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Elemental gradients associated with hollow heart in potato

Studies using X-ray microanalytical techniques

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Studies using X-ray microanalytical techniques

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FOREWORD AND ACKNOWLEDGMENTS

This study was carried out during the author's sabbatical work transfer to the University of California's Davis campus, October 1980 to September 1981. I am indebted to the following for making the study possible: Dr. Arthur Spurr of the Vegetable Crops Department for his collaboration throughout the study and the instruction and help in preparing the tissue samples; Dr. Philip Fenn of the Geology Department for sharing his expertise on X-ray microanalysis and use of equipment; Dr. Herman Timm of the Vegetable Crops Department for supplying the tuber samples from his field experiments and for helpful discussions; and Dr. Lawrence Rappaport, Chairman of the Vegetable Crops Department, for providing laboratory and office facilities.

SUMMARY

Electron microprobe and X-ray fluorescence techniques were used to study elemental gradients in potatoes with symptoms of the physiological disorder hollow heart. The initial work was carried out on mature tubers, with and without advanced symptoms in the form of large hollow areas. Results showed that potatoes have elemental gradients along the tuber's length and width. Lengthwise gradients included an increase in K and a decrease in Cl from stem to apical end. Calcium was lower in the stem end than elsewhere in the tuber. Radial gradients were associated mainly with two contrasting tissues, the central pith (low in starch, high in moisture) and the surrounding perimedulla (high in starch, low in moisture) making up the bulk of the tuber. Elemental levels in hollow heart and control tubers were similar, when the same tissue type was used in the comparison.

The results with mature tubers suggest that if the disorder is caused by an elemental deficiency, as proposed by some workers, such a deficiency is temporary and no longer exists in mature tubers with advanced hollow heart. The results also demonstrate the necessity in studies of this kind of restricting the tests to one tissue within the tuber.

Subsequent work was carried out on tubers with incipient stages of hollow heart. The symptoms originated in and were confined to the central pith tissue. The tuber samples were obtained from plants subjected to temperature stress occurring naturally under field conditions as well as from plants subjected to temperature stress induced under laboratory conditions.

In the field-stressed material, the first visible symptom of the disorder was a small dark necrotic spot 2-3 cells in diam in the central pith. A slightly more advanced, but still early, stage was manifested by a patch of several such (or larger) dark spots, but always confined to the pith. Magnesium levels were much higher in lesion pith tissue than in non-lesion pith tissue. The "halo" zone immediately surrounding the lesion had even higher levels of Mg than the lesion itself. There were no significant gradients between lesion and non-lesion tissue in the other elements (K, Cl, S, P) detected by the electron microprobe. However, the "halo" immediately around the lesion again had higher levels than the lesion itself, at least in K and Cl, for which complete line scans were run. Healthy portions of the pith of tubers with incipient hollow heart had elemental levels similar to those of the pith of control tubers.

Lesions in tubers of the laboratory-stressed plants were of the "brown center" type. K, Cl, S, and P tended to be higher in lesion than non-lesion tissue, but there was no appreciable Mg gradient.

Of the two lesion types encountered in these experiments, it was considered that there was more certainty that the "small isolated dark" type produced under the field-stress conditions was symptomatic of incipient hollow heart.

The results suggest that a nutrient imbalance triggers the onset of the cell necrosis that characterizes the initiation of this disorder in potato. A localized Mg toxicity or Ca deficiency due to high Mg-to-Ca ratio is probably involved.

RESUME

Dans des tubercules de pommes de terre présentant des symptômes du trouble physiologique du coeur creux, les gradients d'éléments chimiques ont été étudiés à l'aide d'une microsonde électronique et de la radiofluorescence. Le travail initial a été conduit sur des tubercules mûrs présentant ou non des symptômes avancés de la maladie sous la forme de grandes zones creuses. Les résultats ont fait ressortir la présence chez les pommes de terre de gradients chimiques sur la longueur et la largeur du tubercule. Parmi les gradients longitudinaux, on note un accroissement de K et une baisse de Cl du talon à la couronne; la concentration en calcium était plus basse du côté du talon (attache du stolon). Les gradients radiaux intéressaient principalement deux tissus contrastants, la moelle centrale (teneur en amidon basse, teneur en eau élevée) et le tissu périmédullaire (teneur en amidon élevée, teneur en eau basse) qui constitue la plus grande partie du tubercule. Dans un même type de tissu, les concentrations en éléments chimiques des tubercules atteints par le coeur creux et des tubercules témoins étaient semblables.

Les résultats des travaux sur les tubercules mûrs suggèrent que si le désordre est causé par une carence en éléments, comme l'ont proposé certains chercheurs, une telle carence est temporaire et n'existe plus dans les tubercules mûrs présentant des symptômes avancés du coeur creux. Les résultats mettent en évidence en outre la nécessité, dans des études de ce type, de restreindre les analyses à un seul type de tissu du tubercule.

Par la suite, des travaux ont été réalisés sur des tubercules aux stades initiaux de la maladie du coeur creux; les symptômes partaient de la moelle centrale et s'y confinaient. Les échantillons de tubercules ont été prélevés sur des plantes exposées à un stress thermique naturel en conditions normales de culture, ainsi que sur des plantes soumises à un stress thermique induit en laboratoire.

Le premier symptôme visible de l'altération physiologique sur les tubercules produits au champ était une petite tache nécrotique noire de 2 à 3 cellules de diamètre, dans la moelle centrale. Un stade légèrement plus avancé, mais encore précoce, se manifestait par la présence d'une plage de plusieurs taches noires de ce type (ou plus grosses), toujours dans la moelle. Les concentrations en Mg étaient beaucoup plus élevées dans les tissus médullaires lésés que dans les tissus sains. La zone en halo entourant directement la lésion avait une concentration en Mg encore plus élevée que la lésion elle-même. Aucun gradient significatif n'a été décelé à la microsonde électronique entre les tissus avec lésion et les tissus sains pour les autres éléments (K, Cl, S, P). Cependant, le halo entourant directement la lésion présentait encore des concentrations supérieures à la lésion elle-même, du moins en ce qui concerne le K et le Cl, pour lesquels des lignes complètes de balayage ont été établies. Dans des tubercules présentant des symptômes initiaux de la maladie du coeur creux, les portions saines de la moelle contenaient des concentrations en éléments semblables à celles de la moelle des tubercules témoins.

Les lésions des tubercules des plantes traitées en laboratoire étaient du type à "centre brun". Les concentrations en K, Cl, S et P avaient tendance à être plus élevées dans les tissus lésés que dans les tissus sains, mais on n'observait pas de gradient en Mg appréciable.

Des deux types de lésions observées au cours de ces expériences, il semble que les taches du type "petit, isolé et noire" produites dans des conditions naturelles de culture seraient davantage symptômatiques du stade initial de la maladie du coeur creux.

Ces résultats portent à conclure qu'un déséquilibre nutritif déclenche la nécrose des cellules, premier symptôme de ce trouble chez la pomme de terre. Une toxicité magnésienne localisée ou une carence en Ca causée par un rapport Mg/Ca élevé seraient probablement en cause.

Elemental gradients associated with hollow heart in potato: Studies using X-ray microanalytical techniques

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INTRODUCTION

Hollow heart is found in potatoes grown throughout the continent and accounts for substantial losses in some years. This physiological disorder is usually the result of growing conditions favoring very active, sudden vine growth after the tubers have set. A greatly increased moisture supply following a dry period, wider plant spacings, later plantings, smaller plant populations, K fertilization level, removal of foliage, lower yields, and large tubers have been linked to the disorder (Crumbly and Nelson 1970a, b; Edmundson 1935; Kallio 1960; Krantz 1947; Krantz and Lana 1942; Moore 1925, 1926; Nelson 1970; Nelson, Jones, and Thoreson 1979; Nelson, Thoreson, and Jones 1979; Werner 1926, 1927.

It appears that the rapid vine growth and rapid tuber expansion phase alters the physiological balance of the plant, and that tops are produced at the expense of the tuber. Under these conditions certain important nutrients may become temporarily insufficient, leading to the death of some pith cells. Levitt (1942) described in detail the histological events associated with hollow heart, from the first visible sign manifested by a few dark necrotic cells in the pith, to the hollow area extending radially into the perimedullary area and characterizing advanced stages. Some results on elemental gradients in potatoes with advanced hollow heart compared with healthy tissue have been reported (Arteca et al. 1980; Levitt 1942; Macklon and De Kock 1967). The present work extends these studies and takes into account the large compositional differences between pith and perimedullary tissue, a point frequently overlooked. In addition, most of our work was focused on incipient rather than advanced stages. We felt that this was critical to an understanding of nutrient balances related to the disorder's inception. Studies were carried out on tubers with hollow heart formed as a result of the natural occurrence of stress under field conditions and of stress induced under laboratory conditions. Electron microprobe, X-ray fluorescence, and atomic absorption techniques were used in determining the elemental gradients and differences.

EXPERIMENTAL

A.0 MAJOR TISSUE ZONES OF POTATO TUBER

A.1 Object

Initial tests in this work were aimed at determining compositional differences between the major tissue zones of hollow heart and healthy tubers.

A.2 Methods

Potatoes used in these tests came from the 1981 varietal trials in California's Central Valley, coordinated by the University of California, Davis. Mature tuber samples were held in cool storage until January 1982, when the analytical tests were conducted. All tubers were cut longitudinally and those revealing hollow heart were retained. Nine such tubers were available for this test series: four tubers of cv. Belchip, two of cv. NDD47-1, and three of cv. AD7508-1. For each hollow heart tuber found, a healthy tuber (with no hollow heart) of the same variety and comparable in size, shape, and pith dimensions was also retained.

Blocks of tissue from the four main tissue zones of the tuber shown in Figure 1 were removed from each tuber, using both cut halves. Care was taken during this and subsequent steps to avoid elemental contamination of the cut tissue surfaces from hands, scalpel, and other objects. Fresh weights were recorded. The tissue samples, in perforated envelopes, were dried at 19^{o}C, the dried weights recorded, and each sample ground to a fine powder using a mortar and pestle.

