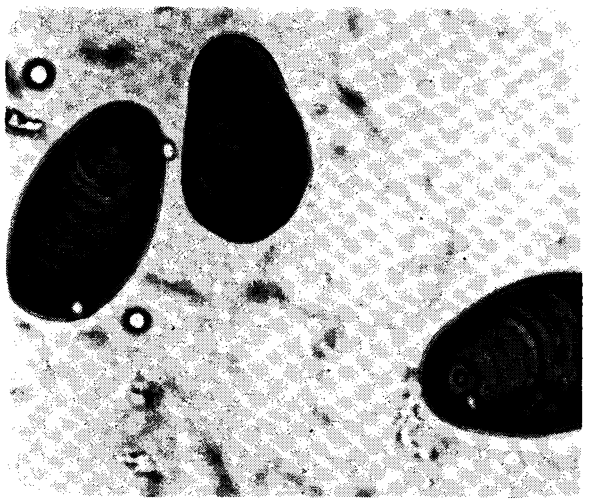

FIG. 1. Photomicrograph (400×) of typical starch grains of tubers of potato plants grown under conditions of constant light and temperature.

Electric radiation meter. The air temperature was 63° ± 2°F. The humidity was not controlled. The same variety of potato was grown under the varying light and temperature conditions of out-of-doors in northern Maine during the summer of 1952.

It was not possible to duplicate the "conditions" of the experiment as performed by Van de Sande-Bakhuyzen (*4*), since he notes that the temperature was "kept as constant as possible" but does not give figures for the range of temperature. He also states that "the temperature decreased for periods of 2 hr and 2½ hr during the time of the experiment 7° and 5°C, respectively." Regarding the light, he writes "under constant illumination by Mazda C lamps." Since no distance is given, it was not possible to ascertain the amount of illumination received by the plants.

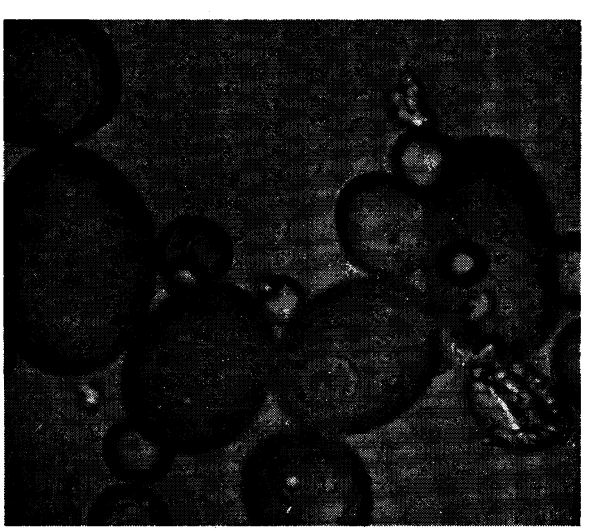

FIG. 2. Photomicrograph (400×) of typical starch grains of tubers of potato plants grown in Maine under field conditions.

Approximately 1000 starch grains from the tubers of plants grown under the controlled conditions of light and temperature and a like number from those grown under uncontrolled conditions out-of-doors in Maine were examined. In all instances, the "lamellations" or "layerings" were present. Previous examinations of the starch grains of potatoes grown out-of-doors at Yonkers, N. Y., were similar in appearance.

Starch grains formed in potato tubers of plants grown under a constant light source and a temperature of 63° ± 2°F showed lamellation superficially indistinguishable from starch grains formed in tubers grown under normal field conditions.

Figures 1 and 2 are typical photomicrographs.

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Received December 21, 1953.

Glucuronic Acid Conjugates of Aspartic and Glutamic Acids in Urine[1]

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It is known that amino acids are excreted in the urine in both free and combined forms. Woodson, *et al.* (*1*) found that only 1 percent of the aspartic acid and 10 percent of the glutamic acid of urine were present in the free state. Whereas peptide combinations may account for some of the bound amino acids in urine, conjugates of various other kinds are also present (*1*).

Although conjugation with glucuronic acid is one of the major detoxifying mechanisms in various species, glucuronic acid is also conjugated with certain normal metabolic products—for example, steroids. We present evidence here that aspartic and glutamic acids occur in urine, at least in part, as glucuronic acid conjugates.

One-hundred-milliliter portions of urine were dried as far as possible in vacuum on a water bath at 56°C.

