pending on the temperature. In addition, the significant effect of high pressure confirms the previous observations that these changes proceed with an increase in volume. The latter observations are of some importance, since they indicate that with pressure treatment the intermediate decomposes to the original, normal state, which cannot revert to the intermediate after the pressure is removed. In this respect, the results are quite similar to those obtained with ultraviolet treatment followed by high pressure.[2]

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Localization of Protein-bound Radioactive Iodine by Filter Paper Electrophoresis

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The serum of an euthyroid patient with a Hurthle cell carcinoma of the thyroid with metastases was analyzed by filter paper electrophoresis, using a modification of the method of Kunkel and Tiselius (*1*), 1 hr after a dose of 40 mc of I^{131} and at daily intervals thereafter for 10 days. One tenth ml of serum was placed on two thicknesses of Whatman #3 filter paper strips held between glass plates. The ends of the paper strips were placed in veronal buffer, pH 8.6 and ionic strength 0.05. A current of 1 ma/strip was passed through at a potential of 300 v. At the conclusion of the electrophoresis, the bottom strip was stained with bromphenol blue, dried, washed with acetic acid, and cut into numbered strips. With the dye elution method the protein fractions were localized and their quantities determined. The top strip was cut into numbered segments, and the relative radioactivity determined in a bell-type Geiger counter.

The total protein-bound I^{131} after electrophoresis was compared with the total raidoactivity of a comparable amount of serum before electrophoresis. These values were identical after 3 days. The radioactivity was only 2% protein-bound after 1 hr and was freely distributed among all the serum proteins. At the end of 30 hr the radioactivity was 33% protein-bound, and there was definite evidence of concentration in the albumin and in the α-2 globulin. At 48 and 72 hr, the concentration at the latter site was more clearly evident. Over 80% of the activity was concentrated in and just beyond the α-2 globulin area at 72 hr and thereafter.

A typical radioelectrophoretogram of the 7-day serum is shown in Fig. 1, together with a graph of the

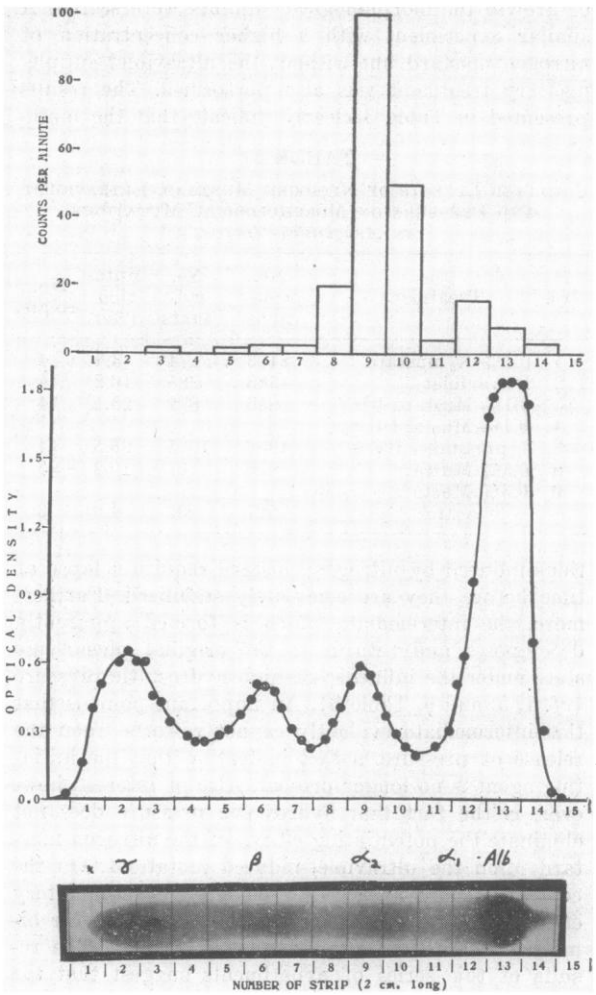

FIG. 1. Top graph shows the radioactivity of the 7-day serum on the segments of filter paper. Center graph shows the relative quantities of protein on similar segments. Photograph of the corresponding stained strip is shown at the bottom.

quantities of protein fractions as determined by the bromphenol blue elution method, and a photograph of the stained strip. The standard electrophoretic pattern of the same serum is shown in Fig. 2.

This method is reported as a new approach to the study of the nature of the circulating thyroid hormone as well as other substances which can be conveniently traced. It is not assumed that this is conclusive evidence of the behavior of normal thyroid hormone, since the subject under investigation had a carcinoma of the thyroid which was possibly in the functioning category. Some evidence that this serum was not completely normal can be seen in the standard electrophoresis in which a small abnormal peak is seen just beyond the α-2 area, especially in the ascending limb.

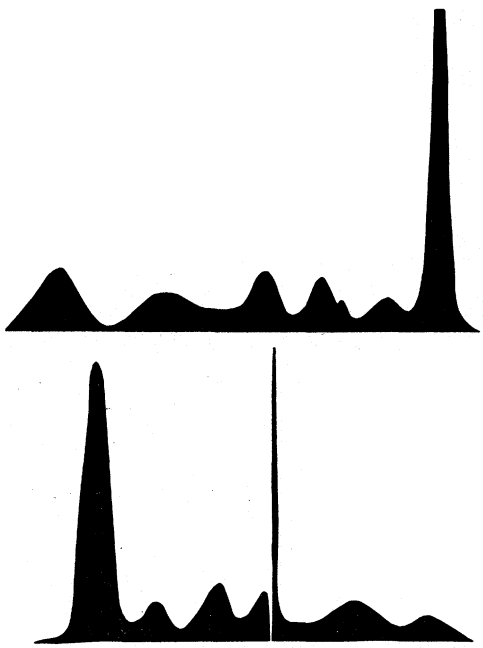

FIG. 2. Standard electrophoretic pattern of the 7-day serum. (Veronal buffer pH 8.6, ionic strength 0.1, 15 ma, 180 min.)

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.