For X-ray fluorescence microanalysis, 6 mm diam spheres of powdered sample were attached to Mylar film held in place in plastic photographic slide holders. A clear aerosol spray was used as a protective coating to effect adhesion of the powder to the Mylar. Sheets of paper with spherical cutouts placed over the Mylar slide facilitated precise application of the aerosol spray, followed immediately by the powdered sample. For each sample, two successive applications of spray and powdered sample were made to ensure a uniform distribution of sample over the 6-mm sphere area. Powdered samples of National Bureau of Standards orchard leaves were similarly prepared for use as standard reference material. Blanks consisted of two coatings of the aerosol spray on Mylar film. The samples were analyzed in a Kevex 0700 energy dispersive spectrometer with the use of a rhodium tube source and a direct excitation mode, a Si(Li) detector, and secondary targets Gd, Sn, Ag, Ge, Fe, and Ti. Data were recorded in counts per second (cts/sec).

Powdered samples were also analyzed for the elements of interest with the use of standard atomic absorption methods.

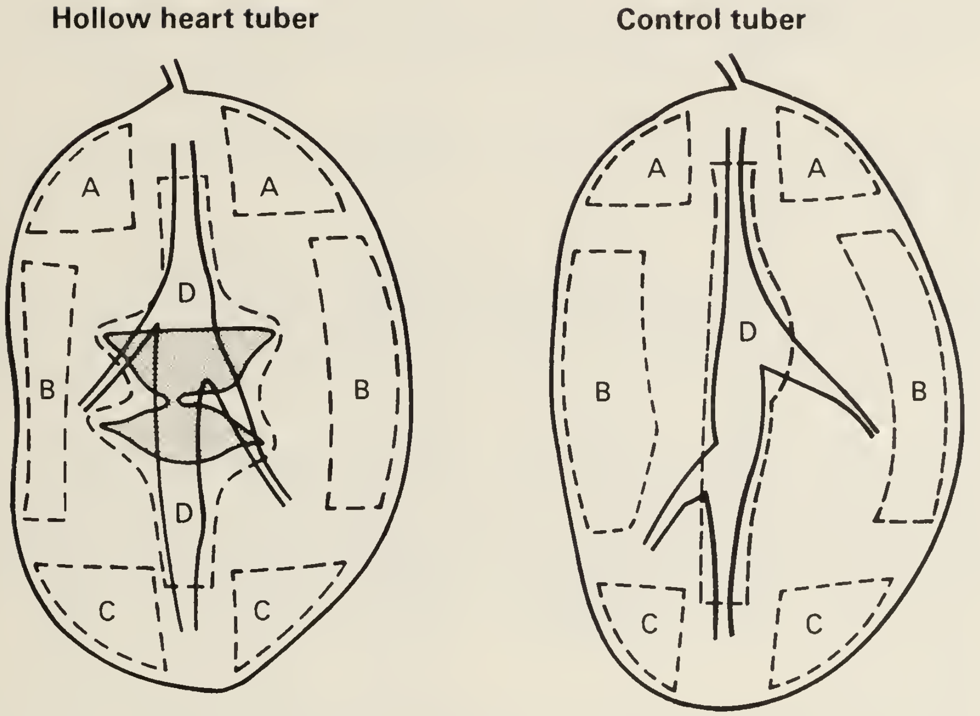


Figure 1. Hollow heart and control potato tubers showing major tissue zones (broken lines) sampled.

A.3 Results

Dry-matter content of pith tissue (zone D) was much lower than that of the surrounding perimedullary tissue (zones A, B, and C) (Table 1, Figure 2). It changed relatively little from stem to bud end of the tuber's perimedullary tissue, although it was slightly lower in the bud (C) end than in the stem (A) or mid (B) regions. Dry-matter content of hollow heart tubers and healthy tubers was similar.

Elements Cl, K, and Ca were detected using the X-ray fluorescence method (Table 1, Figure 3). Other elements were not present in sufficient quantity to be detected by this method. K, Ca, Mg, Na, and P were determined with the use of the more sensitive atomic absorption method (Table 2). Results for K and Ca, which were determined by both methods, showed similar trends.

From stem to bud end of the tuber's perimedullary tissue (i.e., A to B to C), there was a decrease in Cl and an increase in K. No gradients were found in the other elements tested.

The central pith differed strikingly in composition from the perimedullary tissue. Its elemental content (most elements) was higher than that of the other tuber zones, when calculated on a dry-weight basis. Calculated on a fresh-weight basis, however, this difference was reduced considerably.

Hollow heart and healthy tubers were almost the same in elemental composition and dry-matter content. However, dry-matter content of the pith may have been one minor exception, possibly being a little lower in hollow heart tubers than in the controls (presumably because of desiccation of tissue adjacent to the hollow areas).

A.4 Conclusions

Potatoes have elemental gradients along the tuber's length as well as its width. The latter are mainly associated with two contrasting tissues, the central pith (low in starch, high in moisture) and the surrounding perimedulla (high in starch, low in moisture), which make up the bulk of the tuber.

Mature tubers with advanced hollow heart (manifested by large hollow areas extending from the pith into the surrounding perimedullary tissue) do not differ significantly in elemental composition from healthy, mature tubers.

Each powdered tissue sample used for elemental analysis by the X-ray fluorescence and atomic absorption methods used in this experiment represents a relatively large volume of fresh tissue. This was necessary in order to obtain enough sample, after drying, for the analyses. In order to get results on more localized tissue sites, the electron microprobe method will be used in further work.

Table 1. Cl, K, and Ca content of major tissue zones of potato tubers with and without hollow heart, determined by X-ray fluorescence analysis*

Determination for	Expressed as	Hollow heart tubers				Control tubers			
		A	B	C	D	A	B	C	D
Cl	cts/sec	27.3	16.5	9.8	35.7	24.1	16.6	8.0	30.6
	% of dry wt	0.52	0.32	0.19	0.68	0.46	0.32	0.15	0.58
		(±0.02)	(±0.03)	(±0.01)	(±0.03)	(±0.03)	(±0.02)	(±0.02)	(±0.04)
	% of fresh wt	0.12	0.08	0.04	0.09	0.11	0.08	0.03	0.09
K	cts/sec	267.2	332.6	506.9	589.6	255.1	328.3	448.0	519.4
	% of dry wt	1.71	2.13	3.25	3.78	1.64	2.11	2.87	3.33
		(±0.12)	(±0.10)	(±0.22)	(±0.19)	(±0.12)	(±0.18)	(±0.11)	(±0.22)
	% of fresh wt	0.39	0.50	0.67	0.49	0.38	0.49	0.61	0.50
Ca	cts/sec	10.1	22.6	20.8	36.7	11.5	16.6	16.6	34.4
	% of dry wt	0.06	0.13	0.12	0.22	0.07	0.10	0.10	0.20
		(±0.01)	(±0.02)	(±0.01)	(±0.03)	(±0.02)	(±0.03)	(±0.03)	(±0.02)
	% of fresh wt	0.01	0.03	0.03	0.03	0.02	0.02	0.02	0.03
Dry wt	% of fresh wt	23.0	23.5	20.7	12.9	23.4	23.4	21.3	14.9
		(±0.42)	(±0.56)	(±0.64)	(±1.51)	(±0.91)	(±1.06)	(±0.90)	(±0.59)

*(1) See Figure 1 for location of zones A, B, C, and D in tuber.
(2) % element on dry wt basis calculated from cts/sec, with reference to counts obtained for National Bureau of Standards orchard leaves.
(3) Values reported are means of nine determinations using nine different tubers. Standard errors are given in parentheses.

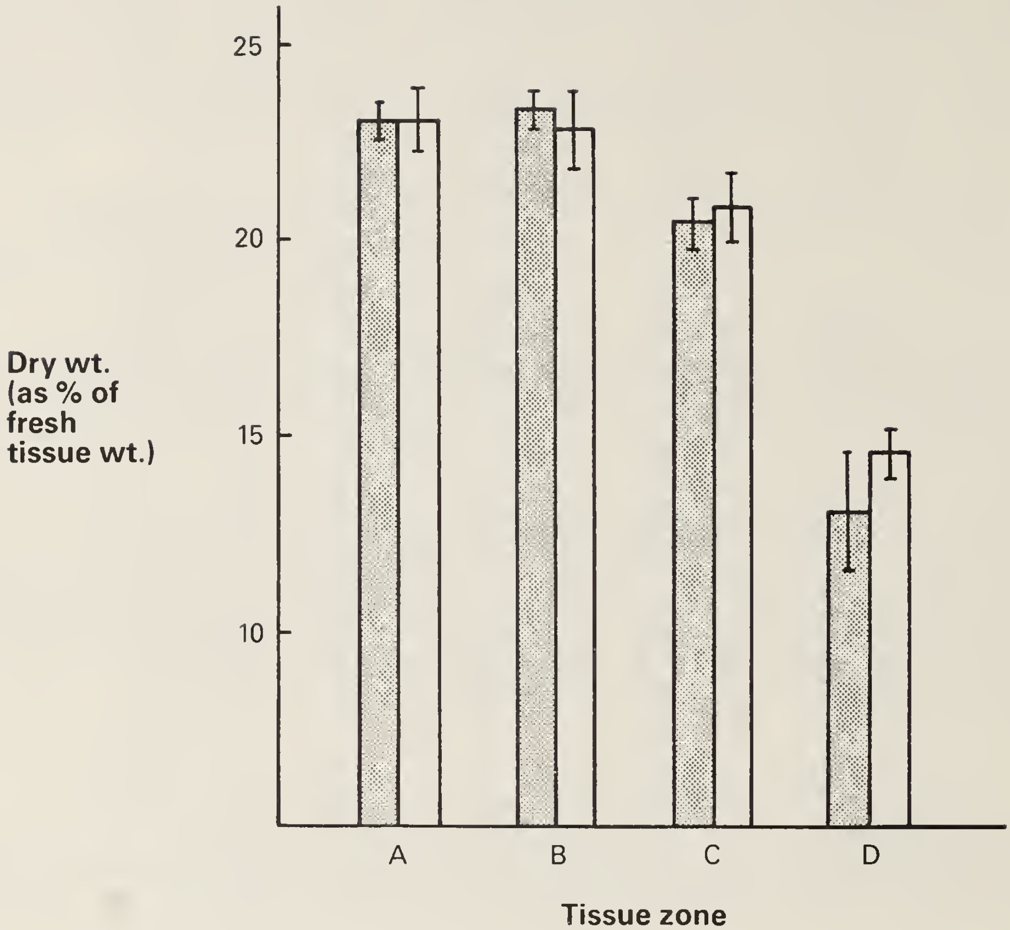


Figure 2. Dry weight content of major tissue zones of hollow heart and control potato tubers.

