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AN AUTORADIOGRAPHIC DEMONSTRATION OF THE PENETRATION OF WATER INTO WHEAT DURING TEMPERING

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ABSTRACT Cereal Chemistry 53(2): 141-149

Autoradiography was used to study the movement of temper water into the bran, germ, and starchy endosperm of wheat prior to milling. At short time intervals after damping, water was heavily concentrated in the germ and bran layers. There appeared to be a rapid entry of water where the germ met the bran on the dorsal side of the grain. Subsequent movement of water occurred mainly in the dorsal region of the grain and only later in the central crease regions, although diffusion from the bran was occurring in all regions. The rate of penetration varied for different cultivars but the mode of movement was essentially the same.

The mode of entry of water into the wheat grain during tempering has in the past created considerable interest because of its importance in milling. Ideally the grain should be in a state where the bran (including aleurone) remains sufficiently moist to avoid fragmentation during milling, yet the endosperm must also be sufficiently moist to produce a satisfactory flour moisture content but not so moist as to adversely affect the friability of the endosperm. The bran/starchy endosperm interface should also be in a condition that allows easy separation.

Most of the previous work on the penetration of water into wheat has been based on total immersion of the wheat grains in water. Those studies reviewed by Bradbury *et al.* (1) indicated that water entered the grain first and most abundantly through the germ end and later through the bran and beard end of the grain. Immersion of the grains is not comparable to present commercial tempering practice, however, except in cases where short immersion times are used in a washer/whizzer. In a majority of cases, tempering is carried out by the addition of water to wheat in a worm-screw, followed by a time interval before milling. Under those conditions, the water would be expected to be quickly bound by the bran, followed by its diffusion inwards.

Seckinger *et al.* (2) have studied the moisture penetration during tempering of a hard red winter wheat using an iodine staining technique and Campbell and Jones (3) relied upon the variation of endosperm density with moisture content to study the movement of moisture into Manitoba wheat during tempering. Both of those methods relate only to the endosperm, however, and give no indication of water movement in either the bran or germ. Recently an autoradiographic technique was used to follow the rate of movement of tempering water into grain (4). In the present work, this technique has been used to follow water movement during tempering in more detail, with particular reference to initial movement through the germ and bran layers. Subsequent movement of moisture through the endosperm in various grain regions was also investigated. A range of wheat types representative of those currently grown in the U.K., was examined.

MATERIALS AND METHODS

Samples of the cultivars Sappo and Maris Dove, both hard English spring wheats, and West Desprez and Maris Nimrod, both soft English winter wheats, were studied (Table I). The protein content was determined by the Kjeldahl method (N × 5.7), moisture content by oven drying at 130°C for 1 hr, and grain hardness as grinding resistance (5).

The method of Butcher and Stenvert (4), with minor modifications, was used to follow the movement of water. Cleaned wheat (10 g) was placed in a small flask and conditioned with the appropriate amount of tritiated water (100 mCi/ml) to produce a final moisture content of 15.5 and 16.0% for the soft and hard wheats, respectively. Temper time ranged from 1 to 12 hr at 20°C before snap-freezing with liquid CO_2 (−80°C) to prevent any further movement of water. Individual grains were embedded in Tissue Tek O.C.T. compound (Ames C, Miles Laboratories), immediately refrozen with liquid CO_2, and sectioned on a Cambridge rocking microtome. The sections were removed and the surface of the wheat grain remaining in the embedding medium was placed in contact with stripping film (Ilford Type K2). After remaining in contact with the film for 72 hr (−30°C), the section was removed and the film developed.

The autoradiographs produced were assessed both on a macroscopic and microscopic level—macroscopically to gain an indication of the gross movement of water through the various regions of the grain and microscopically to investigate movement in the germ and bran layers. The advantages of tritiated water in following moisture movement into grain are that its rate of penetration is only marginally slower than normal water and it is a weak β-emitter, which allows good resolution in the autoradiographs. Control autoradiographs were carried out with each batch consisting both of a nonradioactive section on unexposed film and a radioactive section on exposed film. Results in all cases were negative, indicating that no artifacts were introduced by either pressure or chemography, nor was negative chemography a problem.

