ber of fruits) contains rind and flesh in the same proportion as the whole oranges. The authors' samples are composed of eighth segments, each of which is obtained by making two longitudinal cuts at right angles, followed by an equatorial cut. All oranges are oriented similarly for corresponding cuts, and the personal bias is corrected by selecting left and right pieces alternately for individual oranges.

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STARCH GRANULE FORMATION

Development of Starch Granules in Corn Endosperm

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With the aid of the electron microscope, starch granules are observed in corn endosperm as early as 4 days after pollination. Granules seem to originate in amyloplasts, which often rupture as the granules mature. Central cavities are rarely observed in undried granules and lamellae are seen in less than 15% of the granules. Granules, approximately 15 cells from the aleurone, are largest and the size decreases toward either the center or periphery.

STARCH GRANULES have been examined extensively with the light microscope, and their origin and development have been described (*1, 3, 4, 6, 11, 14*). Most investigators believe that starch granules originate in the amyloplasts (*4, 6, 9–11, 15, 16, 22, 25*). Weier (*22*) and Guilliermond (*10*) consider that amyloplasts are derived from the chrondriosomes and Badenhuizen (*4*) considers that the chrondriosomes are filiform. Duvick (*7*) and Shaw (*20*) conclude that amyloplasts contain phosphorylase, an enzyme needed for the synthesis of starch.

Duvick (*6*) describes large-knobbed filaments (plastids) which give rise to starch granules. The largest knob in any given cell is the first to form starch, but not all such knobs form starch.

Numerous previous investigators consider starch granule formation to begin some 10 to 15 days after pollination (*4, 6, 11, 12, 14, 25*).

Badenhuizen (*4*) assumes that starch chains of various lengths are present in the amyloplast at the time of starch granule formation. When a given number of chains of sufficient length are available, they precipitate, thus forming the young granule. The region of the amyloplast, immediately surrounding the granule, is thus depleted of carbohydrates. As the supply of carbohydrate material to the amyloplast is not constant, the whole amyloplast may become exhausted. This deficiency is overcome, possibly, by diffusion. Badenhuizen concludes that these processes could explain the origin of lamellae.

Another less satisfactory theory considered by several workers (*2, 13*) is that a starch coacervate crystallizes *in toto* to produce a granule. Rhythmic crystallization in the droplet could lead to lamellae.

Reichert (*17*) describes fissures or central cavities in mature corn starch granules and such cavities were observed in ultrathin sections by Whistler and Turner (*23*).

Methods

Corn kernels were obtained from common yellow field corn, single-cross Wf-9x38-11, grown on the Purdue University Agronomy Farm and pollinated by hand.

The methods of collecting, sorting, transporting, subdividing, and killing were those used by Sass (*19*). Kernels were collected every other day starting at 4 days after pollination and ending at 72 days.

Samples were removed from the killing solution and placed in an osmium tetroxide solution for 6 hours. This solution consisted of 1% osmium tetroxide in a veronal-acetate buffer at pH 7.4. After removal from the osmium tetroxide solution, samples were washed in cold distilled water for 1 hour.

For dehydration, samples were stored successively for at least 8 hours in 10, 25, 50, 75%, and anhydrous dioxane, with three changes of the latter.

Samples were impregnated and embedded in methacrylate (20% methyl methacrylate in *n*-butyl methacrylate, containing 0.4%, 2,4-dichlorobenzoyl peroxide as a catalyst). For impregnation, samples were stored successively for 8-hour periods in 10, 25, 50, and 75% solutions of methacrylate in dioxane and finally in 100% methacrylate, with three changes of the latter. After the final 8-hour storage, the mixture was polymerized in No. 0 gelatin capsules at 45° to 48° C. for 48 hours.