Shaded bars represent hollow heart tubers; empty bars represent control (healthy) tubers.

See Figure 1 for tuber zone sites.

Each bar is the mean of 9 tuber replicates, with standard error shown at top of bar.

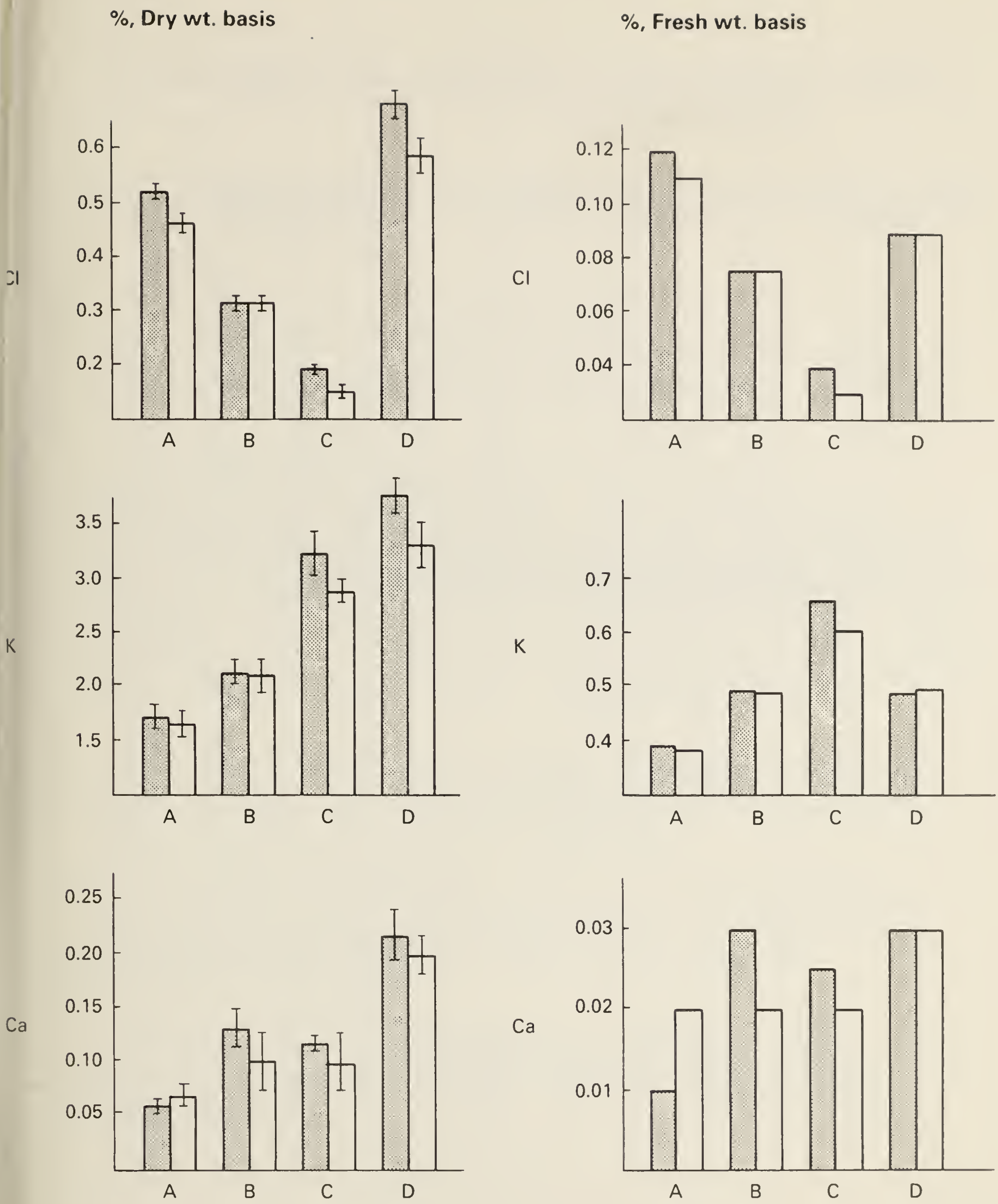


Figure 3. Cl, K, and Ca content of major tissue zones of potato tubers with and without hollow heart, determined by X-ray fluorescence analysis.

Shaded bars represent hollow heart tubers; empty bars represent control (healthy) tubers.
See Figure 1 for location of A, B, C, D tuber zones.
Each bar is the mean of 9 determinations using 9 different tubers, with standard error indicated at top of bar.

Table 2. Elemental content of major tissue zones of potato tubers with and without hollow heart, determined by atomic absorption analysis*

Element	Hollow heart tubers				Control tubers			
	A	B	C	D	A	B	C	D
K	0.89	1.38	2.08	2.73	0.85	1.21	2.10	2.43
Ca	0.03	0.03	0.04	0.05	0.03	0.03	0.04	0.03
Mg	0.08	0.07	0.08	0.12	0.07	0.07	0.06	0.10
Na	0.26	0.34	0.35	0.22	0.36	0.29	0.29	0.18
P	0.30	0.30	0.37	0.56	0.30	0.29	0.36	0.43

* (1) See Figure 1 for location of zones A, B, C, and D in tuber.
(2) Values reported are means (%, dry wt basis) of nine determinations using nine different tubers.

B.0 ADVANCED HOLLOW HEART: manifested by large hollow area in the mature field-grown tuber.

B.1 Object

To assess elemental gradients in tissue near the hollow center of tubers with the advanced hollow heart disorder, relative to tissue from comparable locations of healthy tubers.

B.2 Methods

Tubers used for this experiment were the same as those used for Experiment A. Because the volume of tissue required for each sample in the present experiment was much smaller than in the previous one, and had to be taken very precisely, the samples were taken immediately upon halving the tuber, before taking the larger blocks of tissue required for Experiment A.

An approximately 25-mm cube of tissue from the central part of the tuber, containing the region of interest, was sectioned on a sliding microtome to produce slices 400 µm thick. Before sectioning the slice used for subsequent sampling, the knife blades were cleaned with distilled water and wiped dry. The slice was placed on filter paper. Using a clean scapel, strips approximately 2 mm wide and 12 mm long were removed at the sites shown in Figure 4. These were adjacent to the hollow center, in the case of the hollow heart tuber, extending outwards from the hollow (A) radially into the perimedullary region, (B) along the central pith in the direction of the stem end, and (C) along the central pith in the direction of the bud end. Strips were also taken at comparable sites in healthy control tubers.

To assist in identifying and orientating the samples, one end (shown in Figure 4) of each strip was cut on an angle. Any handling, by means of forceps, was always done at this tapered end of the strip. Caution was exercised throughout the preparation and the handling of samples to avoid elemental contamination from hands or other objects.

The tissue strips were allowed to air dry during their preparation. They were mounted on 25-mm diam round glass microprobe slides, using an electrical circuit copper print to effect adhesion. The mounted samples were then placed in a 30^{o}C oven for final drying and held in a desiccator until analyzed.

Elemental analysis was carried out in an ARL EMX/SM electron microprobe, with the operating voltage 15 kV, beam current 300 nA, and beam diam 35 µm. Spectra were collected on a Kevex 7000 energy dispersive detector system for 100 sec live time. Test sites along the strip were as indicated in Figure 4.

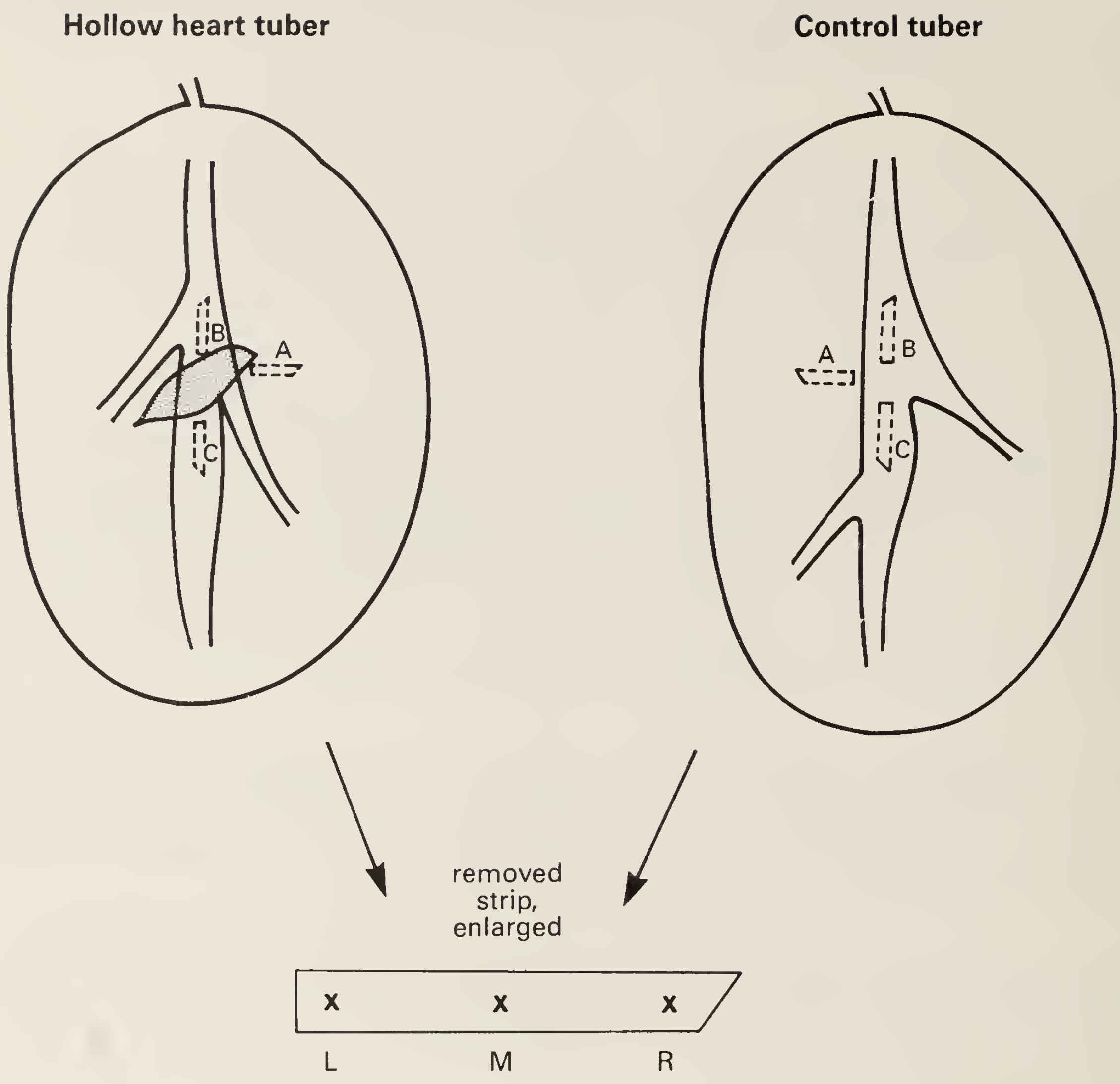


Figure 4. Diagram showing where tissue strips were taken from tubers for electron microprobe analyses, and test sites on strip.