TABLE I
Protein Content, Moisture Content, and Grain Hardness of Two Hard Spring (H.S.) and Two Soft Winter (S.W.) Wheats

Cultivar	Protein %	Moisture %	Grinding Resistance sec
Sappo, H. S.	11.9	12.5	48
Maris Dove, H.S.	12.2	11.8	46
West Desprez, S.W.	13.0	12.0	26
Maris Nimrod, S.W.	8.5	12.5	23

RESULTS AND DISCUSSION

Microscopic Entry of Water

Examination of a longitudinal section (L.S.) through the germ, 1 hr after tempering the cultivar Sappo (Fig. 1), indicates that there has been considerable

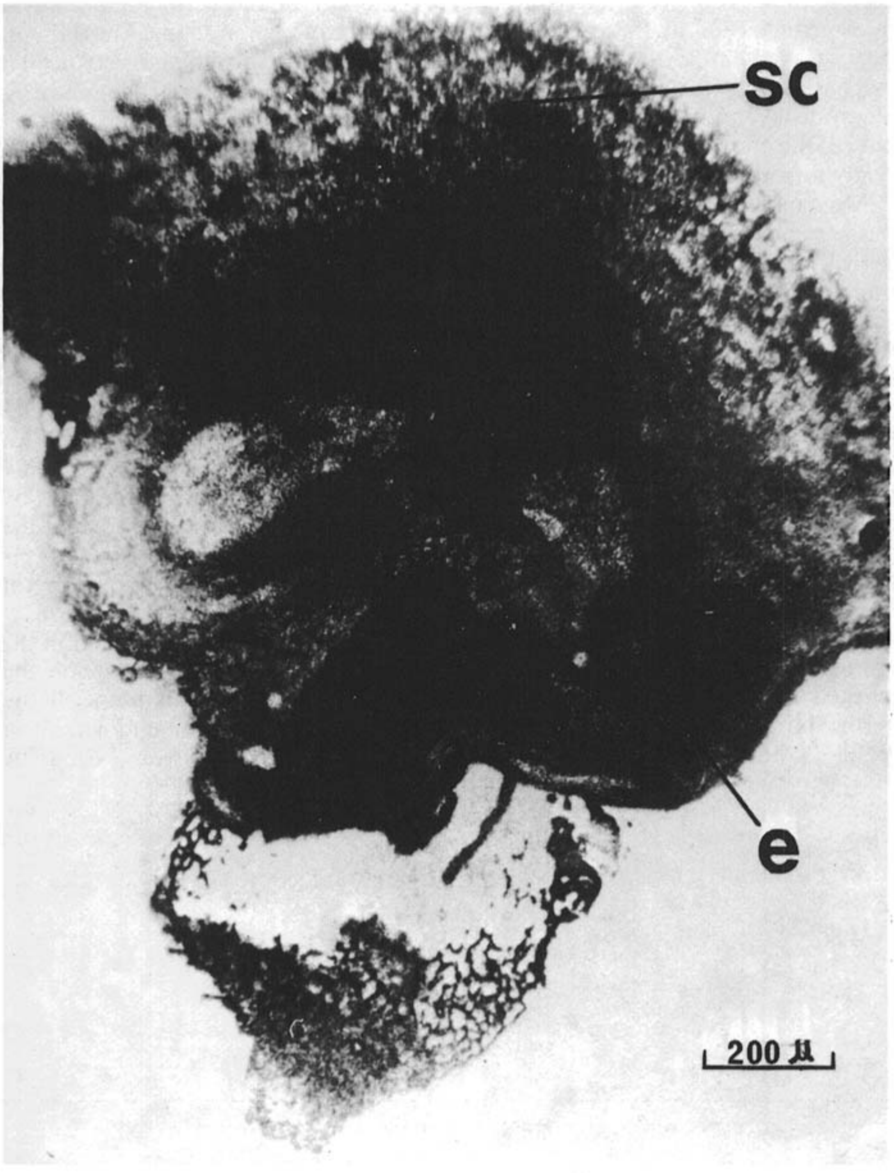

Fig. 1. Location of water in the germ of the cultivar Sappo 1 hr after damping, Longitudinal Section Dorsal. sc = scutellum; e = embryo.

penetration of water. Both the scutellum and embryo are well defined but there has, as yet, been very little movement from the center of the scutellum into the starchy endosperm, although this has occurred at the boundary of the bran and germ. This rapid movement of water into the germ as a whole can be explained by the microscopic structure of the grain since the bran is structurally altered in this region (6,7). Numerous intercellular spaces occur in the germ region where intermediate cells are flattened and cross cells are irregular in shape. The thinnest portion of the outer layer of the testa occurs over the embryo and the nucellar layer is also absent over the greater part of the germ. Furthermore, a modified aleurone layer of thin walled cells extends over the edge of the scutellum and over part of the embryo. Thus, conditions over the germ region are such as to allow a ready absorption of water into the germ.

