This thesis is dedicated to my family,

particularly to my late mother,

Mama Vida, my great aunt, Auntie Carie,

and to my father, "Daddy".

STUDIES ON METABOLIC CHANGES ACCOMPANYING IMBIBITION AND VERNALISATION OF A SPRING AND A WINTER WHEAT

BY

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TABLE OF CONTENTS

ABSTRACT

Changes in protein content, amino acid, fatty acid and phosphate metabolism were followed in the morphologically distinct parts and whole grains of Marquis (spring) and Rideau (winter) wheats during imbibition and vernalisation.

Vernalisation had a negligible effect on the protein content of the grains of either wheats. However, large increases in the total alcohol soluble amino acid and amide fractions were observed in the grain parts (embryo-scutellum and endosperm) of vernalised wheats. The percentage distribution varied both between grain parts and between spring and winter wheats. The duration of the imbibition period prior to vernalisation was another important variant.

Despite these variations, the embryo fractions of both wheats consistently contained larger amounts of nine amino acids following vernalisation.

After vernalisation the ratio of acidic to total basic amino acids decreased in the grain parts.

Three methods of computing the amino acid data were used in all cases; namely, per protein content, dry weight or grain part. Whereas the general trends outlined were consistent throughout, differences in the magnitude of change were observed to be dependent upon the base from which the data were calculated.

With regard to fatty acid composition, it was observed that vernalisation increased the degree of unsaturation in the plumule, radicle and scutellum of Marquis grains (largely as a result of the increased proportions of oleic and linoleic acids), while under the same

conditions the degree of unsaturation in these three grain parts of Rideau grains were decreased (largely as a result of a decrease in the proportion of linoleic acid). The major fatty acid components of the Marquis endosperm and all the grain parts of Rideau wheat were oleic and linoleic acids. The proportions of these acids did not change with vernalisation in the endosperm of either variety. Only traces of linoleic acid were noted before and after vernalisation in all the grain parts of both varieties except in the plumules of the partially and fully vernalised Marquis wheat.

^{32}P-orthophosphate taken up by both varieites during imbibition was incorporated largely into water-soluble organic phosphates and this incorporation continued during vernalisation.

Chilling accelerated the uptake of ^{32}P into the total nucleic acids of the grain parts and whole grains of Rideau wheat, relative to the uptake of ^{32}P into the nucleic acids of the controls. Incorporation of ^{32}P into the total nucleic acids of Marquis whole grains was low and varied only slightly during vernalisation.

The ^{32}P incorporated into the phospholipids of the various Rideau and Marquis grain parts was concentrated in the "lysophosphatidic acid" and lecithin components. In all the Rideau grain parts, the uptake of ^{32}P into lecithin increased with vernalisation; simultaneously, the incorporation of ^{32}P into the "lysophosphatidic acid" component decreased.

The ^{32}P incorporation data suggest that the scutellum of both varieties act as a "sink" for the phosphate metabolites from the other grain parts.

From the results obtained in these studies, a hypothesis has been suggested to account for the mode of action of vernalisation in winter wheat grains.

RESUME

L'influence de la vernalisation et de l'imbibition sur la teneur en proteines et sur le metabolisme des acides amines, des acides gras et des phosphates est studiee dans deux varietes de grain de ble, Marquis (ble de printemps) et Rideau (ble d'hiver).

La vernalisation n'altere pas la teneur en proteines du grain de ble. Cependant elle augmente la quantite d'acides amines solubles dans l'embryon et l'endosperme. La distribution de ces composes dans les differentes parties du grain et dans les differentes varietes de ble n'est pas la meme. De plus la distribution varie avec la duree de la periode d'imbibition.

Le rapport acides amines basiques/acides amines acides decroit au cours de la vernalisation. Ce rapport pourrait donc etre employe comme un indicateur de vernalisation.

Trois facons sont employees pour exprimer la teneur en acides amines du grain de ble, soit micromoles par mg de proteine, par mg de poid sec ou par partie du grain. Les resultats varient quelque peu selon la methode de calcul utilisee mais les tendences generales demeurent les memes.

La vernalisation augmente le degre d'insaturation des acides gras de la plumule, la radicelle et le scutellum du grain de ble Marquis et le diminue dans le ble Rideau. Les acides gras principaux de l'endosperme du grain de ble Marquis et de toutes les parties du grain de ble Rideau sont l'acide oleique et l'acide linoleique. La proportion de ces acides dans l'endosperme des deux varietes de ble ne varie pas durant la vernalisation.

Le ^{32}P-orthophosphate absorbe durant l'imbibition des grains de ble est incorpore surtout dans les phosphates solubles dans l'eau. La vernalisation accelere cette incorporation.

Le froid accelere aussi l'incorporation du ^{32}P dans les acides nucleiques du grain de ble Rideau. Le taux d'incorporation du ^{32}P dans les acides nucleiques du grain de ble Marquis est relativement bas et n'est que legerement modifie par la vernalisation.