B.3 Results

Elements detected in significant quantity by this method were K, Cl, S, P, and Mg. The results are presented in Table 3 and Figure 5.

The "A" strips (running radially from the central region of the tuber towards and into the perimedullary tissue) showed a considerable decrease in level of all elements along the strip, test site L to test site R. No clear difference between the hollow heart and control samples was evident except perhaps in the L test site, i.e., towards the middle of the tuber, close to the edge of the hollow in the case of the hollow heart samples. Here, elemental levels (except for P) were higher in hollow heart samples than in controls.

In "B" and "C" strips (entirely within the central pith, running from the central region towards the stem and bud ends, respectively), there were no such gradients, at least at the M and R test sites. This probably reflects, to a large extent, the uniformity of the tissue tested, i.e., all of the strip was taken from the central pith tissue, which is characteristically low in starch and high in moisture. Test sites restricted to pith tissue (L, M, and R in "B" and "C" strips) or very close to pith tissue (L in "A" strips) had much higher elemental levels than those in the high-starch, lower moisture perimedullary tissue (M and R in "A" strips).

In the "B" and "C" strips, as in the "A" strips, differences between hollow heart and control samples were relatively small and considered insignificant.

B.4 Conclusions

By the time the disorder has reached the advanced stage, a large hollow area extends beyond the central pith into the surrounding perimedullary tissue. Differences in elemental levels evident in this experiment were associated with these two contrasting tissues. These levels are much lower in the perimedulla than in the pith. This important aspect of tuber histology must be considered in any attempt to study differences between hollow heart and healthy tubers.

There was little or no difference in elemental levels between hollow heart and control tubers when the same kind of tissue was compared in these mature tubers. These results, and those of Experiment A, which also dealt with mature tubers, suggest that if this disorder is caused by an elemental deficiency, as has been suggested by some workers, the deficiency is temporary and does not exist in mature tubers with advanced hollow heart.

Future experiments should concentrate on immature tubers that have incipient rather than advanced hollow heart symptoms, restricted to the pith where the disorder originates.

Table 3. Electron microprobe analyses for the localization of elements in hollow heart and control tubers*

	Hollow heart tubers			Control tubers		
	L	M	R	L	M	R
(A) Strips running radially into perimedulla (⇨ in Fig. 4)						
K, cts in '000s/100 sec	13.6	7.5	7.3	12.5	8.5	8.3
Cl, "	3.3	1.4	1.3	1.2	1.1	1.2
S, "	1.3	0.6	0.5	1.1	0.8	0.7
P, "	1.7	1.4	1.2	2.1	1.5	1.4
Mg, "	0.8	0.2	0.2	0.5	0.3	0.1
(B) Strips along pith towards stem end (▯ Fig. 4)						
K, cts in '000s/100 sec	13.2	14.6	14.3	13.4	11.2	12.0
Cl, "	2.4	2.0	1.5	1.4	1.3	1.2
S, "	0.8	1.1	0.9	1.1	1.0	1.0
P "	1.5	2.2	2.0	1.9	1.9	1.9
Mg, "	0.3	0.5	0.4	0.2	0.1	0.3
(C) Strips along pith towards bud end (▯ Fig. 4)						
K, cts in '000s/100 sec	18.9	19.1	17.2	12.6	14.5	13.0
Cl, "	2.5	2.7	2.2	1.3	1.8	1.8
S, "	1.0	1.1	1.0	0.5	1.4	1.0
P, "	2.1	2.2	2.3	2.0	2.1	2.2
Mg, "	0.4	0.4	0.1	0.1	0.3	0.3

* (1) See Figure 4 for tissue test sites.
(2) Values reported are means of 11 tubers for (A) strips, 7 tubers for (B) strips, and 3 tubers for (C) strips.

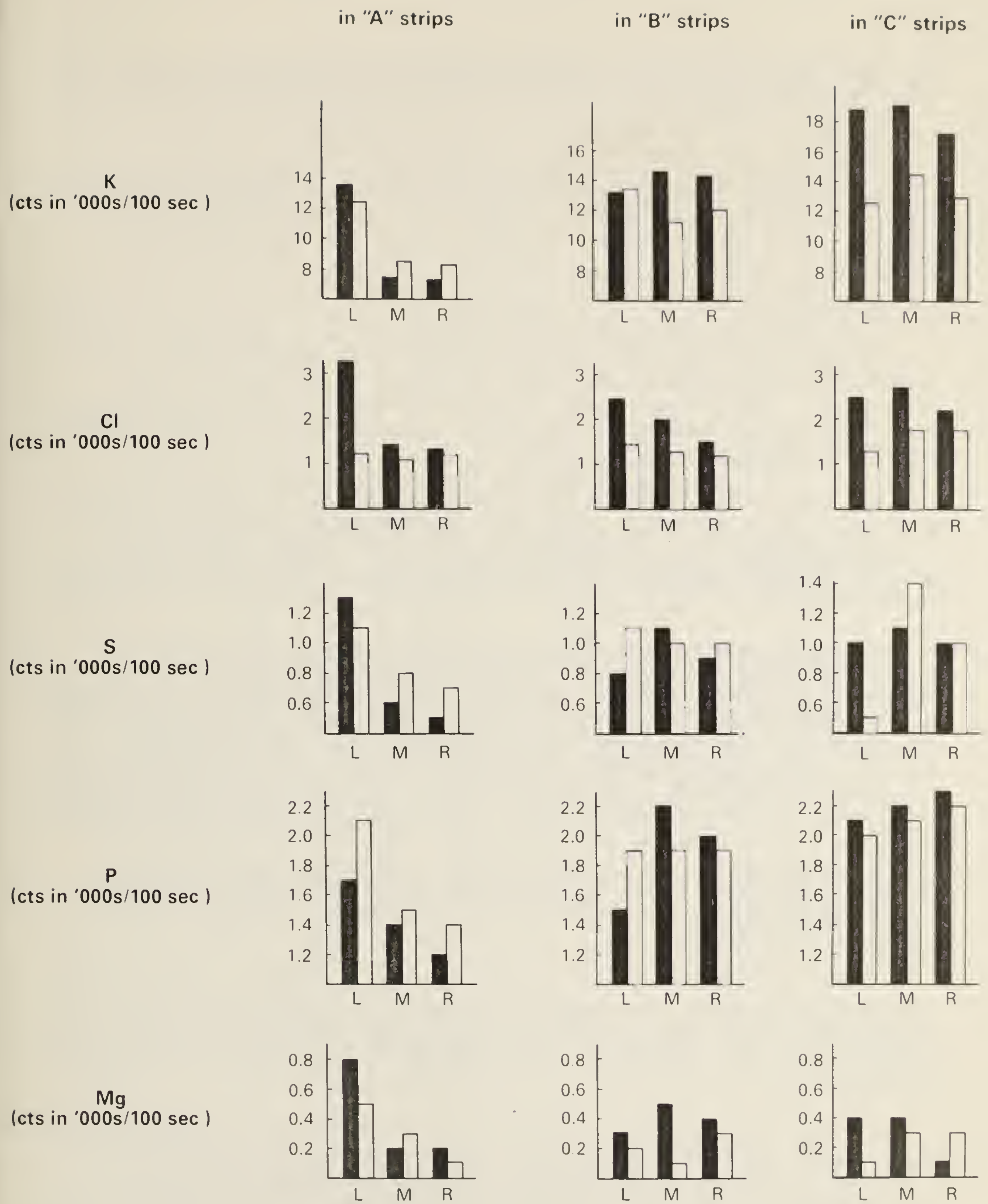


Figure 5. Electron microprobe analyses for the localization of elements in hollow heart and control tubers.

See Figure 4 for location of tissue strips A, B, C in the tuber and test sites L, M, R on the strip.
Shaded bars represent hollow heart tubers; empty bars represent controls.
Each bar is the mean of 11 tubers for "A" strips, 7 tubers for "B" strips, and 3 tubers for "C" strips.

C.0 INCIPIENT HOLLOW HEART, OCCURRING UNDER FIELD CONDITIONS: manifested by a small dark lesion or lesions in the central pith of the immature tuber.

C.1 Object

To determine elemental gradients in the region of the very small dark lesion or lesions occurring in the central pith, characterizing the first visible symptom of hollow heart in potato tubers maturing under field conditions.

C.2 Methods

In May 1981 the Bakersfield area of California received sudden high temperatures while potato crops were still at an early stage of tuber development. Tuber samples obtained of these naturally stressed potatoes were of particular interest in our study. Several of these tubers revealed very early symptoms of hollow heart.

The test material was mainly of the varieties Red La Soda and Pontiac, from the Jacobsen and Nikel farms. For each hollow heart tuber found, a corresponding control was selected, i.e., a tuber of the same variety, grown in the same location, and of comparable size, shape, and internal tissue anatomy.

An approximately 25-mm cube of tissue from the central part of the tuber, containing the region of interest, was sectioned on a sliding microtome to produce slices 400 μm thick. Before sectioning the slice used for subsequent sampling, the knife blades were cleaned with distilled water and wiped dry. The slice was placed on filter paper. Using a clean scalpel, strips approximately 2 mm wide and 18 mm long were removed at the sites shown in Figure 6. These were taken along the longitudinal length of the central pith traversing the small dark lesion or lesions characterizing the first visible symptoms of hollow heart. The entire strip was taken carefully from within the central pith core, avoiding the surrounding perimedullary tissue (which had much lower elemental levels, reported in the previous experiment). Strips were also taken at comparable sites in healthy control tubers.

