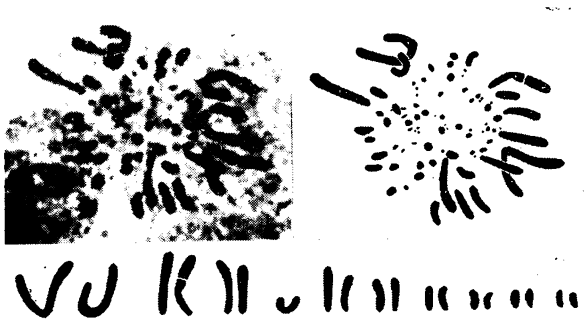

FIG. 1. Chromosomes of a cell in metaphase from a pin feather of a golden pheasant hen. Top left, photomicrograph. Top right, camera lucida drawing of the same cell. Bottom, serial alignment of major elements.

Preculturing technique. It has been found that a brief culturing of growing feather tissue in a hypotonic balanced salt solution results in a marked separation of the chromosomes. This procedure seems to result in an increase in the number of dividing cells, particularly of cells in late prophase. This increase is probably caused by an inhibition or slowing down of cells in metaphase and anaphase, as shown by the work of Hsu and Pomerat (*4*), who studied the chromosomes of mammalian material in hypotonic media (*4, 5*). The addition of paradichlorobenzene to the salt solution appears to enhance the spreading effect, as previously noted for guayule and numerous other plants by Meyer (*6*).

In actual practice, the procedure has been as follows. Holtfreter's solution is saturated with *p*-dichlorobenzene and filtered, then diluted to half strength with distilled water and heated to 41°C. Air or oxygen bubbles are passed through the medium while culturing. The latter process has been found to improve the resulting preparation, probably by supplying sufficient oxygen and by increasing the diffusion of toxic waste products through the stirring action of the rising bubbles. The temperature can be held constant by the use of a water bath.

Tissue is prepared for culturing in the following manner. The sheath of a freshly plucked pin feather is slit lengthwise for a distance of ½ to 1 cm. With a clean scalpel, the proximal millimeter or two of the feather base is gently scraped off the feather sheath and placed in the culture medium.

After culturing for 10 to 20 min, the material is transferred to a deep depression slide containing aceto-orcein, and is stained from 1 to 2 hr. Small pieces of this stained specimen can then be removed and prepared for examination.

In addition to the increased spreading of the chromosomes and the increase in the number of mitotic configurations, the chromosomes appear to be shorter and thicker. Stainability is probably somewhat reduced but not seriously so. The majority of dividing cells appear to be in late prophase. Although the prophase chromosomes are typically rod-shaped, the primary constrictions of the larger ones are clearly evident. A number of excellent metaphase configurations also can be found. These are generally better for study than cells in prophase, because in the latter the chromosomes are spread apart to such an extent that those of adjacent cells often overlap.

The chromosomes of a cell in metaphase from a pin feather of a golden pheasant hen are shown in Fig. 1. The tissue had been cultured for 10 min and was examined as a fresh aceto-orcein squash preparation. A Leitz apochromatic oil-immersion objective (60× N. A. 1.4) was used. The photograph suggests the presence of a helix in some of the chromosomes, due probably to the fixation.

The technique is definitely usable and will, undoubtedly, be improved by further experimentation. It is to be hoped that these methods will facilitate an elucidation of the cytogenetic affinities of recent avian species.

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Received February 5, 1954.

The Appearance of Starch Grains of Potato Tubers of Plants Grown Under Constant Light and Temperature Conditions

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The external markings of the starch grain have been described as "striations" (*1*), "lamellae" (*2*), "striations" (*3*), "laminations or rings" (*4*), "lamellae or layered" (*5*), "striations or lamellations" (*6*), "layerings" (*7*), and "layers and laminations" (*8*).

Van de Sande-Bakhuyzen (*4*) was the first to ascribe these markings to the effect of external conditions. He noted that when the external conditions, illumination and temperature, are constant, "lamination" of wheat starch grains did not occur. Statements found by the writers which claim that these markings are absent in starch grains of plants growing under constant conditions of light and temperature refer only to the work of Van de Sande-Bakhuyzen (*4*).

For this study, Dr. John M. Arthur, of Boyce Thompson Institute of Plant Research, kindly offered to grow plants of the Katahdin variety of potato in a Constant Condition Light Room. These plants were under an AH-9 G.E. 3000-w lamp, which burned continuously from November 12, 1952 to June 3, 1953. The light intensity at soil level (about 42 in. from the lamp) was 450 ft-candles, as measured by a General Electric light meter. At a distance of 24 in. from the lamp tube, the meter reading was 650 ft-candles of 0.15 gram-cal/cm² min, as measured by a General

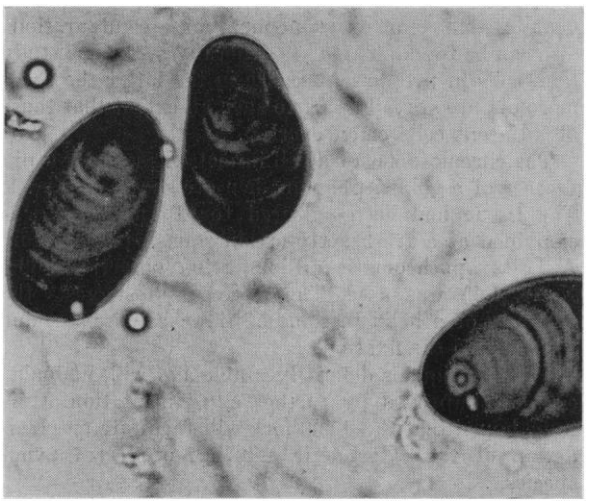

FIG. 1. Photomicrograph (400×) of typical starch grains of tubers of potato plants grown under conditions of constant light and temperature.

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.

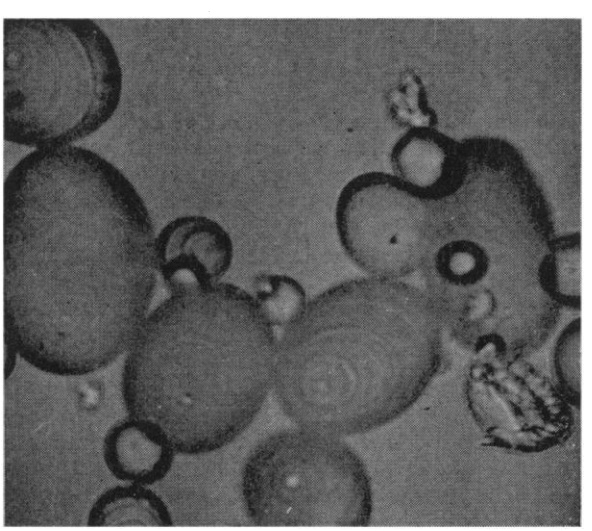

FIG. 2. Photomicrograph (400×) of typical starch grains of tubers of potato plants grown in Maine under field conditions.

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.