Le ^{32}P incorpore dans les phospholipides des grains de ble Marquis et Rideau est concentre dans les lecithines et dans l'acide lysophosphatidique. Dans toutes les parties du grain de ble Rideau, l'incorporation du ^{32}P dans les lecithines augmente avec la vernalisation, tandis que l'incorporation dans "l'acide lysophosphatidique" decroit.

Les resultats obtenus au moyen du ^{32}P suggerent que le scutellum agit comme un sink des composes du phosphate provenant des autres parties du grain.

On suggere une hypothese pour expliquer le mode d'action de la vernalisation chez le grain de ble d'hiver.

VI. INTRODUCTION

(A) Literature Review

(1) General review

(a) Vernalisation - In temperate countries, the grains of winter cereals must be planted before the end of winter in order that these cereals will flower and fruit within ten months of sowing. Spring cereals will flower in mid-summer after a spring sowing. Lysenko (1928) established that slight imbibition of water made the cereal susceptible to the action of cold without inducing excessive germination that could prevent the use of a sowing machine. He termed this action of cold "Jarovization", which in English is known as vernalisation.

The degreee of imbibition should be sufficient to allow vernalisation of winter and spring cereal grains to occur, but insufficient for morphological growth (Lysenko, 1962; Gregory and de Ropp, 1938). Oxygen is also required for vernalisation (Chouard, 1960).

In winter cereal grain a cold treatment (-1 to 5°C), that is, vernalisation, followed by a return to normal temperatures, induces or accelerates a more rapid onset of flowering. The chilling treatment affects the meristematic cells of the embryo (Konovalev, 1937; Konovalev and Rogalev, 1937; Gregory and Purvis, 1938a; Purvis, 1947). Specifically, mitotic cells have been shown to "perceive" the cold stimulus (Wellensiek, 1964 and 1965).

Excised cereal embryos and small fragments of the embryo containing portions of the plumule can be vernalised successfully if they are exposed to water (Purvis, 1940; Nutman, 1941) and sugars normally found in the endosperm (Chouard, 1960). In some winter cereals, mineral ions enhance effects of the chilling process (Gregory and Purvis, 1938).

Although vernalisation invariably accelerates flowering in winter cereals, this is not always the case with spring cereals. Wort (1939 and 1940) showed that in spring wheats, flowering was accelerated in some instances by vernalisation and was either retarded or not affected in other instances.

Certain physico-chemical characteristics are common to all vernalisation phenomena. As a result of vernalisation the cells of the embryo are less readily coloured by ferric chloride and potassium ferrocyanide (Bassarskaja, 1934, 1936). This is one of the empirical tests for the completion of vernalisation. A shift in the iso-electric point of proteins towards acidity following vernalisation has been demonstrated by Ritcher (1934) and Wort (1941). Filippenko (1936) and Chestakov and Serveev (1937) independently reported a slight increase in the coagulability and permeability of proteins. In addition, Purvis and Gregory (1952) observed that the vernalising action modified the pH of the "cell liquids" (vacuolar sap). A reduction in the auxin level has also been noted by Czailachjan and Zdanova (1938) and by Sereiskii and Sluckaja (1954).

To avoid repetition, the words "cold treatment (cold treated)" and "chilling (chilled)" will be used as synonyms of "vernalisation (vernalised)".

(b) Imbibition - The early stages of imbibition relate to the purely physico-chemical phenomenon of hydration of hydrophilic colloids.

Ermilov (1960) showed that the hydration of the component organs in the maize grain is a sequential process. He demonstrated that the ingress of water into the endosperm is temperature independent and is completed before the entry of water into the embryo starts. He also showed that the subsequent hydration of the embryo was dependent on temperature and upon the "activation" of the scutellum. Mayer and Poljakoff-Mayber (1963) stated that the chief components which exhibit active imbibition are the cell wall celluloses, mucilages and pectic substances and the hydrophilic proteins in the aleurone layer and in the embryo proper. Ingle et al (1964) observed that rapid uptake of water by these components facilitated hydration of carbohydrates and mobilization of reserve materials (fats and sugars) and their utilization for axis growth in corn. They noted that the mode of entry of water into the seed or grain, as well as the sequence initiated by this entry were difficult to define. Ku (1965) observed that in winter (Rideau) wheat grains, imbibition of water was complete after 14 hours at 24°C; she also noted that in spring (Marquis) grains, imbibition was complete after only five hours.

The effect of water on protein reserves in seeds and grains is much better understood. The hydration of grain proteins leads to complex changes, five types of which are relevant to the present study:

(i) the de novo synthesis of such enzyme systems as proteases (Briggs, 1963) and amylases (Paleg, 1960; Yomo, 1961; Drennan and Berrie, 1962; Menshall and Goodwin, 1964) controlled by DNA-RNA synthesis (Naylor, 1966; Chrispeels and Varner, 1967);

(ii) the activation of dehydrated enzyme systems (Tazakawa and Hirokawa, 1956);

(iii) the associated breakdown of certain storage materials (Tazakawa and Hirokawa, 1956);

(iv) the synthesis of new cellular material from the breakdown products so formed (Oota et al, 1953);

(v) the transport of lower molecular weight substances from one part of the grain to another, especially from the endosperm to the embryo (Mer et al, 1963; Oaks, 1965) and subsequently from the embryo to the aleurone layer (Chrispeels and Varner, 1967).