Movement of water in the bran layers can be gauged from Fig. 2a. The bran is in the region close to the germ in the same L.S. as above. It is apparent that the pericarp region has readily absorbed water as would be expected from its rather porous structure, but a number of dark projections are also evident where the water has moved into the aleurone cells from the pericarp. This movement of water is not uniform, suggesting less permeability in certain areas, possibly because of slight variations in structure. It is not possible to determine accurately whether it was the testa or hyaline layer offering the initial resistance to moisture movement, although from the work of Hinton (8) the testa is more likely. An interesting point is that there was a considerable quantity of water already present in the starchy endosperm in this region, 1 hr after damping, caused by diffusion inwards from the bran and movement of water from the edge of the germ region. A similar situation existed in the bran nearer the beard end of this section (Fig. 2b) where the aleurone cells are again becoming well defined, but it is apparent that much less water has entered the starchy endosperm.

After 3 hr (Fig. 3, L.S. through germ), the water is evenly distributed in the bran (near center) as a whole and is diffusing inwards. It is interesting to note the marked boundary between bran and starchy endosperm at this point. If the permeability of the aleurone layer were similar to the starchy endosperm, a regular gradient from the aleurone to the starchy endosperm would occur. A

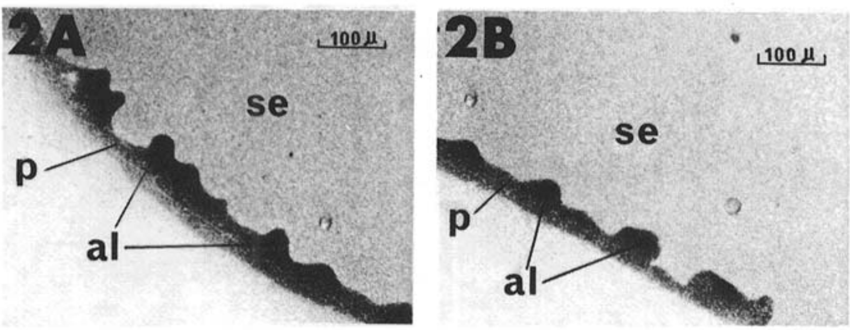

Fig. 2. Location of water in the bran layer and starchy endosperm near the a) edge of the germ and b) center of the grain for the cultivar Sappo 1 hr after damping, Longitudinal Section Dorsal. se = starchy endosperm; al = aleurone cell; p = pericarp.

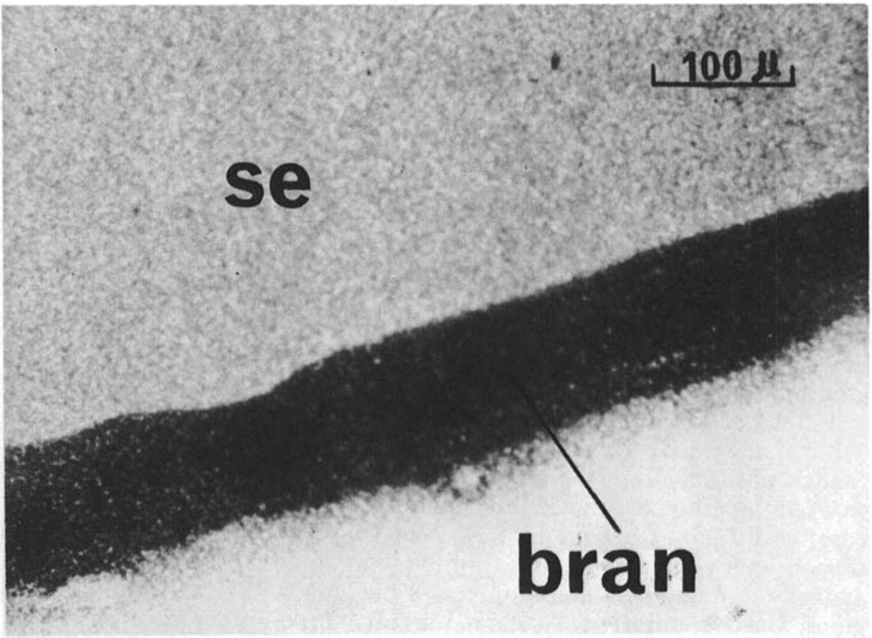

Fig. 3. Location of water in the bran layer and starchy endosperm near the center of the grain for the cultivar Sappo 3 hr after damping, Longitudinal Section Central.