To assist in identifying and orientating the samples, one end of each strip was cut tapered. Any handling, by means of forceps, was done at the tapered end of the strip. Caution was exercised throughout the preparation and the handling of samples to avoid elemental contamination from hands or other objects.

The tissue strips were allowed to air dry while being prepared. They were mounted on 25-mm diam round glass microprobe slides, using electrical circuit copper print as the adhesive. The mounted samples were placed in a 30°C oven for final drying and held in a desiccator until analyzed.

Elemental spectra at a few sites on the strip were obtained using an ARL EMX/SM electron microprobe, with the operating voltage 15 kV, beam current 300 nA, and beam diam 15 μm. X-ray counts for 100 sec live time were collected on a Kevex 7000 energy dispersive detector. This gave preliminary information on the elements present and their relative levels over a few different spots on the strip. Test sites along the strip are shown in Figure 6.

For more critical analysis, line scans were taken along the entire length of some strips (Figure 7) to analyze for selected elements. This was done with the electron microprobe set up with wavelength dispersive crystal spectrometers with the use of RAP (Mg $K\alpha$), ADP (Cl $K\alpha$), and LiF (Ca $K\alpha$) crystals.

C.3 Results

The energy dispersive method of analysis detected elements K, Cl, S, P, and Mg in significant amounts in this series of samples.

By restricting the test tissue to the central pith, it was clear that the problem of various elemental levels in various tissues of the tuber had been overcome. Note the uniformity of elemental composition throughout the length of control strips, Tables 4 and 5.

In this group of tubers, many were found that revealed incipient symptoms of hollow heart, from a very small (2-3 cells in diam) dark spot characterizing the first visible symptom to a slightly more advanced but still early stage consisting of a patch of these small dark lesions. They were always within the central pith, usually about half way between stem and bud ends of the tuber. Elemental analyses by the energy dispersive method are shown for the single lesion type (Table 4) and for the multilesion type (Table 5).

Mg levels were much higher in and around the lesion or lesions than in healthy tissue farther away from the lesion area. In addition, the "halo" zone immediately surrounding the lesion (M2 in Table 4) and light-celled areas between lesions in the case of the multilesion samples (M2 in Table 5) had much higher Mg levels than the lesion (M1) itself.

K and Cl gradients in the region of the lesion resembled those for Mg, with the "halo" zone registering higher counts than the inner part of the lesion. However, there was little or no gradient in K or Cl between the lesion area and healthy tissue at either end of the strip, as was the case with Mg.

Line scans using the more precise and detailed wavelength dispersive method of analysis confirmed the above trends. The line scan for Mg, shown in its entirety in Figure 8, is a typical result. The important segments of a rerun on the same sample strip are shown in Figure 9. Other tissue strips of the same variety, and of other varieties tested, revealed the same gradient for Mg so there was no question as to the validity of

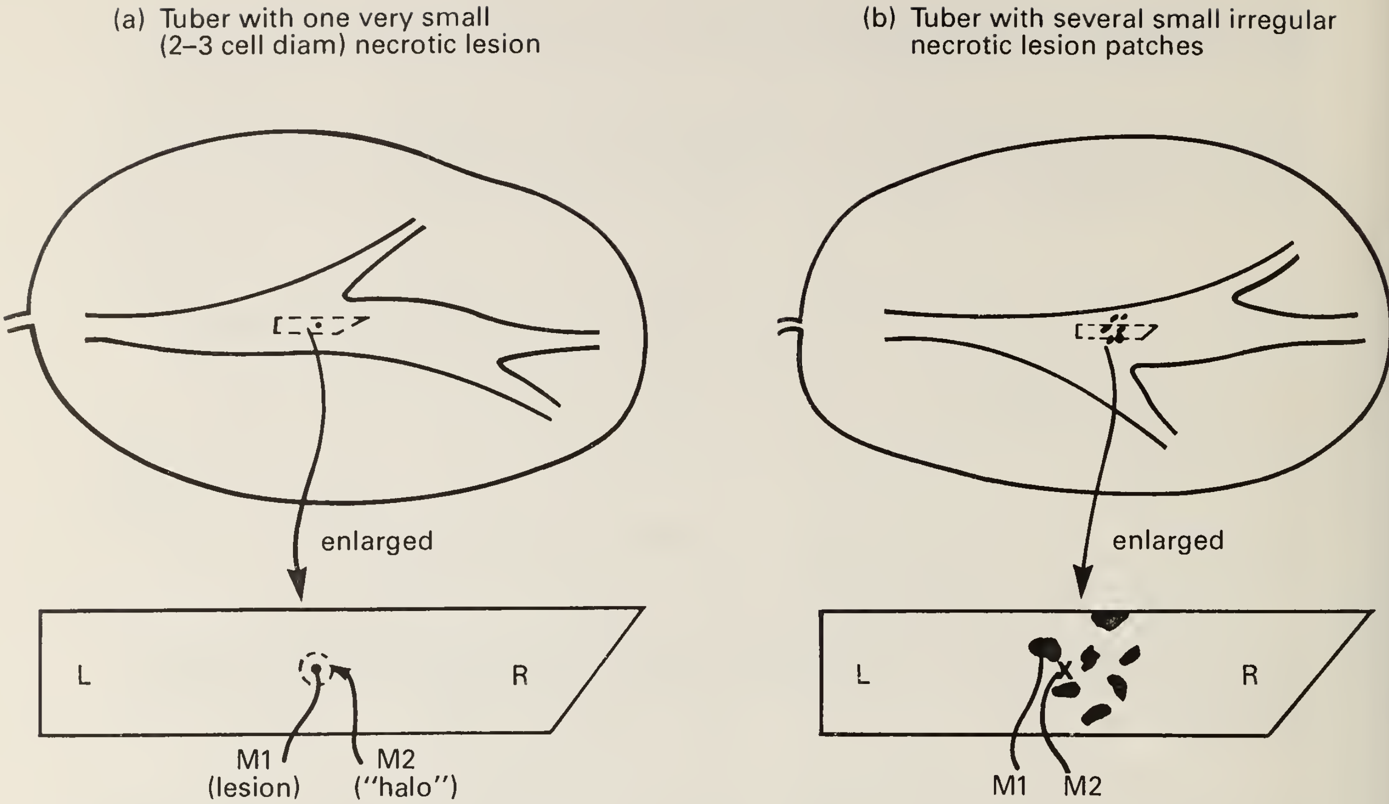


Figure 6. Diagram showing where tissue strips were taken from tubers with incipient hollow heart for electron microprobe analysis via energy dispersive detector system, and test sites on strips.

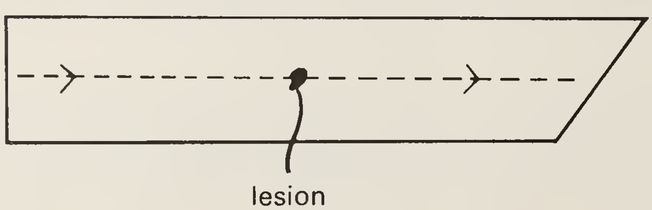


Figure 7. Path of line scan along tissue strip analyzed using wavelength dispersive analysis system with electron microprobe.

Table 4. Electron microprobe analysis for elements in tuber with incipient hollow heart (one small dark necrotic spot), compared with control tuber*

	Hollow heart tuber				Control tuber		
	L	M1	M2	R	L	M	R
K, cts in '000s/100 sec	11.3	10.0	13.4	15.6	13.2	13.3	12.6
Cl, "	1.5	2.7	3.3	2.7	2.5	2.5	2.2
S, "	1.1	1.1	1.1	1.1	1.0	0.8	1.0
P, "	2.8	1.9	2.9	3.6	2.6	2.8	2.6
Mg, "	0.5	1.0	1.7	0.6	0.5	0.4	0.5

* (1) See Figure 6(a) for test sites. Note that M1 site is within the single small necrotic lesion present in this tuber, and M2 is the "halo" region immediately surrounding the lesion.
(2) Typical counts for one cultivar are reported. Other cultivars tested showed similar gradients, although levels varied from one cultivar to another.

Table 5. Electron microprobe analysis for elements in tuber with incipient hollow heart (several small dark patches), compared with control tuber*

	Hollow heart tuber				Control tuber		
	L	M1	M2	R	L	M	R
K, cts in '000s/100 sec	12.9	8.0	17.3	17.5	13.7	13.9	14.8
Cl, "	3.7	2.3	5.4	4.1	3.1	3.5	3.4
S, "	1.0	0.7	0.7	0.7	1.1	0.9	1.0
P, "	2.5	2.9	3.7	3.5	2.7	3.0	3.3
Mg, "	0.6	1.0	1.2	0.4	0.5	0.6	BDL

* (1) See Figure 6(b) for test sites. Note that M1 site is within the dark necrotic spots and M2 represents the light-celled regions between M1.
(2) Typical counts for one cultivar are reported. Other cultivars tested showed similar gradients, although levels varied from one cultivar to another.
(3) BDL = below detectable limit.

the examples presented here. The Mg level was higher in the lesion area than in healthy tissue at either end of the strip and it was dramatically higher in the "halo" immediately surrounding the lesion than in the center of the lesion itself.

Line scans for K (Figure 10) and Cl (Figure 11) indicated that the level of these elements was also higher in the "halo" surrounding the lesion than in the lesion itself. Unlike Mg, however, their levels were not clearly higher than those of healthy tissue further removed from the lesion area.

The level of Ca present was generally below detectable limits by either analytical method. However, one line scan run on one tuber sample showed a small rise in Ca level (i.e., above "background") corresponding with the lesion (Figure 12). Although not a typical result, it is shown here because of our interest in any detectable Ca, in view of the well-known interaction between Mg and Ca.

A typical result for control tissue is shown in Figure 13, for Mg in this instance. There is no gradient along the length of the strip, and the general level is the same as in the non-lesion ends (L and R) of the tissue strips containing incipient hollow heart lesions. (The rhythmic pattern simply denotes variations in Mg level as the beam passes over and impinges on an individual cell or junction between cells.) Results on control tissue for the other elements were similar in that there was no appreciable gradient along the length of the strips.

A typical line scan on a multilesion tuber specimen of incipient hollow heart is shown in Figure 14, in this case for Mg. The gradients are as would be expected on the basis of those found for samples containing a single lesion per tuber. The light-celled regions between the lesions had much higher levels than the lesions themselves, and both these areas contained more Mg than healthy tissue farther away from the lesions.