The increased metabolism resulting from imbibition leads to the emergence of the embryo through the pericarp. This, in turn, causes a major change both in the immediate environment of the young plant and in its subsequent development. The increased metabolic activity required for vacuolation, cell division, and the synthesis of new material is sustained by the mobilization of the reserves in the endosperm. This increased metabolism is initiated by the release of gibberellic acid from the embryo (Chrispeels and Varner, 1967); the acid activates the amylases and proteases in the aleurone layer of the endosperm.

Wellington (1965) observed that the time required to complete each intermediate stage in the germination process depended not only upon the hydration of colloids, but also upon the physiological maturity of the grain.

Brown (1965) pointed out that the information on the intermediate steps of germination, their sequence and interdependence was relatively meagre. This information is usually inferred from the effects of specific environments, treatments and chemicals on the end response

of the seed or grain. The maturing grain on the ear does not differ significantly from the grain at the end of maturity with respect to its morphology, anatomy or cytology. The onset of cell growth or division, or both, are prerequisites for seedling growth. However, they are relatively late events in the germination sequence.

Obviously then, primary changes must be looked for on the macromolecular level, as well as in the enzyme-substrate-hormone relationships. Such biochemical studies, in general, have been confined to the metabolic processes that are concerned with the breakdown of storage material (Ingle et al, 1964) and those that provide the cell with energy. A major problem lies in the difficulty of distinguishing primary from secondary metabolic responses to treatments, both of which affect the course and result of germination.

The ability of a grain to germinate may be attained relatively early in its development, i.e. long before ripening and soon after fertilization (Grabe, 1956). Hyde et al (1959) observed that in both grass and legumes the ability to germinate could be related to a distinct developmental stage occurring when the moisture uptake abruptly levels off and ceases to parallel dry weight accumulation.

The usual optimal temperature range for grain germination is around 15 to 30°C. Ku (1965) reported that the rate of germination for spring and winter varieties of Triticum vulgare (Rideau and Marquis respectively) was greatest at 24.0 ± 1.0°C. The main overall effect of temperature on imbibed seeds relates to the "growth potential" of the embryo. Does low temperature directly or indirectly affect the metabolism of the seed or grain ? Stone (1957) suggested that the cold induced increase in "growth potential" of the embryo of Pinus lambertiana

is the result of the conversion of substrates to intermediates required for growth. This is supported by the fact that cold increases the efficiency of this embryo in utilizing sucrose for root growth.

(2) Protein and Amino Acid Changes

Amino acid and protein changes accompanying cold hardening and vernalisation have been followed by numerous authors. Pauli and Mitchell (1960) reported that Pawnee wheat plants grown previously at 70°F and then cold hardened at 35°F (1.6°C), i.e. adapted to resist frost damage, contained more soluble protein nitrogen than unhardened (non-adapted) plants grown at 70°F (21.1°C). Pauli et al (1961) correlated the increase in soluble nitrogen of winter wheat varieties with their cold hardiness. Zech and Pauli (1960) did not notice any change in the non-soluble protein nitrogen content of winter wheat leaves during the hardening process in field plantings. They did note, however, that soluble protein nitrogen increased gradually in these leaves with the onset of winter. Trione (1966) observed that the soluble protein fraction increased threefold in winter wheat varieties (vs. spring varieties) grown at 2°C. Durzan and Chalupa (1968) reported that the soluble protein content of the jack pine embryo decreased during germination, while dry weight and amide content increased; they also noted that many soluble proteins disappeared with germination.

Markowski et al (1962), working with winter and spring wheats grown at 1.5°C, stated that the total amino acid content as well as the qualitative composition of the free amino acid fraction were similar in both wheat varieties. However, conflicting evidence was suggested by the work of Smirnova-Ikonnikova and Feofanova (1963) who demonstrated that the free amino acid content in the embryo and endosperm did change

with vernalisation. The latter report was confirmed by St. Grzesiuk and Kulka (1963) who worked with rye at 4°C.

(3) Changes in DNA, RNA and Related Compounds

From experiments performed with microsomal particles of Vigna seeds and seedlings, Oota and Osawa (1954) concluded that there was a relationship between the ratio of RNA to protein and the seedlings' ability to synthesize protein.

It is now known that protein synthesis occurs on the ribosomes and that such synthesis is DNA-RNA directed (Nirenberg and Leder, 1964; Byrne et al, 1964; Crick, 1966).

Keys (1963) showed that during the first two days following the germination of wheat grains, soluble nucleotides remained at a low level in the endosperm whereas a fifteen-fold increase of these nucleotides was observed in the embryo. Keys and Cornelius (1965) demonstrated that, for embryos of the same dry weight, the level of soluble nucleotides was much lower at 0 - 5°C than at 25°C.

Nitsan (1962) reported an accumulation of an electrophoretically homogeneous macromolecular component in the vernalised embryos and seedlings of Petkus winter rye. This component was absent in non-vernalised embryos.