Sections were made with a wedge-

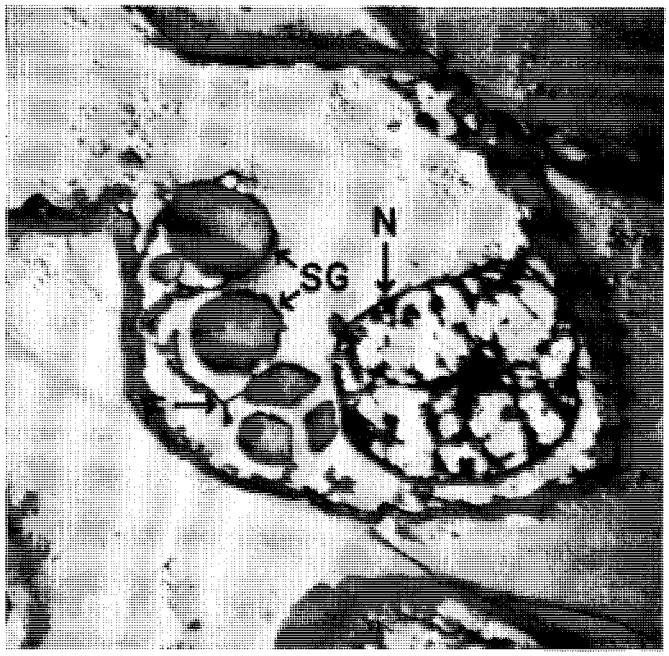

Figure 1. Cross section of kernel 4 days after pollination

Heavy line is cell wall
SG. Starch granule
F. Knobbed filaments
N. Nucleus

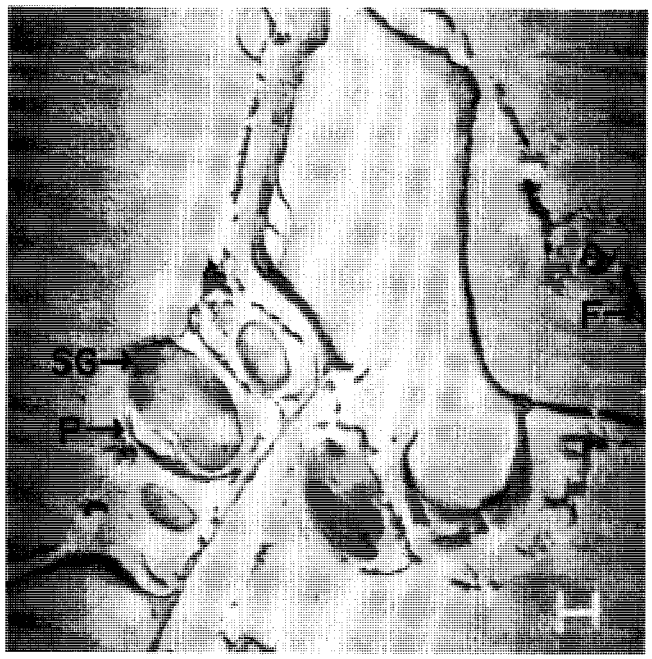

Figure 2. Section at 6 days

SG. Starch granule
P. Plastid membrane
F. Knobbed filaments

modified Spencer Model 820 rotary microtome, using glass knives mounted in a rocking knife holder adapted for this microtome from the design of Dempsey and Lansing (*5*). Sections, 0.1 micron thick, were cut perpendicular to the long axis near the distal end of the kernel approximately 15 cells in from the aleurone in the floury endosperm. Cut sections were placed on grids with a silica membrane. These silica coated grids were prepared by evaporating an approximate 100 A. layer of silica on collodion-covered grids, from which the collodion was later dissolved by immersing in acetone.

The specimen on the supporting silica coated grid was submerged in toluene for 2 minutes to remove the embedding material and was then shadowed lightly with chromium at an 18° angle.

Specimens prepared in this manner were observed in the R.C.A. Model EMU-2D electron microscope.

Results

Starch granules are observed in corn endosperms 4 days after pollination (Figure 1) and are therefore possibly present even earlier. The presence of

