STARCH-SUGAR INTERCONVERSION IN *SOLANUM TUBEROSUM* L.

II. INFLUENCE OF MEMBRANE PERMEABILITY AND FLUIDITY[1]

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Abstract

A strong relationship was found between membrane permeability and starch to sugar conversion in stored potatoes. Tissue from tubers stored at low temperature had higher sugar content and more permeable membranes than from that of tubers stored at high temperature. Tubers, moisture stressed during growth and stored at 5.5 C, accumulated more reducing sugars and had more permeable membranes than tubers from normally irrigated plants when stored at the same temperature. The basal portion of Russet Burbank tubers accumulated more sugars and was also found to have significantly more permeable membranes than the apical portion. Examination of membrane order with spin-labeled lipid probes showed that the order parameter of amyloplast membranes was less at low temperatures than when measured at higher temperatures. Membranes examined with spin-labeled lipid probes from tubers stored at 5.5 C and 15.5 C showed similar order parameters at all temperatures measured between 0 and 25 C with a linear decrease in S values at higher temperatures. These results strongly indicate a physical change of membranes with changing temperature, thus influencing sugar accumulation, which is also reversible, as indicated by loss of sugars during the reconditioning process.

Resumen

Una fuerte relación fue encontrada entre la permeabilidad de la membrana y la conversión de almidón a azúcares en papas almacenadas. Tejidos de tubérculos almacenados a bajas temperaturas, tuvieron mayor contenido de azúcares y más membranas permeables que aquellos tubérculos almacenados a altas temperaturas. Tubérculos sometidos a stress

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de humedad durante el crecimiento y almacenados a 5.5°C acumularon más azúcares reductores y tuvieron más membranas permeables que los tubérculos regados normalmente y almacenados a la misma temperatura.

La porción basal de tubérculos Russet Burbank, acumuló más azúcares y se le encontró también poseer significativamente más membranas permeables que en la porción apical. Un exámen del orden de la membranas, con ensayos de lipidos spin-labeled mostró que el parámetro de orden de membranas de amiloplastos fue menor a bajas temperaturas que cuando era medido a altas temperaturas. Las membranas examinadas de tubérculos almacenados a 5.5°C y 15.5°C bajo pruebas de lípidos spin-labeled mostraron parámetros de orden similares a todas las temperaturas medidas entre 0 y 25°C con un descenso linear de valores S a altas temperaturas. Estos resultados indican fuertemente un cambio físico de las membranas con la temperatura, influenciando así la acumulación de azucares que es también reversible, tal como indica la pérdida de azúcares durante el proceso de recondicionamiento.

Introduction

Storage of potato tubers at low temperatures (below 7 C) causes conversion of starch to sugars. Accumulation of reducing sugars by the Russet Burbank tuber is not uniform. The basal portion accumulates two to three times more sugar than the apical portion (5, 6, 7). Moisture stressing of plants during growth also causes increased sugar development with low temperature storage. This is especially true for the basal portion. Such tubers are commonly referred to as sugar-end tubers and show symptoms of senescence of the basal portion (5). Uneven reducing sugar accumulation causes problems in the processing of uniformly colored, frozen French fries. Because of the economic importance of sugar development in potatoes, many studies have been made to determine and control the biochemical and physiological mechanisms involved in starch-sugar conversion. Much effort has been directed to the studying of activities of enzymes involved in starch-sugar interconversion, however, with little success (1, 12, 16, 18, 23). Ohad et al. (15) were the first to suggest and provide evidence that low temperature-induced starch degradation is controlled by changes in the amyloplast membrane surrounding starch granules, allowing degradative enzymes access to the starch grains. Crafts (2) and Isherwood and Kennedy (9) reported that discs cut from tubers stored at low temperature were structurally weak and the membranes were more permeable, allowing faster leakage of inorganic ions into sucrose solutions than that from discs of tubers stored at higher temperatures. Raison et al. (19, 20) provided evidence that at a critical temperature, chill-sensitive plants showed membranes with decreased fluidity resulting in higher activation energy of membrane-bound enzymes, rendering these enzymes less active. Isherwood (10) used electron micrographs to observe the amyloplast membrane of tubers which sweetened due to senescence. He noticed that the membranes were disintegrating; however, other tubers stored at 2 C for 38 days had starch grains still enclosed in a double amyloplast membrane. The present study was initiated to obtain additional information on the relationship between amyloplast membrane and reducing sugar accumulation in potatoes stored at low temperature.