Studies by Salisbury and Bonner (1960) with 5-fluorouracil suggest that nucleic acid synthesis is involved in the vernalisation process. Reports in other fields implicate nucleic acids in hastening the flowering process (Evans, 1959; Chailakayan et al, 1961). Tomita, 1963 obtained evidence that uridylic acid was capable of producing a vernalisation-like effect when painted on to the leaves of winter rye plants.

Highkin (1955) soaked pea seeds in water at 4°C for eighty-five days; this water extract was used to soak non-vernalised pea seeds which were subsequently planted. This treatment led to a shortened flowering time. However, Highkin did not demonstrate whether nucleic acids or related compounds were contained in the water extract.

(4) Changes in Carbohydrates and Lipids

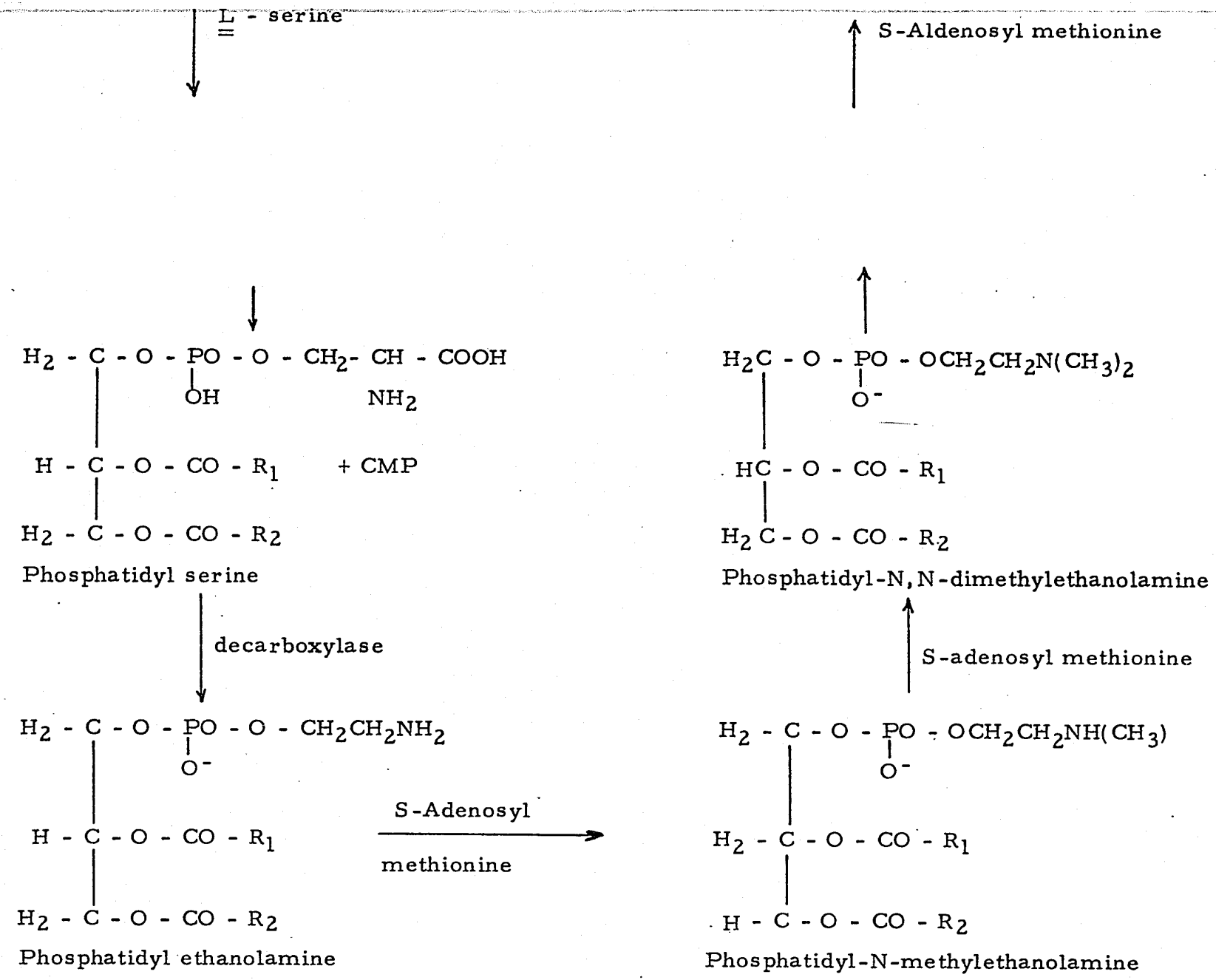
The effect of vernalisation on overall changes in the composition of carbohydrate and lipid fractions have been followed in cereal grains and in a few dicotyledonous plants. Sechet (1949) observed that in the vernalised grains of oat, barley, corn and rye, there was an accumulation of soluble sugars both in the embryo and endosperm following vernalisation. This increase was accompanied by a concomitant decrease in the amount of reserve polysaccharides. Duperon (1950) made a similar observation with respect to the seeds of Raphanus sativus, but noted that later when chlorophyll appeared, control and vernalised seedlings had the same carbohydrate composition. Duperon (1949) reported that the vernalised seedlings of Sinapsis alba contained more soluble sugars and lipids than control seedlings. When the same seedlings were exposed to 20°C for ten days, a decrease in the lipid content and a larger increase in the water soluble carbohydrates were observed. Redshaw and Zalik (1968) detected increases in the total lipids of Sanaste fall rye, Prolific spring rye, Kharkov winter wheat and Red Bobs spring wheat seedlings during six weeks of growth at 4 - 6°C.