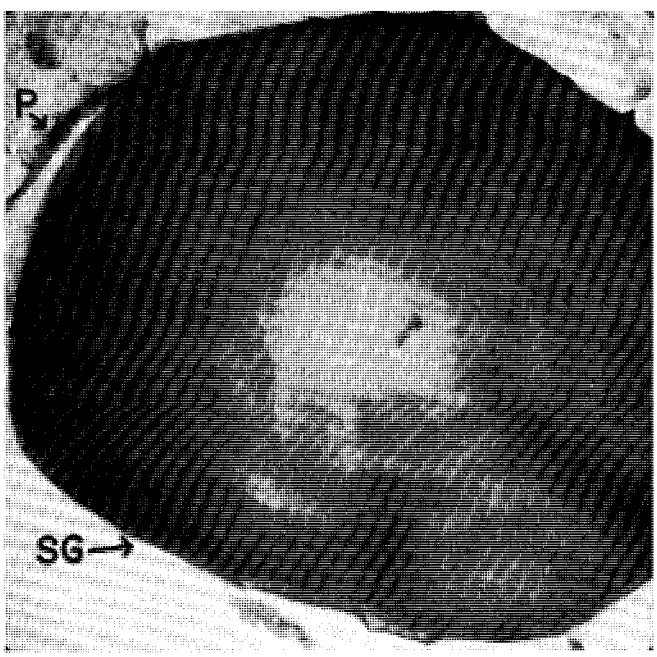

Figure 3. Section at 18 days, 25,000×

SG. Starch granule
P. Plastid membrane

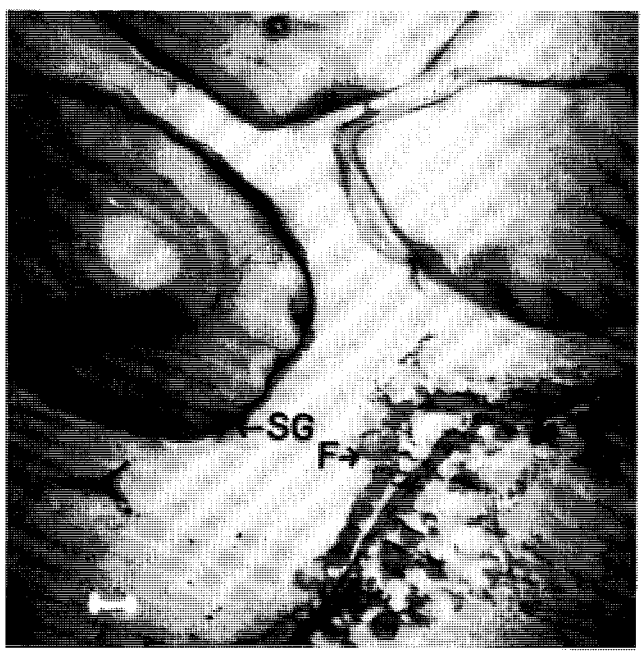

Figure 4. Section at 54 days, rings

SG. Starch granule
F. Knobbed filaments

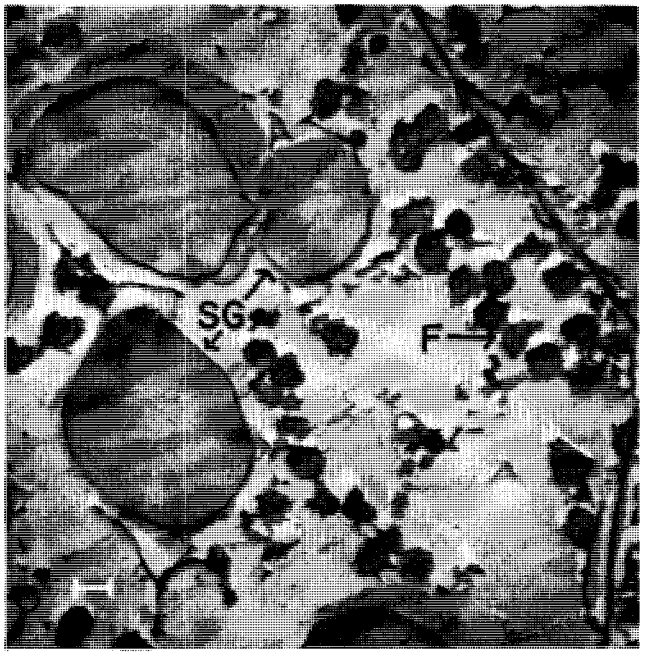

Figure 5. Section at 20 days

SG. Starch granule
F. Knobbed filaments

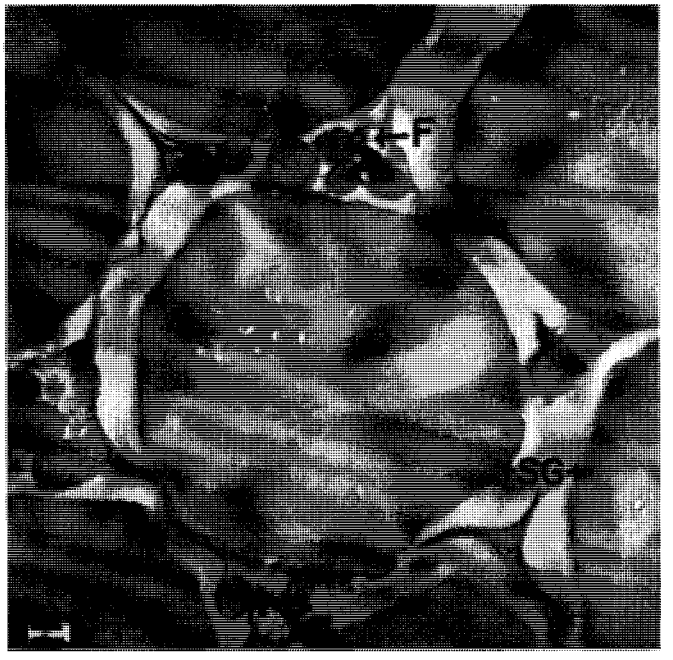

Figure 6. Section at 60 days

SG. Starch granule
F. Knobbed filaments

starch at the 4-day stage is further confirmed by treatment of 4-day sections 1 micron thick with iodine solution and observation of the characteristic blue color. These early formed granules are usually 2 microns or less in diameter and rarely exceed 2 to 3 microns. Consequently the granule, 3 microns in diameter shown in Figure 1, is exceptional. All micrographs were made at 7230 ×, except as otherwise indicated. The measuring bar is equal to 1 micron. Induction of spherocrystal formation by laboratory manipulation of the tissue is not excluded but does not seem likely. The