Materials and Methods

Tubers of the Russet Burbank cultivar were grown at Washington State University at the Othello Research Station during the growing season of 1976. Seed tubers were planted in the middle of April and the crop was harvested in September. Moisture-stressed tubers were obtained by termination of irrigation for two weeks on a portion of the field in the latter part of June when the tubers were initiated and actively growing. The harvested tubers were stored at 5.5 C and 15.5 C for eight weeks. Some of the tubers stored at 5.5 C for five weeks were transferred to 15.5 C for the last three weeks of storage. Such tubers are referred to as reconditioned tubers.

At the time of analysis, the tubers were cut cross-sectionally into apical and basal halves. The skin of each half was removed and reducing sugars determined by the arsenomolybdate method as described earlier (22). Sample size consisted of 10 to 12 tubers blended together with 80% ethanol in a Waring blender, after which an aliquot was taken for analysis. Hydraulic permeability of membranes was measured on potato discs (9 mm in diameter and 1 mm thick). The discs were allowed to equilibrate with 10 ml of tritiated water (10 uci/ml) over night, and were transferred to tritiated free water after blotting them dry. Time-course elution of tritiated water from the discs was followed as described by Thimann and Samuel (24) and Poovaiah et al. (17). Hydraulic permeability of membranes was also measured by following time-course water efflux from potato discs (9 mm in diameter and 10 mm thick) placed in 0.47 M Mannitol solution according to the procedure used by Glinka and Reinhold (3).

Amyloplast membranes were isolated according to the procedure of Ohad et al. (15) and spin label 2 - (3 - carboxyl propyl) - 4 - 4 - dimethyl - 2 - tridecyl - 3 - oxazollidinyloxyl methyl ester, designated here as 5NS, was incorporated into the membranes by placing 25 μgm of the spin-labeled material in chloroform at the bottom of small, glass vials and evaporating the chloroform. Amyloplast membranes suspended in 0.5 M tris chloride buffer of pH 7.4 were added to the vials and the vials were shaken overnight. The membranes were then pelleted and washed twice to remove free spin label. The membranes were then resuspended with a small amount of buffer and transferred to an EPR cell. Electron paramagnetic resonance spectra of the samples were recorded on a Varian E-9 spectrometer operating in the X-band mode. Temperature of the sample was varied between 0 and 25 C, using a Varian temperature controller. Cooling was accomplished by nitrogen gas passed through an ice and salt mixture. Electron paramagnetic resonance spectra of different samples were recorded at different temperatures. Order parameters (S) were calculated according to the formula of Griffith and Jost (4) as a measure of fluidity of the membrane.

Results

Tubers which had been moisture stressed during growth and stored at low temperatures (5.5 C) accumulated significantly more reducing sugars than tubers grown under a normal moisture regime or those stored at higher temperatures (Table I). The tubers which had high sugar accumulation also had the shortest half-time in seconds to reach equilibrium with tritiated water. Tubers stored at 15.5 C took the longest half-time to reach equilibrium. The basal portion accumulated significantly more sugars and had a much shorter half-time to reach equilibrium than the apical portion. This can also be seen in Fig. 1 where an earlier peaking out and leveling off of radioactivity was attained by the basal portion of tubers. A similar type of earlier peaking out of stressed tubers is shown in Fig. 2, indicating greater permeability of these tubers.

TABLE 1. —*Reducing sugar content, half-time to reach equilibrium and percentage weight loss due to osmotic water efflux of discs of tubers as influenced by storage temperature, moisture stress and portion of tuber.*

Storage Treatment	Tuber Portion	Reducing Sugar %	Half-time in seconds	% Weight Loss
5.5 C	Apical	1.88^{cd}*	148^{bc}	17.60^{c}
	Basal	3.75^{b}	119^{cd}	18.94^{b}
Stressed 5.5 C	Apical	2.45^{bc}	140^{bc}	18.58^{b}
	Basal	8.31^{a}	95^{d}	23.23^{a}
15.5 C	Apical	0.33^{e}	184^{a}	15.35^{d}
	Basal	0.95^{de}	172^{ab}	15.89^{d}
Reconditioned	Apical	0.67^{de}	165^{ab}	15.52^{d}
5.5-15.5 C	Basal	3.20^{bc}	134^{bc}	17.16^{c}
Total Average	Apical	1.33^{B}	146^{A}	16.76^{B}
	Basal	4.05^{A}	118^{B}	18.81^{A}

*Means not having a common letter are significantly different at the 5% level according to Duncan's new multiple-range test.