(5) Phosphate Metabolism with Reference to Phospholipids

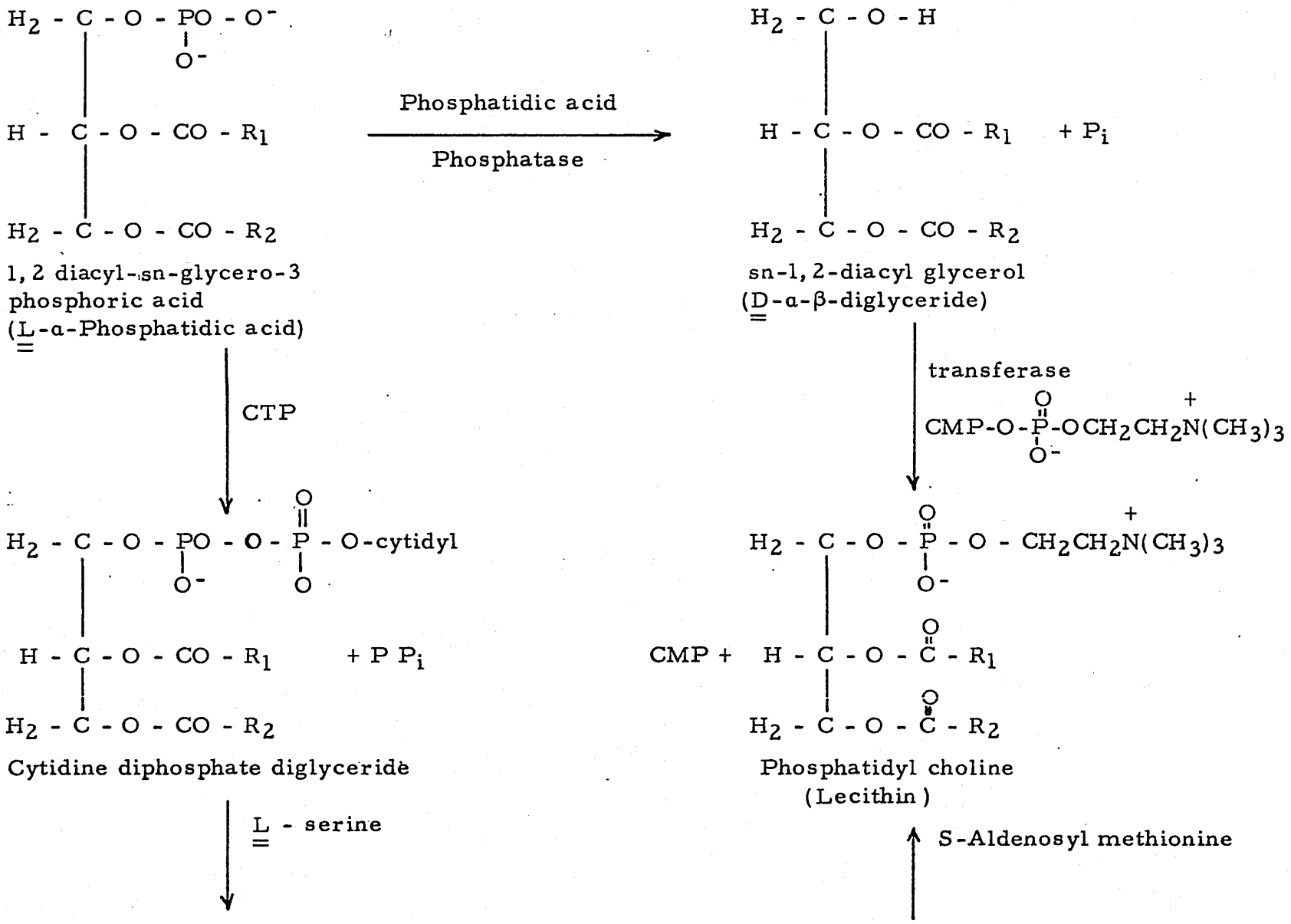
Phospholipids are important structural components of plant cell membranes, particularly those of the chloroplast and mitochondria (Benson, 1964). It has been suggested that phospholipids undergo dynamic changes in fulfilling certain physiological functions such as ion transport (Hokin and Hokin, 1959; Wogt, 1957).