smallness of early granules as well as differences in grain due to season and variety may possibly be the reason why large granules have not been seen previously in corn. Sandstedt (*18*) has observed starch granules in wheat kernels 4 days after pollination.

The young starch granules are surrounded by a membrane which is undoubtedly the membrane of the amyloplast (Figures 1 and 2). As the granules mature, they completely fill the amyloplast and often rupture it. Fragments of the amyloplast membrane may be seen attached to granules at all stages of ma-

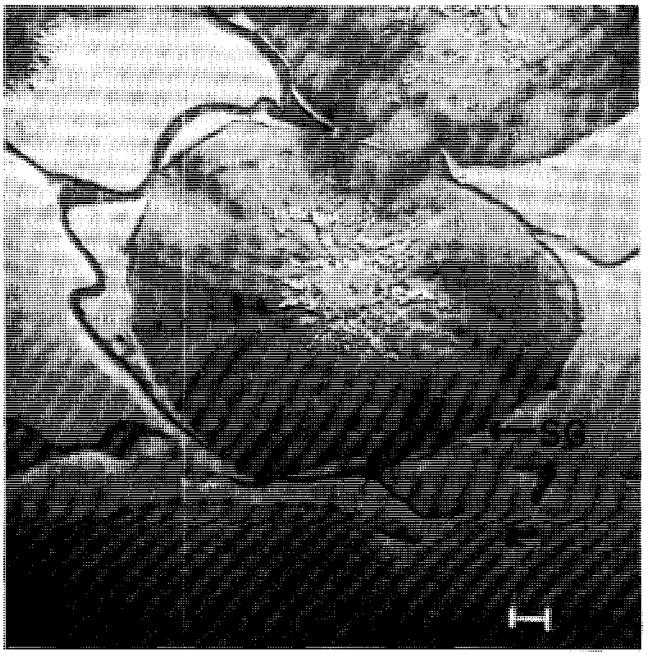

Figure 7. Section at 72 days

SG. Starch granule

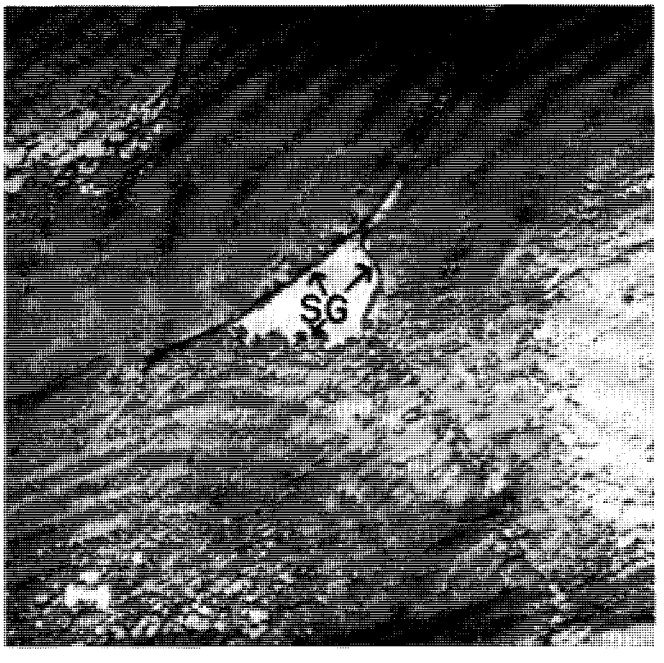

Figure 8. Horny section at 70 days

SG. Starch granule

turity beyond 12 days after pollination (Figure 3). Because of the supporting strength of the embedding material, it seems unlikely that the membrane would be lost, although it might rupture slightly during sectioning. Somewhat similar electromicrographs of older tissue have been shown by Southwick (*21*).