Membrane permeability, as measured by the rate of weight loss of tuber discs due to water efflux in 0.47 M Mannitol solution, showed results very similar to those obtained with the tritiated water method. The basal

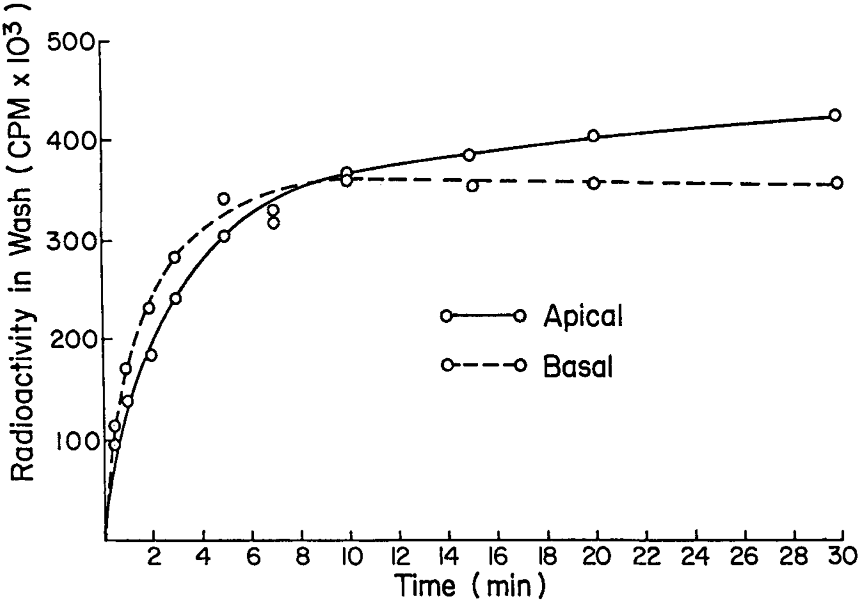

FIG. 1. Time-course elution of tritiated water from apical and basal portions of tubers stressed during growth and stored after harvest at 5.5 C.

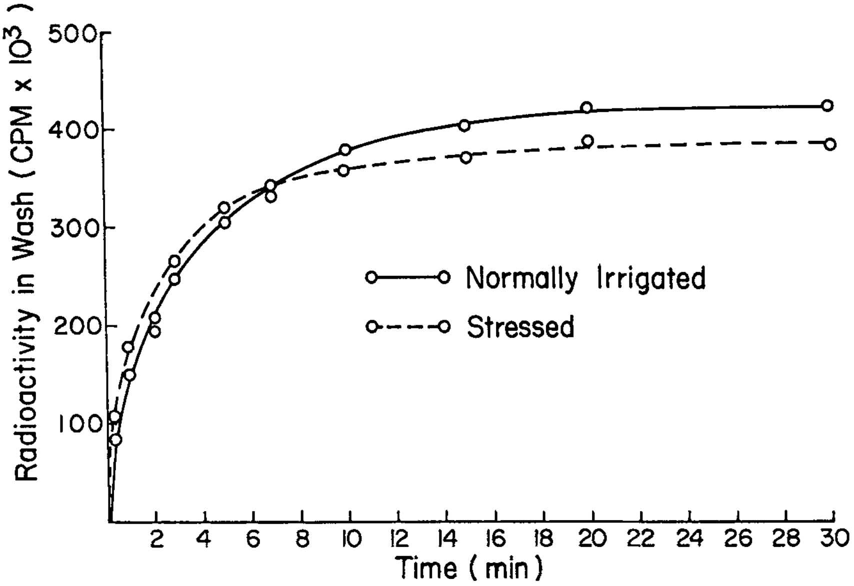

FIG. 2. Time-course elution of tritiated water from tubers of normally irrigated and moisture-stressed plants. Stored eight weeks at 5.5 C.

portion of stressed tubers which accumulated the highest percentage of reducing sugars exhibited the shortest half-time to equilibrium with tritiated water and also had the greatest percentage of weight loss in the Mannitol solution (Table I). This was also true for the basal as compared to the apical portion. The more rapid water efflux for the basal portion, as indicated by weight loss of discs, is shown in Fig. 3. Moisture-stressed tubers also showed more water efflux, which again is an indication of their greater permeability when compared to tubers from normally irrigated plants. (Fig. 4).