Plant lipids have a unique composition which became evident with the discovery and elucidation of the structure of monogalactosyl and digalactosyl diglycerides (galactolipids) by Carter and associates (1956), of phosphatidyl glycerol by Maruo and Benson (1958) and sulphoquinovosyl (sulpholipid) by Benson and co-workers (1959). These four compounds together with phosphatidyl choline (lecithin) and phosphatidyl ethanolamine, are the major lipid components in plant photosynthetic tissues. In non-photosynthetic tissues, glycerides, phosphatidyl inositol, lecithin and phosphatidyl ethanolamine comprise the major lipids (Nichols, 1964).

Kennedy (1961) confirmed the central role played by phosphatidic acid in the de novo biosynthesis of different glycerides and phospholipids. Lecithin, one of the important plant and animal phospholipids, has been shown to be biosynthesized by two different pathways. In the first, (see Scheme I), elucidated by Kennedy (1961) using liver preparations, phosphatidic acid is dephosphorylated to D-α-β-diglyceride. Kennedy and Weiss (1956) and Rossiter et al (1957), using liver preparations and brain tissue respectively, demonstrated that D-α-β-diglyceride reacts with cytidine diphosphate-choline to form phosphatidyl choline (lecithin), the reaction being catalysed by the enzyme cytidine diphosphate-choline transferase.



Scheme I. Pathways for Lecithin Biosynthesis


Phosphatidic acid
Phosphatase
+ P_i
1, 2 diacyl-sn-glycero-3 phosphoric acid
(L-α-Phosphatidic acid)
sn-1, 2-diacyl glycerol
(D-α-β-diglyceride)
CTP
transferase
$CMP-O-P(=O)(O^-)-OCH_2CH_2N^+(CH_3)_3$
-cytidyl
+ P P_i
Cytidine diphosphate diglyceride
Phosphatidyl choline
(Lecithin)
L - serine
S-Aldenosyl methionine
CMP +

The second pathway (see Scheme I), involves the stepwise N-methylation of phosphatidyl ethanolamine to phosphatidyl choline. Bremer and Greenberg (1961) demonstrated that by stepwise acylation, with liver microsomal preparations, phosphatidyl ethanolamine in the presence of methionine is transformed to phosphatidyl choline, these reactions being carried out in the presence of S-adenosylmethionine. Kaneshiro and Law (1964) obtained a purified cell-free soluble enzyme from Agrobacterium tumefaciens which catalyses the transfer of a methyl group from S-adenosylmethionine to phosphatidyl ethanolamine forming phosphatidyl-N-methylethanolamine. The enzymes responsible for the subsequent methylation steps to form lecithin are associated with a particulate system.

Borkenhagen et al (1961) had previously shown (Scheme I) that in the presence of phosphatidyl serine decarboxylase, phosphatidyl serine was broken down to phosphatidyl ethanolamine and carbon dioxide in crude extracts of E. coli.

Carter and Kennedy (1966) showed that phosphatidic acid (Scheme I) reacted with cytidine triphosphate in the presence of phosphatidic acid cytidyl transferase to form cytidine diphosphate-diglyceride in preparations from guinea pig liver. Using similar preparations, Kanfer and Kennedy (1964) demonstrated that cytidine diphosphate diglyceride reacted with L-serine, in the presence of L-serine-CMP phosphatidyl transferase to form phosphatidyl serine and cytidine monophosphate.

Sastry and Kates (1965), using ^{32}P-orthophosphate and ^{32}P-glycerophosphate, obtained evidence suggesting that the pathways described above existed in Chlorella vulgaris.

Using excised tomato root tissue exposed to uniformly labelled ^{14}C-serine, serine-1-^{14}C, serine-3-^{14}C, or ethanolamine-1,2-^{14}C, Willemot and Boll (1967) presented evidence showing the occurrence

of a pathway whereby phosphatidyl serine was decarboxylated to phosphatidyl ethanolamine; the latter was further methylated to phosphatidyl choline.

(6) Changes in Fatty Acid Composition

Fatty acid accumulation in plants is influenced by such environmental factors as light and temperature (Dybing and Zimmerman, 1966) and by the stage of maturity of the plant (Sims, 1963). Ponelleit and Alexander (1965) and Stephansson et al (1961) studied the genetic control of fatty acid composition in Maize seeds (grains) and Rape seeds, respectively, under fixed environmental conditions.

Hardman and Crombie (1958) and White (1958) reported that the overall fatty acid composition of the seeds of Citrullus vulgaris and cotton, respectively, remained relatively constant during germination. Similar observations were made by Huber and Zalik (1963) on the germinating seeds of Linum usitatissimum L. However, Crombie and Comber (1956) noted that oleic acid was preferentially utilised in germinating watermelon seedlings.

Generally, organisms growing at lower environmental temperatures have more highly unsaturated fatty acids (Kates and Baxter, 1962) and more highly unsaturated lipids (Pearson and Raper, 1927; Gaughran, 1947; Bass and Hospodka, 1952; Hilditch, 1956) than those organisms growing at higher environmental temperatures.

Gerloff et al (1966) reported that the fatty acid content of non-hardy Caliverde and hardy Vernal alfalfa root tissue increased approximately two-fold during cold hardening. Redshaw and Zalik (1968) noted an increase in unsaturation in the fatty acid composition of whole seedlings of spring and winter rye during vernalisation.