Rupture of the membrane during growth might explain why lamellae are observed in less than 15% of the granules. Diffusion difficulties could cause lamellae formation in granules surrounded by intact plastid membranes. However, if the membrane were ruptured, starch synthesis would not be hindered by membrane diffusion and a more even granule development could occur. An electromicrograph of a cross section of corn endosperm 54 days after pollination is shown in Figure 4. Lamellae are seen around the center, but not in the exterior portion of the granule. Here, the amyloplast may not have ruptured in the early stages of granule development, but at a later stage; perhaps 25 to 35 days after pollination. In general, lamellae are more often seen in large granules than in very small granules. There is no explanation of this observation at present.

Electron micrographs indicate that starch granules of corn floury endosperm increase in size as the kernels mature (Figures 1, 2, 5, 6, and 7). The outline of the granules does not appear to change appreciably from its general circular pattern. However, granules of the horny endosperm are more polygonal than those of the floury (Figure 8). Perhaps this arises because the granules within the horny endosperm are more tightly compressed, in comparison to the loosely packed granules of the floury endosperm.

The size of the granules, during development, ranged from less than 1 micron at 4 days after pollination (Figure 1) to approximately 30 microns at 72 days after pollination (Figure 5). The average size of mature granules is approximately 15 microns. The size of granules 10 days after pollination (2 to 3 microns) to maturity corresponds to that reported by Wolf and coworkers (*24*) and by Evans (*8*). It is also observed in agreement with Duvick (*6*) that starting at about the 13th cell in from the aleurone, corn starch granules decrease in size and in number, going from this point to the periphery. In fact, the observation holds regardless of the stage of maturity of the corn kernel. This size distribution may be due to the flow of soluble carbohydrate raw material through the kernel after its entrance at the proximal end. While the endosperm is still coenocytic (*6*), the soluble carbohydrates should be fairly evenly distributed through the endosperm. After the cell walls are formed between the nuclei, about 3 to 4 days after pollination (*6*), the soluble carbohydrates must diffuse through the cell membranes from the intercellular spaces. It seems that there should be a concentration gradient of soluble carbohydrates from the proximal to the distal end of the kernel. As the carbohydrate supply moves up the kernel, the main line of transport should be in the region just outside the embryo. Thus a lateral concentration gradient might be expected, and there should be smaller and fewer granules on either side of the region of highest concentration of soluble carbohydrates.

The knobbed filaments described by Duvick (*6*) may be seen in Figures 1 and 5 through 7. These knobs are less than 0.5 micron at 4 days after pollination and increase to about 2 microns at 60 days. The knobbed filaments are well defined up to 38 to 40 days after pollination. Thereafter, in many cases, they appear to aggregate, with the filament between knobs no longer visible (Figure 6). However, well defined knobbed filaments are observed at all stages of maturity.

Yasui (*25*) believes the starch granules of corn endosperm originate as compound granules and later become simple granules. In contrast to this, most of the starch granules observed in the present work are simple granules, although some compound granules occur.