C.4 Conclusions

Potatoes subjected to temperature stress that occurs naturally in the field when the tubers were still at an early stage of development afforded the opportunity to study incipient hollow heart. The first symptom visible to the naked eye was a small dark necrotic spot approximately 2-3 cells in diam in the central pith, about midway along the length of the tuber. A slightly more advanced, but still early, stage was manifested by a patch of several such (and larger) dark spots. These were always contained within the pith.

Restricting the tests to pith tissue overcame inaccuracies that occur in microanalytical studies when more than one tissue type is included in a sample.

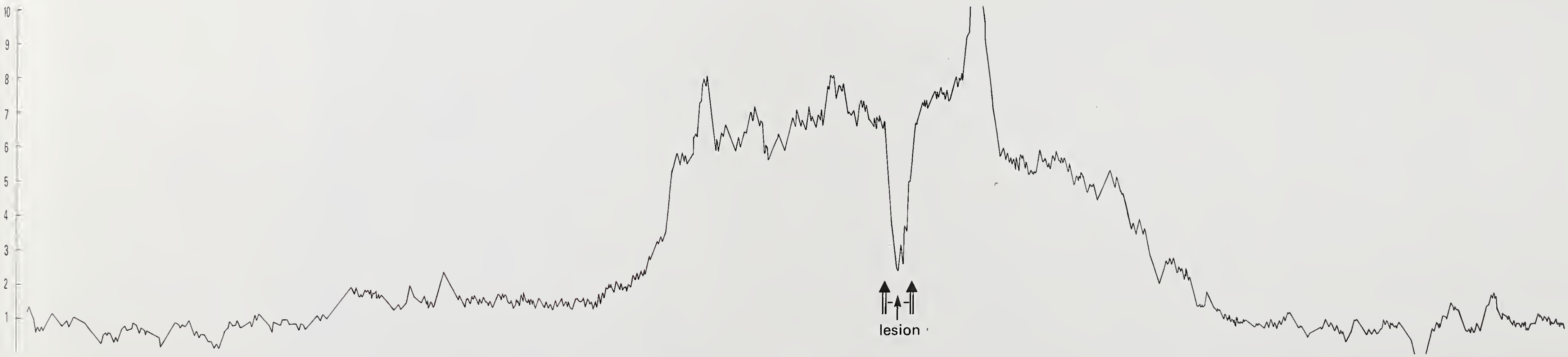


Figure 8. Line scan for Mg along the entire length of a tissue strip taken from the central pith of potato tuber.
(This tissue sample traversed a very small lesion characterizing the first visible symptom of the disorder hollow heart.)

Figure 9. Important segments of a line scan for Mg rerun on the same tissue sample shown (in its entirety) in Figure 8.
The scan runs along the pith tissue traversing a very small (2–3 cell diam) dark lesion characterizing the first visible symptom of hollow heart.

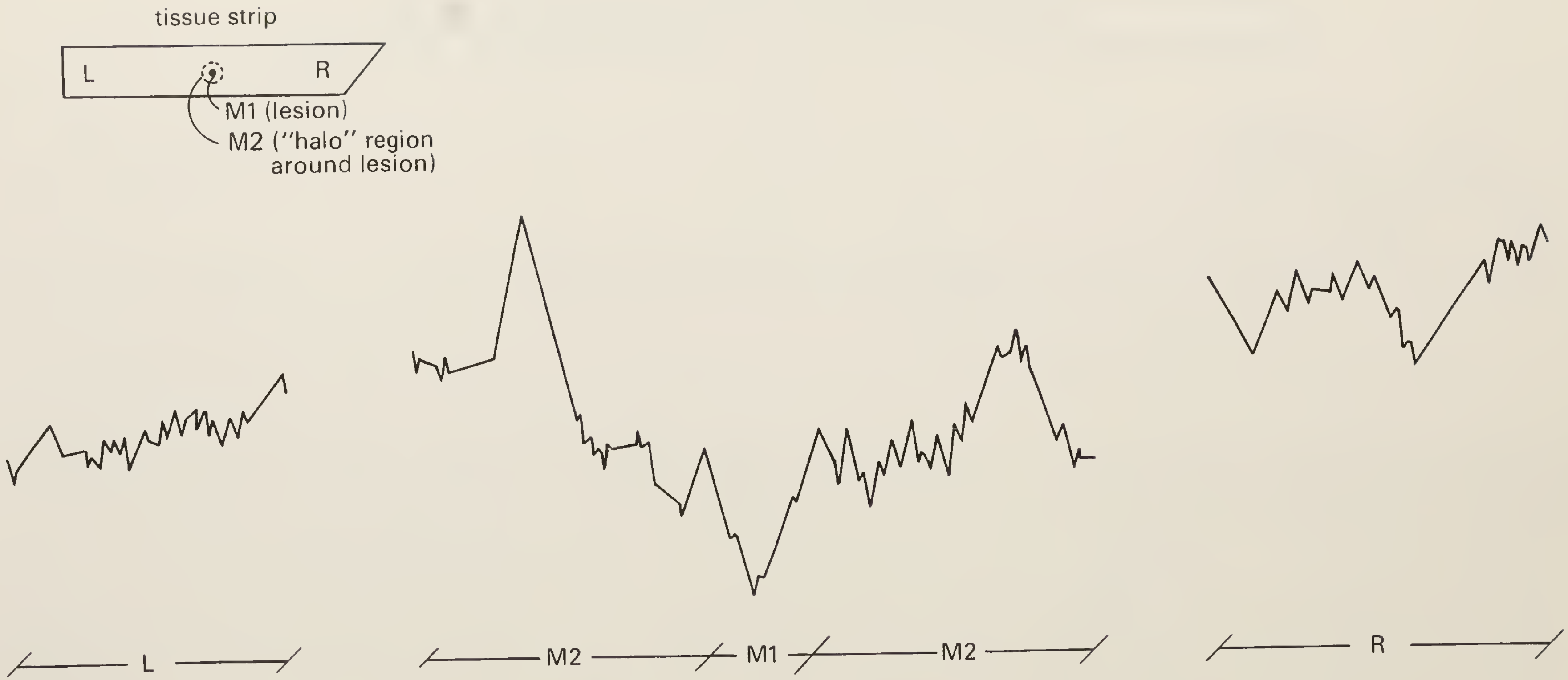


Figure 10. Segments of a line scan for K traversing a small dark lesion characterizing the first visible symptom of hollow heart in potato.

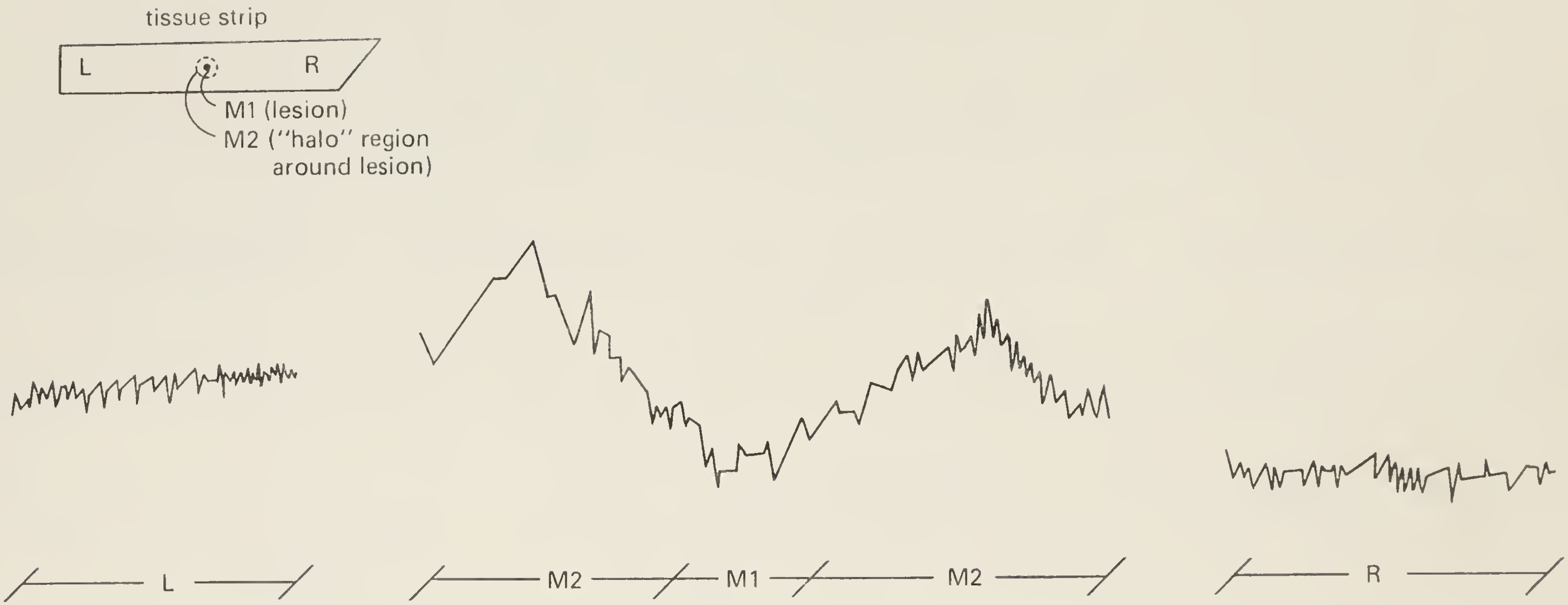


Figure 11. Segments of a line scan for Cl traversing a small dark lesion characterizing the first visible symptom of hollow heart in potato.