Membrane fluidity measurements using 5NS as a probe showed higher S values or more lipid order for membranes of moisture-stressed tubers. No differences were observed in S values of membranes from tubers stored at different temperatures when measured at a constant temperature. When the temperature of the membranes was varied from 0 to 25 C, a decrease in fluidity was obtained with lower temperatures (Fig. 5). No differences in slopes were observed for the differently treated membranes at the temperatures studied.

Discussion

A discussion by Lyons (13) of chilling injury in chill-sensitive plants proposed that at a particular temperature, a phase change occurs. This results in change of physical structure of membranes from a normal, liquid crystalline (more fluid) to a solid, gel (less fluid) state leading to increased permeability of the membranes, decreased activity of membrane-bound enzymes and accumulation of certain substances. The results obtained in these studies indicate that phase changes can occur in potato amyloplasts at low temperatures and are enhanced by conditions which promote senescence, such as moisture stress during growth.

Potato tubers which accumulate reducing sugars in storage have been shown here to have increased membrane permeability. Spin-labeling studies (Fig. 5) have shown that the membranes studied exhibited decreased lipid fluidity with decreasing temperature, but no discontinuities, indicating discrete phase changes could be clearly detected using the 5NS probe. Previous studies have shown that when membranes become rigid, activities of membrane-bound enzymes decrease (13). Isherwood (8) postulated that starch-sugar interconversion is dynamic and continuous in tubers. If this is true, then as membranes become more rigid, and the activity of membrane-bound enzymes, such as amyloplast-bound starch synthetase, may be affected without a significant change in activity of nonmembrane-bound enzymes, such as phosphorylase (major starch degrading enzyme in potatoes). A shift in the balance of starch-sugar metabolism may then occur favoring sugar accumulation.

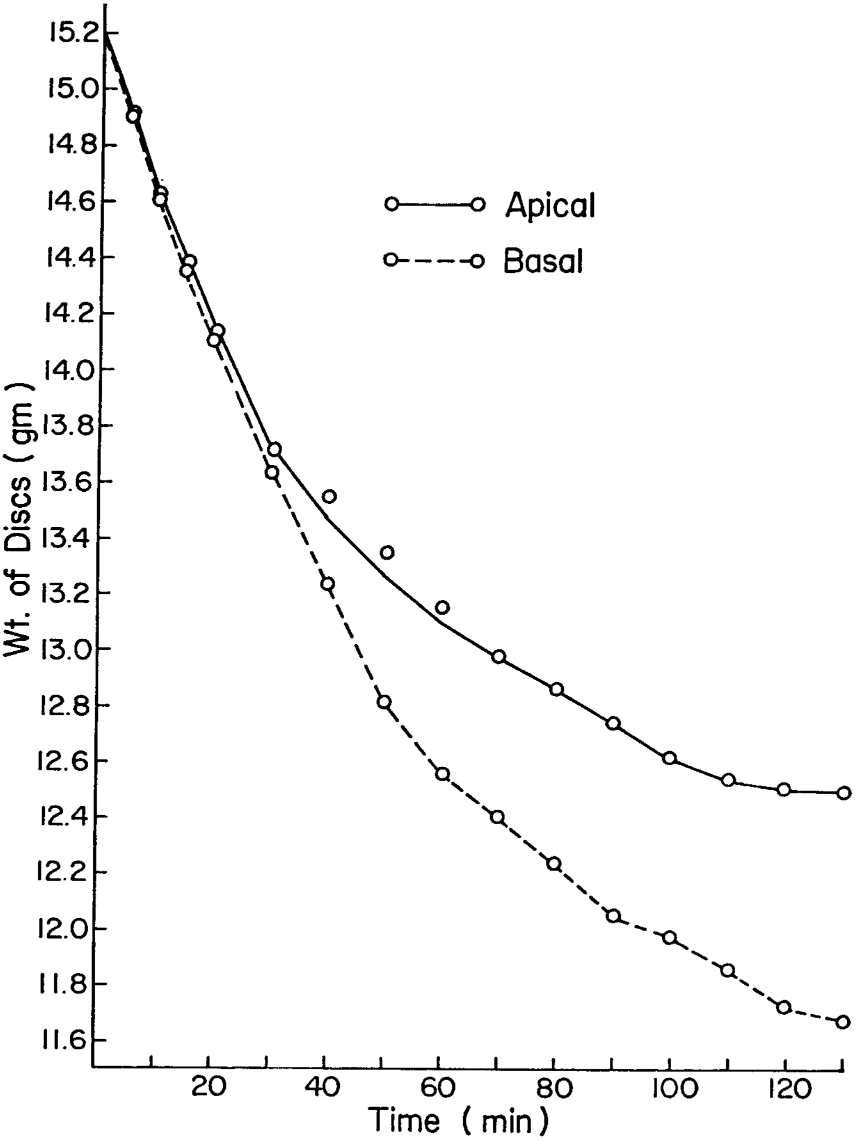

FIG. 3. Time-course osmotic water efflux from apical and basal portions of tubers moisture stressed during growth and stored after harvest at 5.5 C.

