filter paper using butanol-acetic acid-water (50-10-40) as a developing solvent. Fig. 1 shows a papergram after spraying with modified Folin-Denis reagent[2] and treating with ammonia fumes to alkalize the reagent. No. 1 shows separation of the tyrosine and chlorogenic acid. Nos. 2 and 4, from Burbank and Yampa skin extracts, respectively, show high chlorogenic acid but very low tyrosine content. The flesh extracts Nos. 3 and 5 reveal small amounts of tyrosine and a weak test for chlorogenic acid. Fluorescence of the papergram under ultraviolet light with maximum intensity at 3650 A also showed minute quantities of chlorogenic acid in the flesh. Fluorescence of the chlorogenic acid spot from the peeling extract was very pronounced. Fig. 2 shows the ultraviolet absorption spectra from Russet Burbank skin and flesh which were determined on a 50-mg sample of each dissolved in 200 ml distilled water after extraction with petroleum ether as described above. Spectrum No. 1 is typical for chlorogenic acid with high absorption at 324 mµ. Spectrum No. 2 has a strong

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[2] One part Folin-Denis reagent, 1 part water, and 2 parts 95% ethanol.

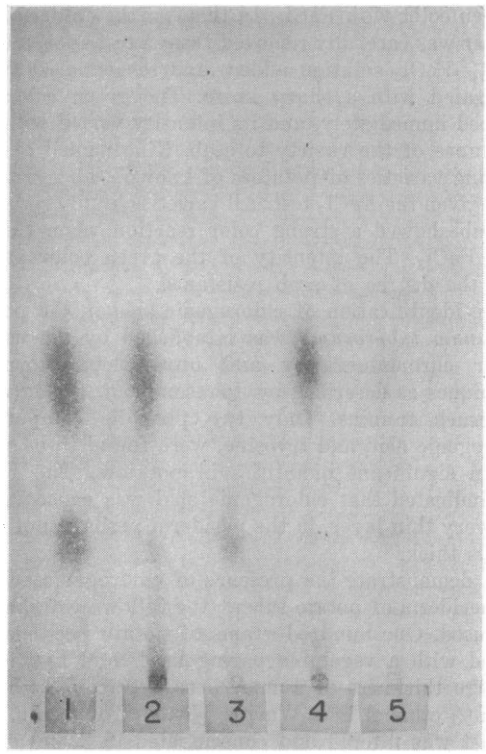

FIG. 1. Papergram sprayed with Folin-Denis reagent: No. 1, separation of chlorogenic acid and tyrosine; Nos. 2 and 4, extracts from Burbank and Yampa skins, respectively; Nos. 3 and 5, flesh extracts from Burbank and Yampa.

band at 268 mμ which is due to tyrosine. A very slight band at 324 mμ indicated traces of chlorogenic acid in the flesh.

One-dimensional paper chromatography, using the above developing solvent, was found to give good separation for chlorogenic acid. Fig. 3 shows the similarity of the ultraviolet spectra of pure chlorogenic acid and potato chlorogenic acid, both extracted from papergrams. This procedure was used as a basis for quantitative estimation of chlorogenic acid in potato peelings. These estimates were determined as follows: 100 g of peelings were weighed into a Waring blendor cup. Three hundred ml 95% ethanol was added to the peelings and extracted for 5 min. After filtration the extract was concentrated under reduced pressure until 50 ml was equivalent to 100 g peelings. Twenty aliquots of 2 μl were placed on a papergram in a row of adjacent spots. After developing for 20 hr, the papergram was dried. The fluorescent chlorogenic acid band was located under ultraviolet light, then removed from the papergram and eluted from the strip with 75% ethanol. The eluate (approx 1.5 ml) was made to 10 ml with distilled water. Optical density was determined at 324 mμ using a Beckman Model D. U. Spectrophotometer. $E^{1\%}_{1\,cm}$ at 324 mμ for anhydrous chlorogenic acid is given by Moores *et al.* (*8*) as 526.

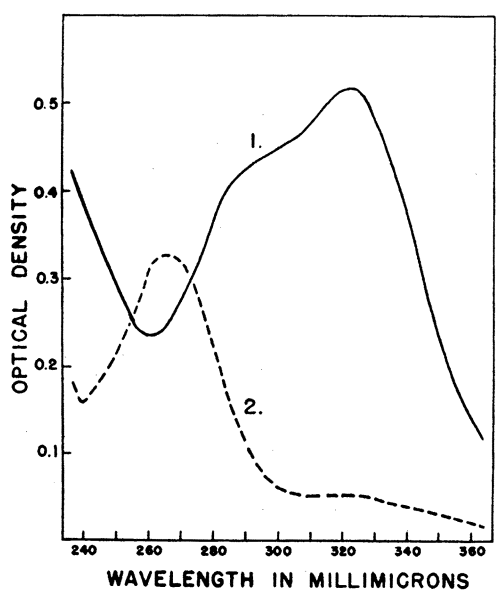

FIG. 2. UV absorption spectra of extracts from Burbank skin (1) and flesh (2).