VI. INTRODUCTION

(B) Statement of the Problem

Many studies have been undertaken to elucidate the phenomenon of vernalisation (Lysenko 1928, 1932; Lang, 1952, 1957; Gregory and Purvis 1936, 1937, 1938, 1948); however, many of the biochemical changes accompanying or underlying this phenomenon still remain obscure. For the most part, relevant biochemical studies on vernalisation have dealt with changes in amino acid content of whole embryos and/or whole grains during germination and growth (Markowski et al, 1962; Smirnova-Ikkonnikova and Feofanova, 1963; St. Grzesiuk and Kulka, 1963). No valid comparative data relating these changes to innate varietal differences, to grain parts, or to specific environmental responses have been published. Furthermore, little or no information has been reported on changes in phosphate compounds during or at the end of vernalisation, although some data are available on nucleic and changes (Konarev, 1954; Finch and Carr, 1956).

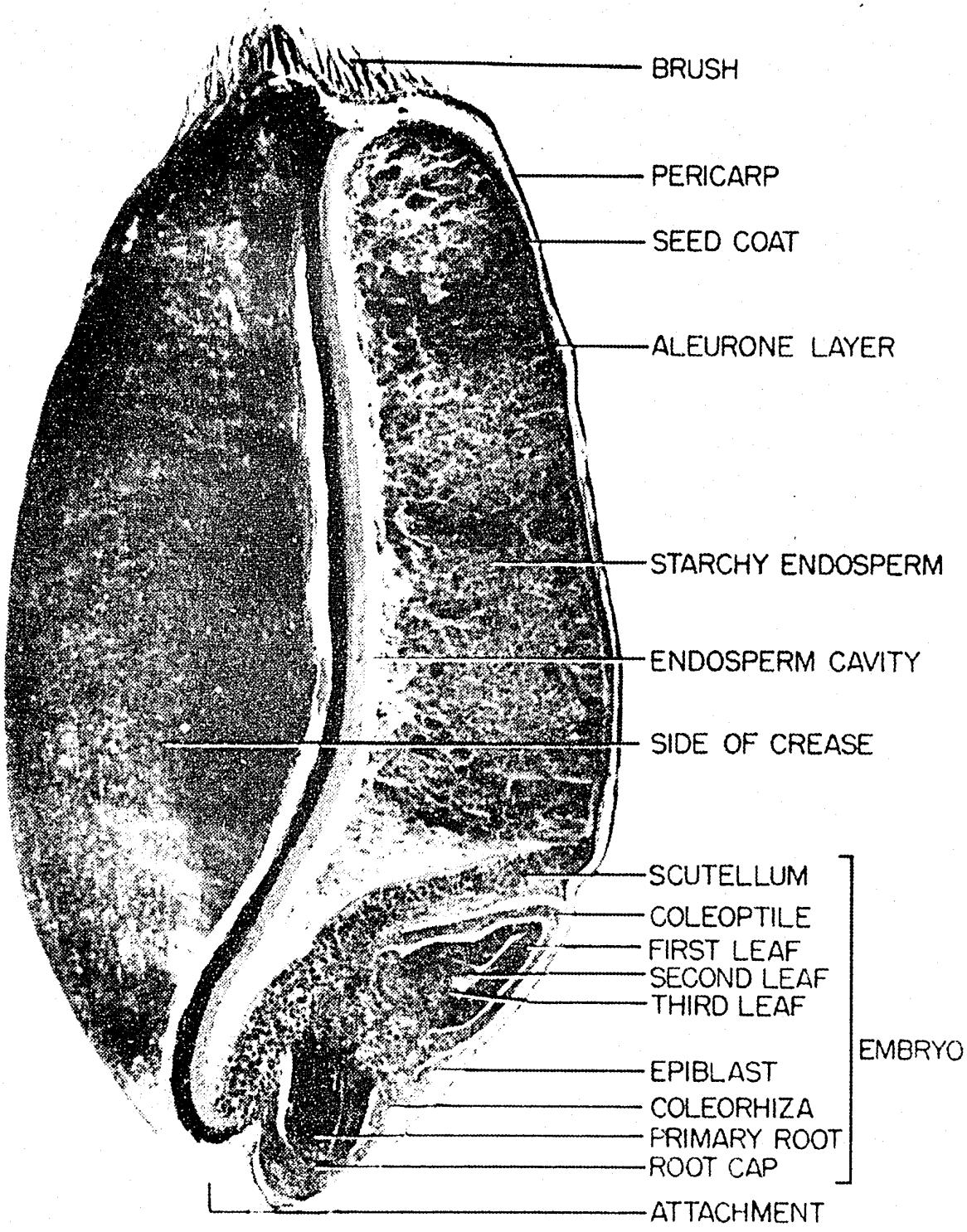
The present study, therefore, was undertaken to obtain a more detailed picture of the metabolic changes occurring the morphologically distinct parts of wheat grains both during the periods of imbibition and vernalisation, and following completion of vernalisation. The investigations were made on the plumule, radicle, scutellum and endosperm-pericarp of both a spring and a winter variety of Triticum vulgare L. (Fig. I). Specifically, changes in total protein content, amino acid and fatty acid compositions were followed; phosphate metabolism with the aid of ^{32}P-orthophosphate was also studied.