Ohad et al. (15) proposed that at low temperatures, the amyloplast membrane is damaged and becomes more permeable allowing degradative enzymes access to the starch. However, Isherwood (10) using electron

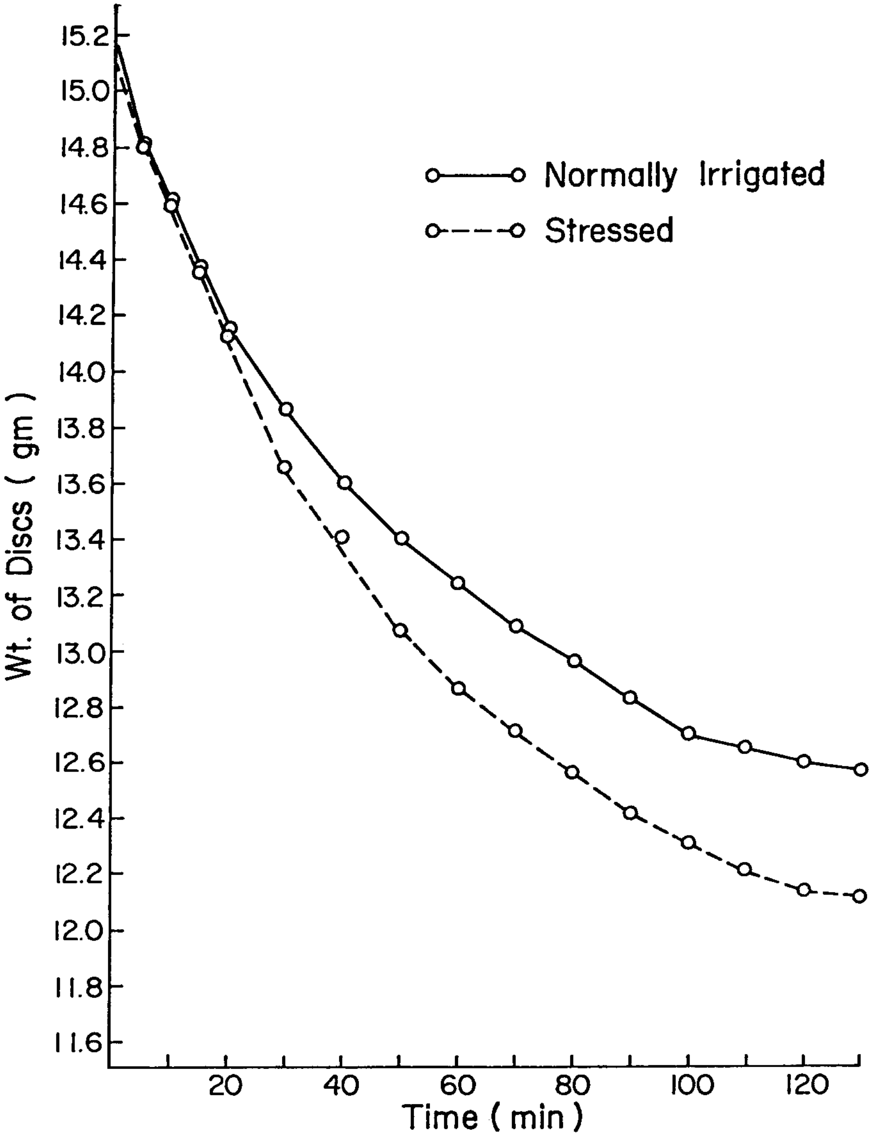

FIG. 4. Comparison of time-course water efflux in 0.47 M Mannitol solution of tubers from plants normally irrigated and moisture stressed during growth. Tubers stored for eight weeks at 5.5 C.

micrographs, was unable to detect any damage to the amyloplast membrane in tubers stored at low temperatures. Changes in the physical struc-