[1] This work was aided in part by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

[2] From a thesis submitted to the faculty of the University of Tennessee in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] We wish to express our appreciation to T. P. Nash, Jr., and Gordon C. Mills for their valuable suggestions and advice and to acknowledge the able technical assistance of Bryan G. Mordecai. Appreciation is extended to the Rohm and Haas Co. for their contribution of the various ion-exchange resins used.

The remainder of the water was removed by azeotropic distillation with ethylene dichloride under vacuum (*2*). A dry but gummy residue (I) was obtained which was exceedingly hygroscopic. While still in the drying flask, residue I was extracted twice, for 1 hr each time, with 50-ml portions of 0.2*N* dl-camphorsulfonic acid (CSA) in dry acetone (*3, 4*).

The extracts (II) containing amino acids and their derivatives possessing free amino groups were decanted from the insoluble components of residue I, and the amino compounds were precipitated by the addition of triethylamine. The supernatant fluid, which retained the triethylamine salt of CSA (*5*), was decanted and the precipitate was washed several times with fresh dry acetone. Residual acetone was removed in vacuum, leaving a dry stable powder (III).

Tests on fraction III were strongly positive for glucuronic acid, both with naphthoresorcinol (*6*) and with the more specific carbazole reaction (*7*).

Fraction III was dissolved in 100 ml of water and passed through an ion-exchange column (IR-4B, Rohm and Haas), which was prepared by the method of Sanger and Tuppy (*8*). The column was then washed with deionized water until the washings were negative to ninhydrin, thus indicating that the column was freed of unadsorbed compounds.

The column adsorbate was eluted with 0.1*M* sodium tetraborate or citrate until the naphthoresorcinol test indicated that the elution of the glucuronic acid conjugates was complete (*9*). The total eluate was evaporated to dryness in vacuum and extracted with diethyl ether to remove interfering substances. Residual ether was removed by evacuation and the remaining solids (IV) were extracted with absolute ethanol. Two fractions were thus obtained, one ethanol soluble (V) and the other ethanol insoluble (VI). Examination of these two fractions by ascension chromatography failed to reveal the presence of free amino acids or free glucuronic acid. However, development of the chromatograms with aniline hydrogen phthalate and alkaline potassium permanganate (*10*) disclosed a reducing substance.

Portions of fractions V and VI were made 1*N* with respect to HCl and were hydrolyzed by autoclaving at a pressure of 10 lb/in.^{2} for 4 hr. The hydrolysates were evaporated to dryness, redissolved in water, and reevaporated. The process was repeated several times to remove residual HCl. Paper chromatography, as well as microbiological assay procedures, established the presence of glutamic acid with only traces of aspartic acid in the ethanol-soluble fraction, whereas by the same techniques aspartic acid was found mainly in the ethanol-insoluble fraction VI.

In preparing dry urine solids (I), there is the possibility that conditions used may have produced the combination of glucuronic acid with glutamic or aspartic acid. However, a portion of lyophilized urine yielded results identical with those obtained on the product from the azeotropic distillation.

The results indicate the presence of a heretofore unreported type of conjugate in urine. Experiments to establish proof of structure, as well as to determine the amounts excreted, are in progress.

References and Notes

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Received January 25, 1954.

Communications

The Brocken Spectre

In your issue of January 29 [*Science* **119**, 164 (1954)], there is a letter from Donald M. Black regarding "The Brocken Spectre of the Desert View Watch Tower, Grand Canyon, Arizona." Mr. Black is evidently under the impression that this phenomenon, commonly called a "glory," is seen very infrequently. In fact, it has become a very usual observation since flying above the clouds became common. All that is required is that the aircraft be between the sun and a cloud and that the observer can see the shadow on the cloud. The aircraft itself is unnecessary except as a carrier of the observer, since the phenomenon is backward scattering from the water droplets in the cloud and really surrounds the line from the sun through the observer's eye.

In the older literature that deals with atmospheric phenomena, the glory is not explained theoretically, but an attempt at an explanation is given by H. C. van de Hulst under the title, "A theory of the anticoronae" [*J. Opt. Soc. Am.* **37**, 16 (1947)]. No definite experimental verification of this theory has been made, but it seems probable that it is correct.

I have taken some small-scale color photographs from an aircraft over the ocean, but a complete check could be made only from an extensive program of