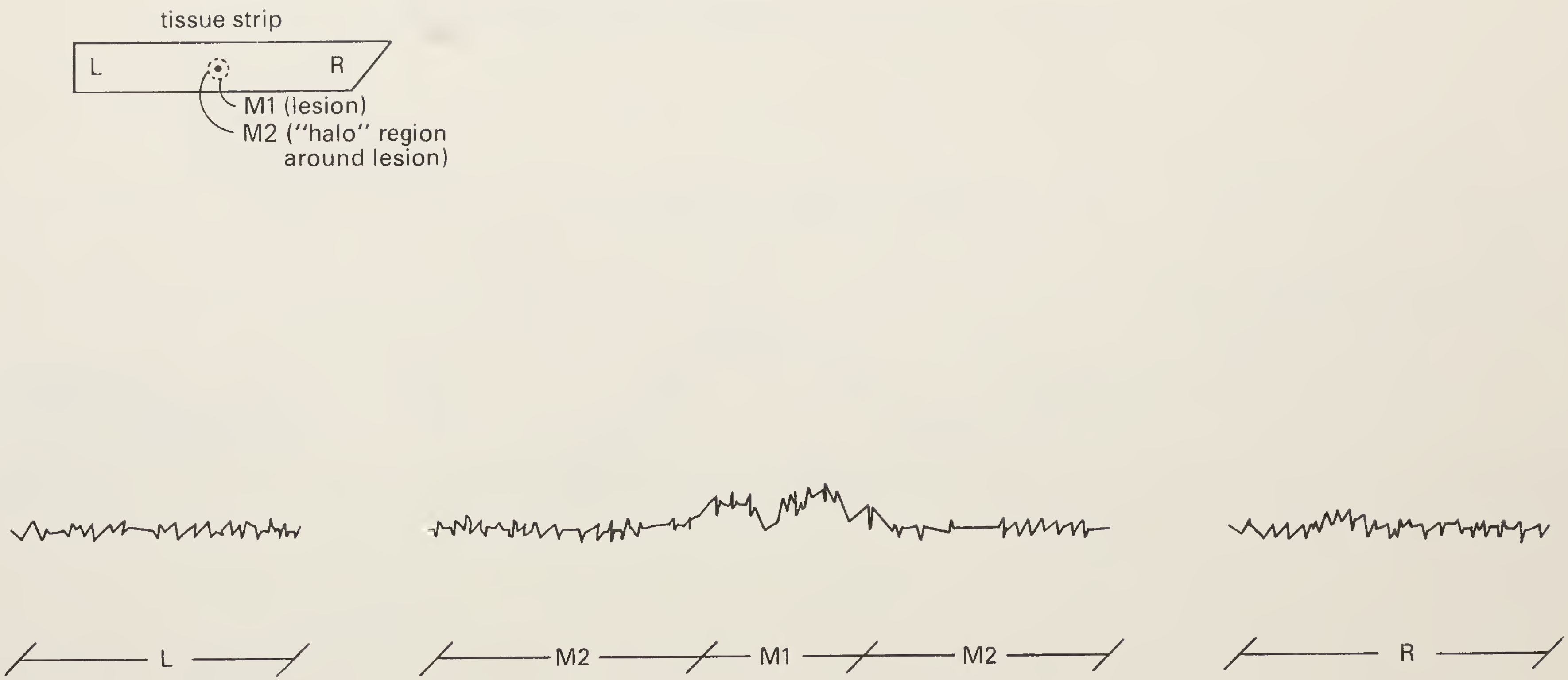


Figure 12. Segments of a line scan for Ca traversing a small dark lesion characterizing the first visible symptom of hollow heart in potato.

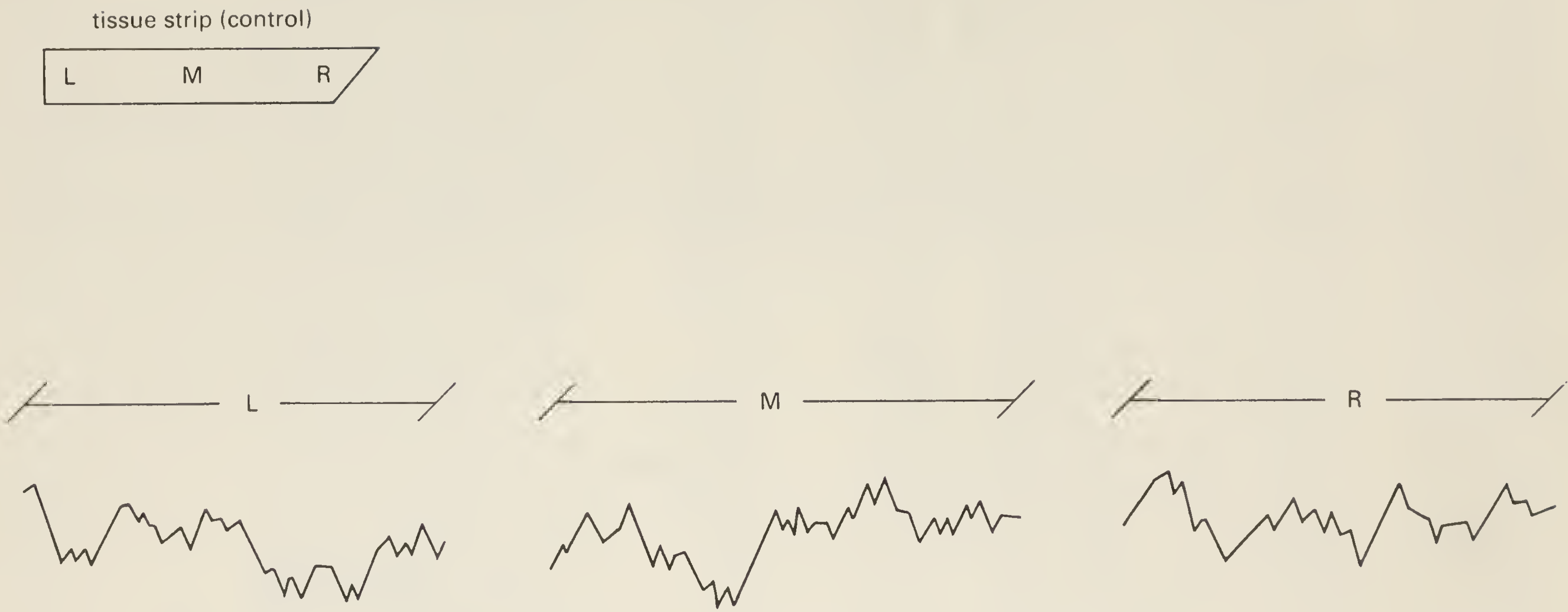


Figure 13. Typical segments of a line scan for Mg along pith of control tuber (region of pith comparable to that sampled for hollow heart tuber, Figure 9).

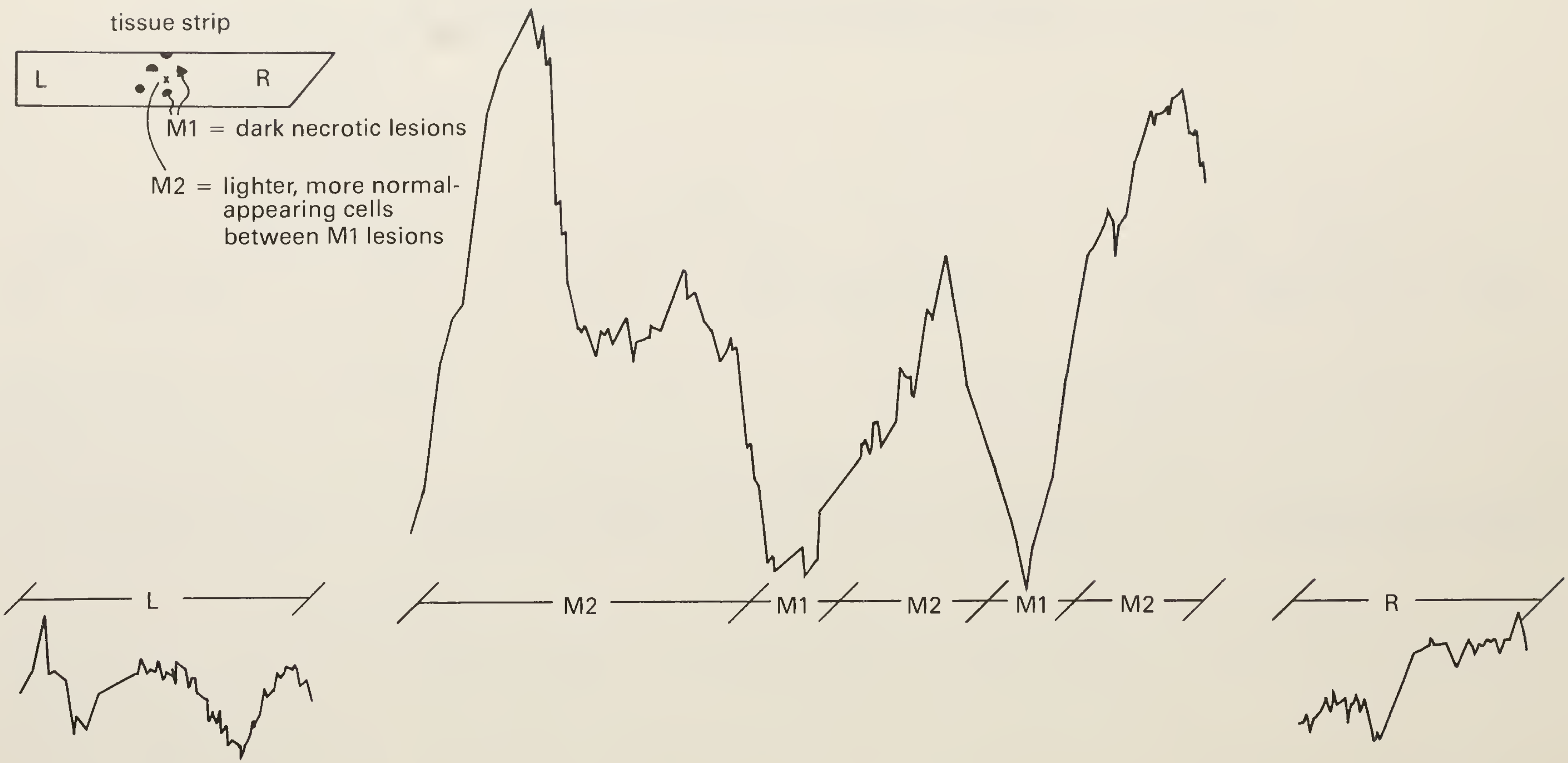


Figure 14. Segments of a line scan for Mg along pith traversing a patch of several small dark necrotic lesions characterizing an early stage of hollow heart in potato.

The elemental microanalyses revealed dramatic gradients in Mg. Levels of this element were considerably higher in the lesion area than in healthy pith (i.e., the healthy portion of the pith of tubers containing incipient hollow heart, as well as the pith of control tubers). The "halo" zone immediately surrounding the lesion had even higher levels than the lesion itself.