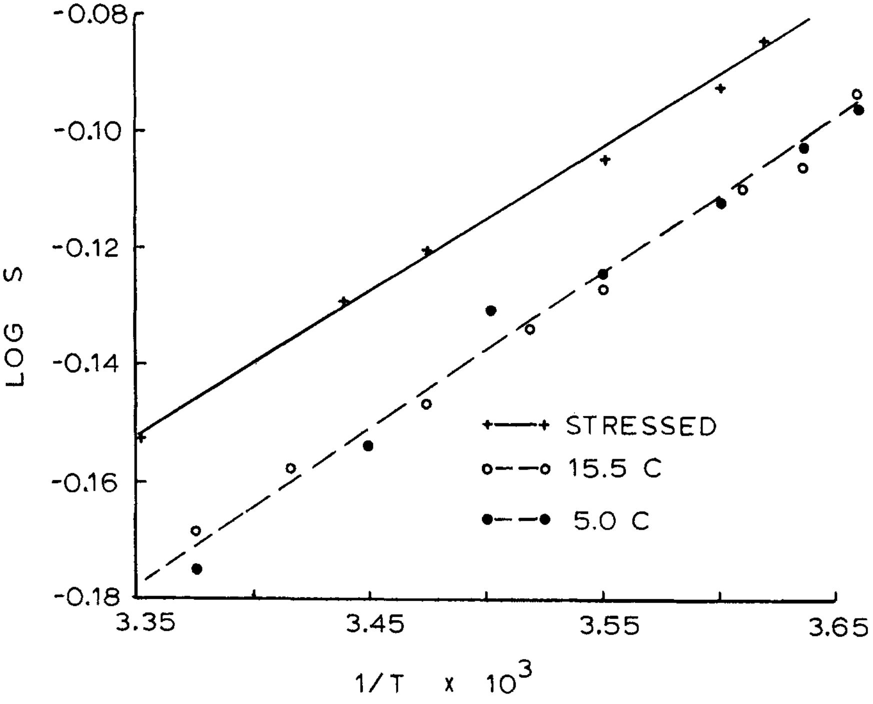

FIG. 5. Changes in order parameter (S) of amyloplast membranes of tubers with change in temperature at the time of measurement. Tuber samples were from plants; stressed during growth and stored at 5.5 C after harvest, from normally irrigated plants and stored at 5.5 C and 15.5 C after harvest.

ture of membranes, particularly lipid protein interactions, are not detectable by the technique employed by Isherwood. Isherwood (10) is of the opinion that transport of metabolites across the amyloplast membrane may be affected by low temperatures, as has been shown with sweetening induced by cyanide and ethylene chlorhydrin. Cyanide is known to inhibit electron transport in membranes and ethylene chlorohydrin is similar to many anaesthetics which are known to cause a change in lipid structure and to affect lipid-bound membranes (11). The results in these studies indicate that starch degradation at low temperatures is very much related to membrane changes. It has been observed that lipids crystallize in membranes of senescent tissues (14, 25). This may very well be the case for the amyloplast membrane surrounding starch granules in moisture-stressed tubers showing symptoms of senescence. Iritani and Weller (7) have shown that tubers from plants which died prematurely also accumulate greater amounts of sugars in storage than tubers from plants which remained alive until time of harvest.

Membranes have the capability of repairing themselves. Razin et al. (21) have shown that detergents induce separation of lipids and proteins, but that removal of the detergent by dialysis leads to restoration of membrane structure. Tubers which had been stored at low temperatures decrease sugar content upon storage at higher temperatures (reconditioning), as shown in Table I. There is a strong possibility that membrane changes are reversible since permeability, as measured by water efflux out of discs, was significantly lower for discs from tubers which had been reconditioned. Half-time to reach equilibrium by the tritiated water method was also higher, however, the differences were not significant. Lyons (13) states that temperature-induced phase change in the lipid portion of membranes of chill-sensitive plants is completely reversible.

The present studies using spin-labeled lipid probes indicate that the low temperatures cause a physical change in membranes which is reversible. As indicated in Fig. 5, membranes from tubers stored at 5.5 C and 15.5 C showed similar S values at all temperatures measured, however, there was an increase in S values (more rigid) at the lower temperatures. The rapid ability of membranes to change with temperatures suggests a physical rather than a biochemical change. On the other hand, membranes from stressed tubers stored at 5.5 C had higher S values than membranes from tubers from normally irrigated plants, indicating an irreversible change had occurred as suggested for senescent tissue by Isherwood (10).

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