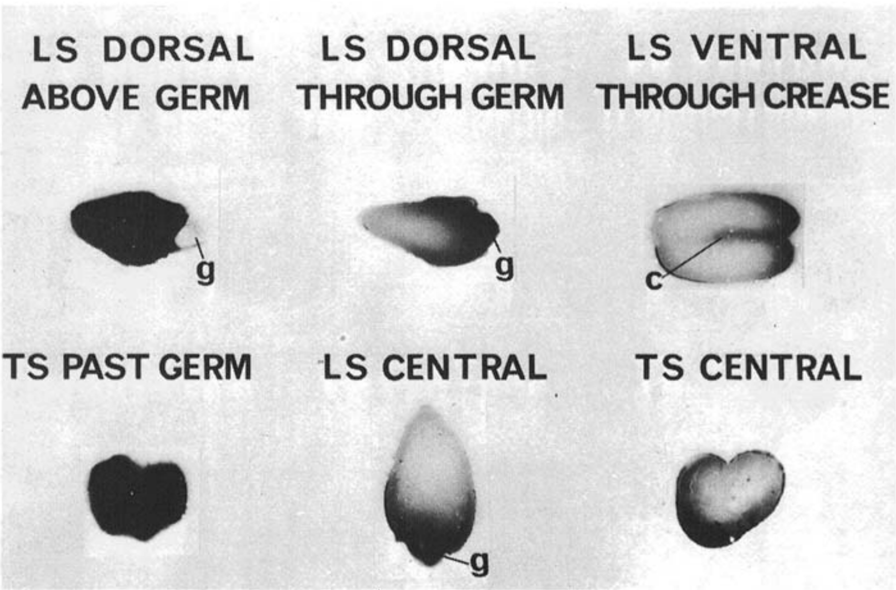

Fig. 4. Location of water in the various portions of the grain 1 hr after damping for the cultivar Maris Dove. g = germ; c = crease.

regular moisture gradient does exist but only in the starchy endosperm, indicating that the movement of water is more rapid in the starchy endosperm than in the aleurone layer. This relates to previous work (9) where it was indicated that the presence in bran of strongly hydrophilic components would regulate the movement of moisture into the starchy endosperm.

Macroscopic Movement through the Grain

Having established the initial mode of entry of water into the wheat grain, the gross movement through the various portions of the grain was examined. The dependence of the movement of water on the position in the grain is shown by Maris Dove 1 hr after damping (Fig. 4). The dorsal movement of water, especially near the upper edge of the germ, is very evident. In the center of the grain there is still some movement into the starchy endosperm from the germ region but this is not as marked. In the crease region there has been very little penetration, and movement here is mainly by diffusion inwards from the bran.

After 3 hr (Fig. 5) the heavy concentration of water dorsally is still evident although diffusion from the dorsal starchy endosperm is occurring into the central endosperm. Moisture is also moving into the endosperm in the center and crease by diffusion from the bran. In 6 hr (Fig. 6) this trend continues with diffusion occurring in all regions from the bran, and moisture moving almost diagonally through the grain, both across the grain and down from the dorsal regions. The concentration of water in the bran is becoming much less marked as equilibrium states are being attained, although this is still evident in the central regions of the grain. After 12 hr (Fig. 6) an equilibrium state appears to have been attained in all parts of the grain.