There was little or no gradient in K or Cl between the lesion area and healthy pith. As with Mg, however, the "halo" immediately surrounding the lesion had much higher levels than the lesion itself.

The healthy portions of pith of tubers with incipient hollow heart and the pith of control tubers had similar elemental levels.

These X-ray microanalytical results obtained in the form of energy spectra and the more detailed line scans were repeatable and conclusive.

The results suggest that a nutrient imbalance triggers the onset of the cell necrosis characterizing the initiation of this disorder in potato. A localized Mg toxicity or Ca deficiency resulting from a high Mg-to-Ca ratio is probably implicated.

D.0 INCIPIENT HOLLOW HEART (PRESUMABLY), LABORATORY-INDUCED BY TEMPERATURE STRESS: manifested by a uniform light brown, oval-shaped area in the central pith of the immature tuber.

D.1 Object

To determine elemental gradients in the region of the uniformly light brown discolored area in the central pith of potatoes subjected to temperature stress under laboratory conditions.

D.2 Methods

The tubers for this experiment were received on 3 August 1981 from Dr. H. Timm of the Vegetable Crops Department, University of California at Davis. They were from an experiment in which Dr. Timm induced hollow heart under laboratory conditions in order to provide material facilitating further study of this disorder. The plants had been subjected to temperature stress by sudden elevation in growing temperature, with water supply kept adequate throughout. The tubers were harvested at an early stage of tuber development, sliced longitudinally, and examined for incipient hollow heart symptoms. Many tubers were found to have a characteristic discoloration, presumed to be incipient hollow heart, though differing from the smaller, very dark type studied in Experiment C.

In the present test series the discolored areas (lesions?), one per tuber and usually oval in shape, were uniformly light brown in color over the entire discolored area. Located about midway along the length of the tuber or sometimes nearer the stem end, they varied in size from roughly 4 mm x 8 mm in some tubers to 10 x 20 mm in others. The smaller ones were usually contained within the pith, but larger ones extended radially slightly beyond the pith into the perimedullary tissue.

Tissue samples of the lesion region of affected tubers and comparable areas of control tubers were taken for X-ray microanalysis (Figure 15). Care was again exercised to include only pith tissue in the sample. Methods used in sampling, mounting, and analyzing by energy dispersive mode are as described in section C.2.

D.3 Results

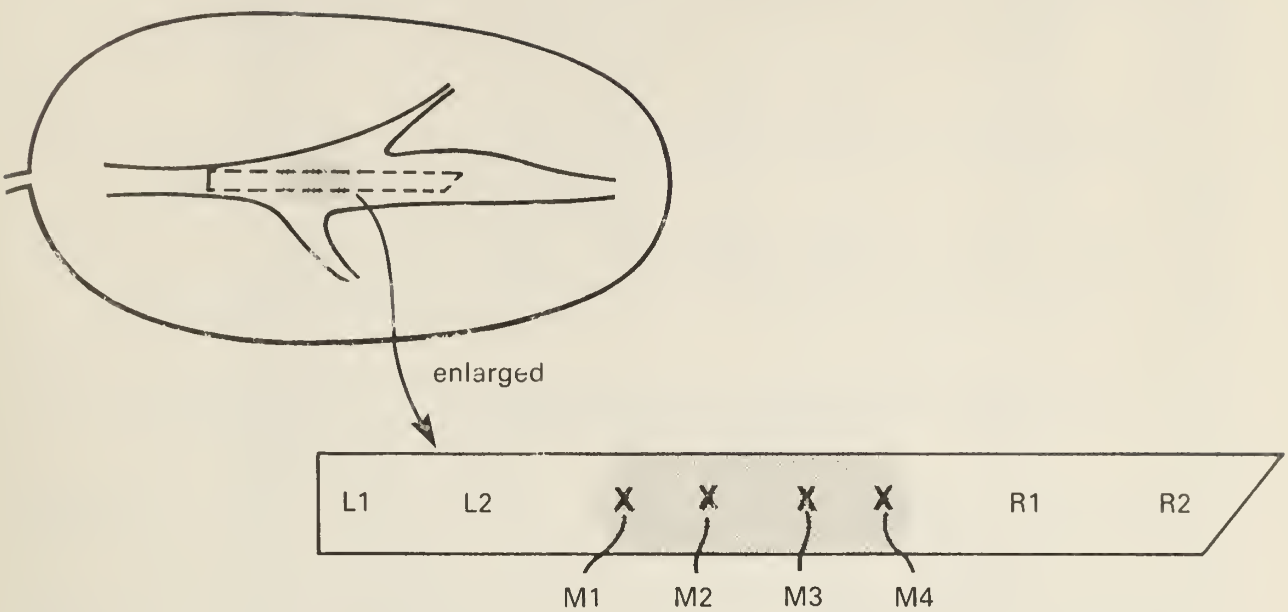
The uniformly light brown discolored areas in the pith of affected tubers in this experiment are thought to be "brown center", a precursor of hollow heart if the disorder continues to develop (Van Denburgh et al. 1979). The cells in these lesions did not appear to be as severely necrotic as those in the smaller, isolated, darker lesions in Experiment C tubers, which resulted from field-induced temperature stress. The latter type of lesion has been commonly associated with incipient hollow heart (see Levitt 1942).

The energy spectra revealed K, Cl, S, P, and Mg in significant quantities. Lesion and non-lesion pith tissue did not vary greatly in elemental composition, but there was a trend towards higher levels of all elements in the lesion area. This trend was most pronounced with K and least pronounced with Mg.

D.3 Conclusions

The "brown center" type of lesion produced under laboratory stress conditions differed from the "small isolated dark" type produced under field stress conditions in elemental composition as well as appearance. Results for Mg, in particular, differed between the two types. The small isolated dark type of lesion dealt with in Experiment C has been more commonly associated with hollow heart. It is concluded, therefore, that the differences in elemental composition found in Experiment C are more relevant to studies on incipient hollow heart than are those of this experiment.

Tuber with lesion



Control tuber

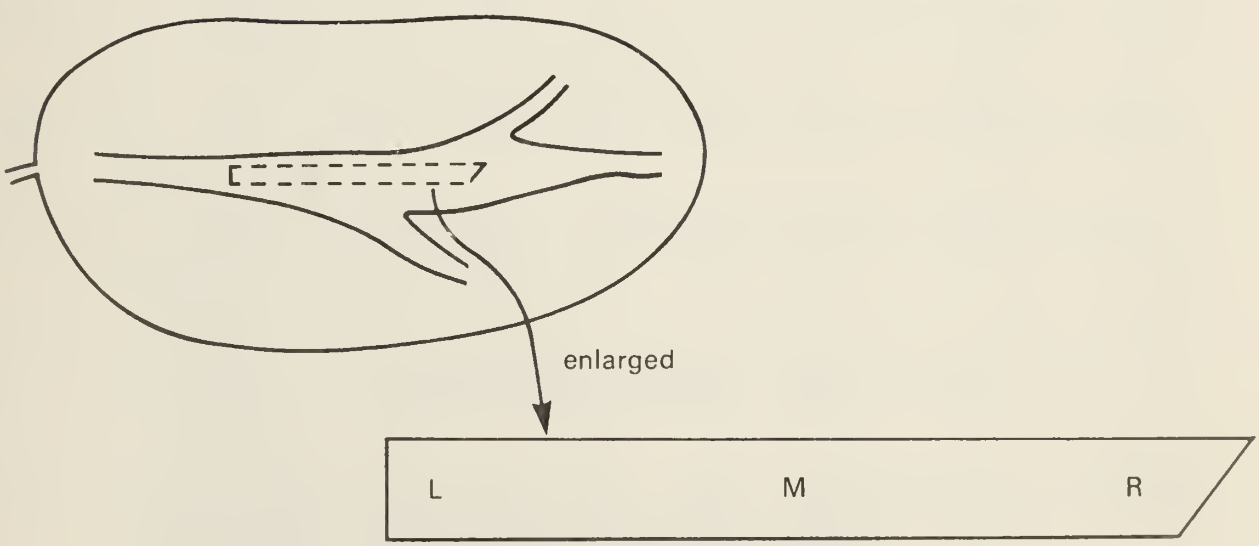


Figure 15. Diagram showing where strips were taken from tubers with and without lesions (potatoes temperature-stressed under laboratory conditions), and test sites on strip.

Table 6. Electron microprobe analysis for elements in tubers with incipient hollow heart in the form of a uniformly light brown discolored area (in potatoes temperature-stressed in the laboratory), compared with control tubers*

	Hollow heart tuber								Control tuber		
	L1	L2	M1	M2	M3	M4	R1	R2	L	M	R
	(counts in '000s/100 sec)										
K	8.5 (±1.1)	10.3 (±1.2)	12.6 (±1.1)	14.9 (±1.2)	16.8 (±1.7)	17.8 (±1.7)	14.5 (±1.6)	13.5 (±0.7)	13.6 (±1.9)	11.7 (±1.3)	14.0 (±1.9)
Cl	0.2 (±0.2)	0.4 (±0.2)	0.5 (±0.2)	0.5 (±0.2)	0.4 (±0.2)	0.6 (±0.2)	0.4 (±0.2)	0.1 (±0.1)	0.8 (±0.2)	0.5 (±0.2)	0.6 (±0.2)
S	1.0 (±0.1)	1.1 (±0.1)	1.2 (±0.1)	1.3 (±0.1)	1.5 (±0.3)	1.6 (±0.2)	1.1 (±0.1)	0.9 (±0.1)	1.1 (±0.2)	0.8 (±0.2)	1.3 (±0.2)
P	1.3 (±0.1)	1.7 (±0.2)	2.0 (±0.3)	2.3 (±0.4)	2.7 (±0.4)	2.6 (±0.3)	2.5 (±0.3)	1.9 (±0.1)	2.4 (±0.4)	2.4 (±0.3)	2.3 (±0.3)
Mg	0.3 (±0.1)	0.3 (±0.1)	0.4 (±0.1)	0.3 (±0.1)	0.4 (±0.1)	0.2 (±0.1)	0.3 (±0.1)	BDL	0.2 (±0.1)	0.2 (±0.1)	0.1 (±0.03)

* (1) See Figure 15 for test sites. Note that M1, M2, M3, and M4 are within the light brown discolored lesion area.
(2) Values reported are means of seven determinations using seven different tubers, with standard errors given in brackets.
(3) BDL = below detectable limit.

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