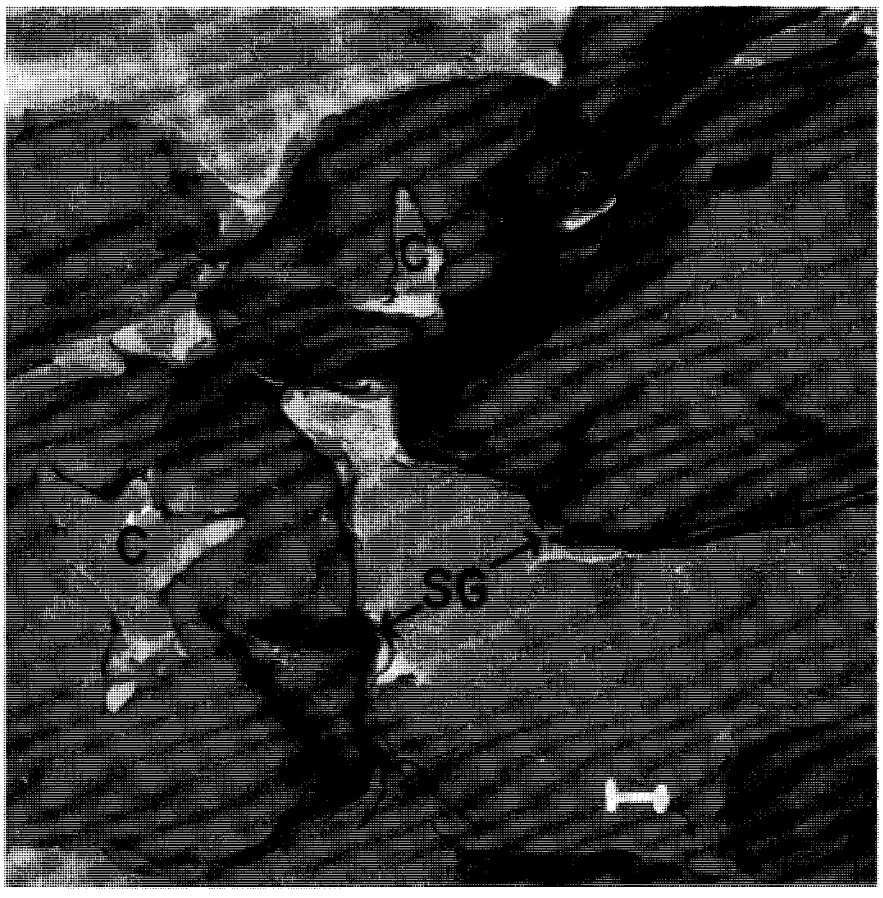

Figure 9. Section of dried mature kernel

SG. Starch granule
C. Central cavities or fissures

The hilum in mature cornstarch is described by Reichert (*17*) as a round spot or irregularly shaped cavity which is usually fissured. Whistler and Turner (*23*) observed cavities of this type in ultrathin sections of mature cornstarch. Such cavities are rarely observed in undried sections of kernels, but are observed in sections prepared from dried mature kernels (Figure 9). Cavities are therefore probably formed as the result of dehydration of the kernel.

Sections, 0.1 micron in thickness, regardless of the stage of maturity, fail to exhibit birefringence when placed under cross Nicols of the polarizing microscope. These ultrathin sections probably do not contain sufficient crystallites for a cross to be observed, since a well-defined cross is observed in 1-micron thick sections.

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FOOD STORAGE EFFECTS

Changes in Light Reflectance and Ascorbic Acid Content of Foods During Frozen Storage

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Reflection measurements made by a General Electric recording spectrophotometer and reduced ascorbic acid values determined by a conventional method of assay on samples prepared in a uniform manner from frozen fruits and vegetables stored at +10°, 0°, and −20° F. for 12 months reveal that the storage temperature had a very definite effect on the capacity of the foods to reflect visible light and on the reduced ascorbic acid content of the foods. The greatest change in reflectance and in vitamin content occurred in foods that had been stored at the higher temperatures. Changes in the reflectance of the foods during frozen storage seem to parallel changes in ascorbic acid content.

Color is recognized as one of the important factors in determining the acceptability of foods to the consumer. Proper color is usually regarded as an indication of the quality of a natural food. While a degree of correlation exists between color and quality in some foods, it appears less definite in other foods. Color is a readily evident characteristic that is widely considered in the quality grading of food. Food control officials, in general, have included color as a quality characteristic in setting up standards for marketable foods. On the other hand, nutritional investigators have associated changes in ascorbic acid content with changes in food quality.

Visual differentiation in food colors is subject to inherent errors which are dependent upon a number of factors, including the grader's ability to detect small color differences and to correlate these differences with the different grades of food. This ability is frequently affected by optical fatigue, lighting conditions, and other factors.

In recent years attempts have been made to eliminate the personal element in the measurement of color changes or color differences in foods. Some devices, as well as their application, have been described (*1–5, 8*). One device which has performed satisfactorily in measuring reflectance directly and color indirectly and has yielded reproducible data under properly controlled operating conditions is the General Electric (Hardy) recording spectrophotometer, used for the reflectance measurements reported here. While the reflectance data recorded by this instrument constitute informative and useful information, this information is not identical with that obtained through visual inspection. The instrument measures physical properties directly, which may be translated indirectly into color com-