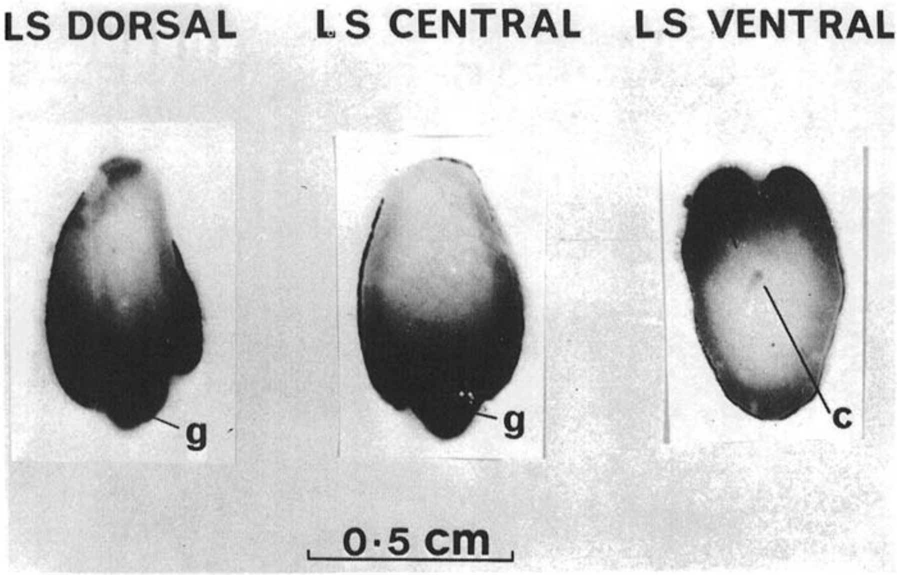

Fig. 5. Position of water in the various portions of the grain 3 hr after damping for the cultivar Maris Dove.

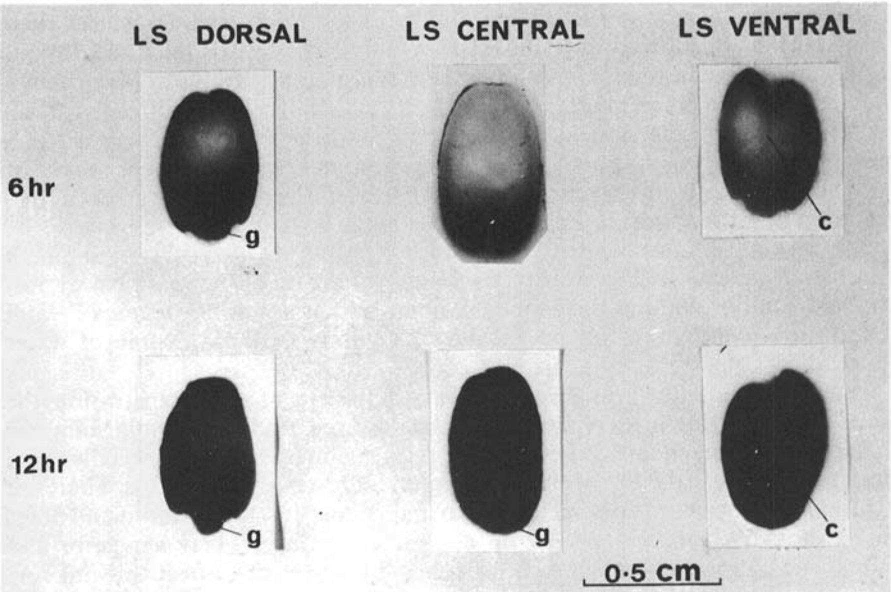

Fig. 6. Position of water in the various portions of the grain 6 and 12 hr after damping for the cultivar Maris Dove.

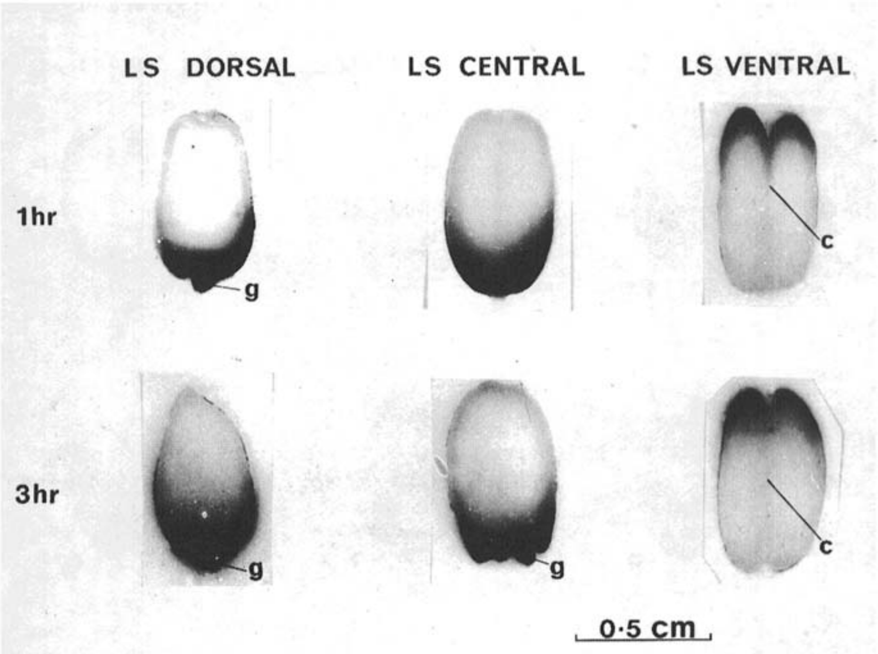

Fig. 7. Position of water in the various portions of the grain 1 and 3 hr after damping for the cultivar West Desprez.