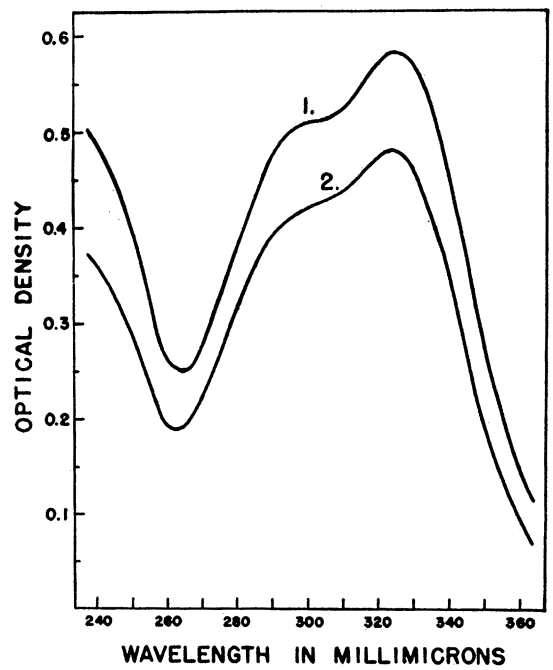

FIG. 3. UV spectra of pure chlorogenic acid (1) and potato chlorogenic acid (2). Both extracted from papergrams.

An unnamed USDA potato seedling highly resistant to scab, which gave a strong $FeCl_3$ test, contained 77 mg chlorogenic acid/100 g peelings of an average thickness of 1 mm. Since the chlorogenic acid is concentrated in a very thin layer, its actual concentration in individual cells was several times the above amount. A seedling variety highly susceptible to scab, which gave a weak $FeCl_3$ test, contained only 40 mg chlorogenic acid/100 g peelings. The ultraviolet absorption spectra of the peeling extracts of the above seedlings are shown in Fig. 4. Both extracts were prepared and diluted in the same manner. These spectral

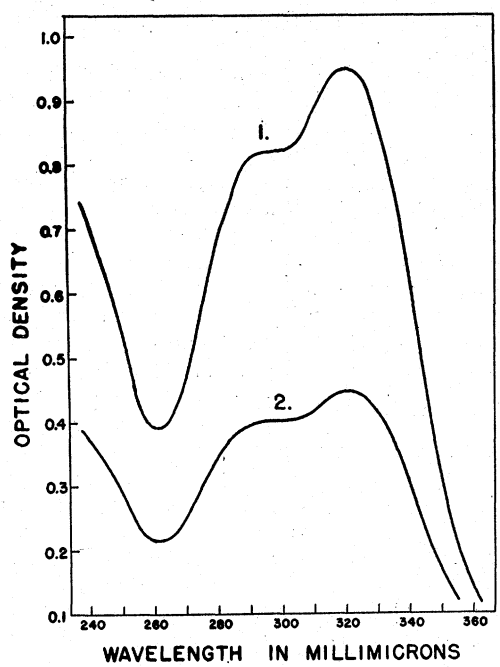

FIG. 4. UV absorption spectra of the peeling extract from a scab-resistant seedling (1) and from a scab-susceptible seedling (2).

curves likewise show much higher concentration of chlorogenic acid in the scab-resistant seedlings as compared to a scab-susceptible seedling.

The total amount of chlorogenic acid is not as important as the concentration in local areas. For example, in some scab-resistant potato varieties, the chlorogenic acid appears to be more heavily concentrated in or near the lenticels, which serve as the natural avenue of entrance for the scab organism. The $FeCl_3$ test also indicates that chlorogenic acid accumulates around a tissue injury, either mechanical or parasitic in origin. This observation, together with the fact that the concentration of the acid in the periderm varies with the degree of scab resistance, suggests that chlorogenic acid is involved in a protective mechanism. The exact nature of this protection has not been determined, but indications are that one or more mechanisms are involved.

It is possible that chlorogenic acid lowers pH of the cells, thereby creating an unfavorable medium for growth of the scab organism. Experiments indicate that when chlorogenic acid was added to unbuffered potato dextrose agar medium, the lowered pH was sufficient to retard growth of *S. scabies*. On buffered agar (pH 6.2) 400 mg chlorogenic acid/100 ml agar failed to retard growth of several physiologic races of *S. scabies*.

Since tyrosinase is also concentrated in the same area as chlorogenic acid, it may be that both are involved in a general protective mechanism. Chlorogenic acid is a good substrate for the polyphenolase fraction of tyrosinase. Upon tissue injury tyrosinase immediately oxidizes chlorogenic acid to the quinone which may be toxic to pathogenic organisms, and this would be in accord with views expressed by Szent-Györgyi and Vietorsz (*9*) as to a function of tyrosinase. The rate of quinone formation would vary with the concentrations of chlorogenic acid and tyrosinase. Quinone formation in the area where chlorogenic acid was concentrated was also found to be greater in the potato varieties resistant to scab. An acidified KI-starch solution was used as a test for quinone formation after macerating the tissue with a sharp knife.

Chlorogenic acid itself or its quinone may be directly or indirectly involved in the formation of cork cambium in a manner similar to action of the wound *hormone* traumatin as described by Bonner and English (*10*). This is believed true because chlorogenic acid added to a freshly cut surface of a tuber speeds up the production of suberized tissue.

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A Modification of the Sudan Black B Technique for the Possible Cytochemical Demonstration of Masked Lipids[1, 2]

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The technique used in the study described here is an adaptation of a routine cytochemical method employed on blood films to demonstrate lipids. This modification is designed to reveal what are commonly called "masked" lipids not demonstrable by routine procedures. It involves the common use of Sudan black B, but on films previously treated with various organic acids.

The routine procedure for the demonstration of masked lipid in blood films is as follows:

1. Fix smears in formol vapor for 2–5 min.
2. Immerse fixed films in a 25% aqueous solution of acetic acid for 2 min. Citric acid (5%), oxalic acid (10%), or formic acid (10–25%) may be used in place of acetic acid.

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[2] Presented in part before The Ohio Academy of Science, April 28, 1950.