The movement of water into the grain of the soft English winter wheat, West Desprez (Fig. 7), shows a very similar pattern to that of Maris Dove. The grains of this cultivar tend to be longer than the more spherical grains of Maris Dove, thus presenting a greater surface area of bran for the absorption of water. It can be seen that the water has largely absorbed into the bran, but there is still a preferential entry around the germ region. Subsequently, movement occurs by diffusion inwards from the bran region and by the initial dorsal movement across the grain and then into the center of the grain.

The very soft English winter wheat, Maris Nimrod, shows a very rapid rate of moisture penetration (Fig. 8). In 3 hr the dorsal region of the grain has almost attained equilibrium and the central region also has a marked degree of water penetration. The crease region, however, exhibits the least degree of water penetration.

Thus, although the various cultivars vary in the rate of water penetration, the mode of penetration in all remains basically the same. An initial binding of water to the bran and an enhanced entry in the germ region occur. The especially rapid entry of water near the top of the germ region indicates an easy access. There are natural lines of cleavage between embryo and endosperm and embryo and bran, and although there appears to be a "cementing layer" between germ and endosperm (10), it appears that treatment with water can affect this link. As indicated in the microscopic examination, the germ as a whole readily absorbs water due to the characteristics of the surrounding layers. This would produce swelling and a probable physical separation from the bran, which is already modified in this region, and would allow an easy entry into the grain and rapid

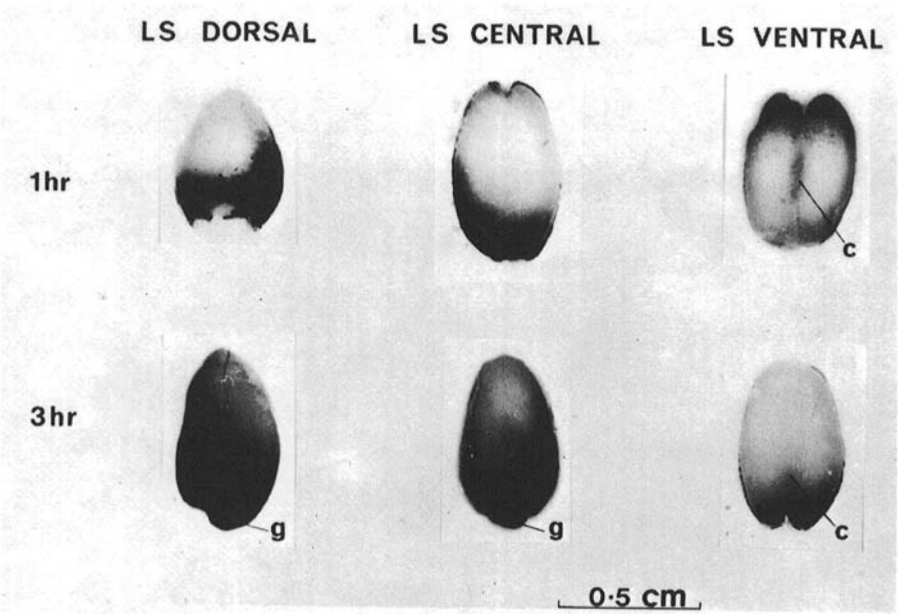

Fig. 8. Position of water in the various portions of the grain 1 and 3 hr after damping for the cultivar Maris Nimrod.

diffusion into the starchy endosperm. This effect would be expected to be more marked the greater the amount of tempering water added, although structural features due to cultivarietal factors could also be significant. The subsequent movement of water is basically in keeping with observations made by previous workers (2,3) who were able to investigate movement in the starchy endosperm, indicating a preferential movement into the dorsal region and only later into the central and crease regions. Although there are marked differences in the rate of penetration of water during tempering, especially with the cultivar Maris Nimrod, it is not clear whether this is due to its soft nature or its low protein content. It would, however, be expected to have a significant effect on its milling behavior compared to the other cultivars at time intervals shortly after damping. The significance of this is currently being assessed in conjunction with a study of the factors responsible for affecting the rate of moisture movement.

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