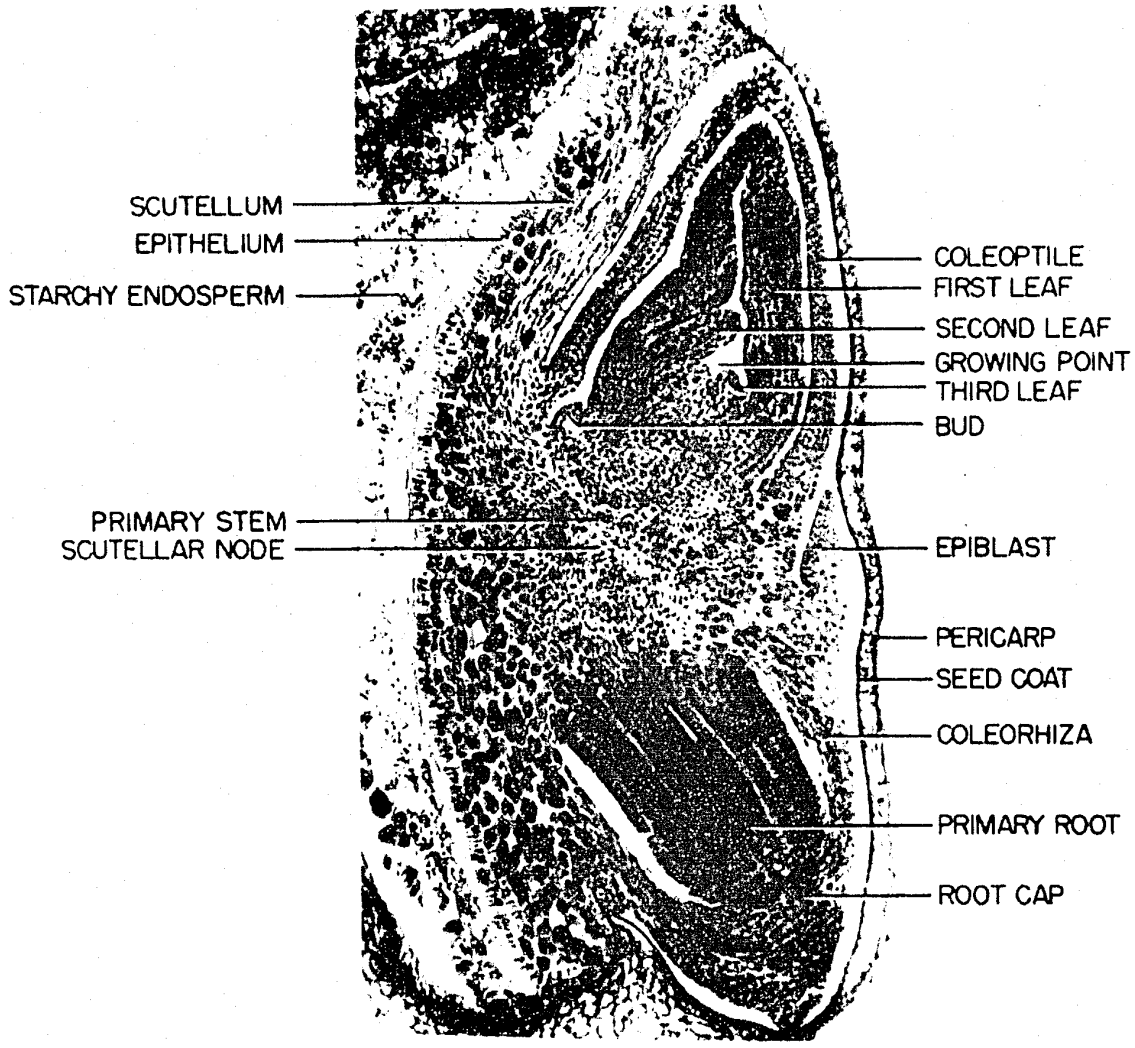
FIGURES I A and I B

Legend

1A. Longitudinal section of a grain of Triticum vulgare L. var. Marquis.

1B. Longitudinal section of the embryo of Triticum vulgare L. var. Marquis.


BRUSH
PERICARP
SEED COAT
ALEURONE LAYER
STARCHY ENDOSPERM
ENDOSPERM CAVITY
SIDE OF CREASE
SCUTELLUM
COLEOPTILE
FIRST LEAF
SECOND LEAF
THIRD LEAF
EPIBLAST
COLEORHIZA
PRIMARY ROOT
ROOT CAP
EMBRYO
ATTACHMENT


SCUTELLUM
EPITHELIUM
STARCHY ENDOSPERM
PRIMARY STEM
SCUTELLAR NODE
COLEOPTILE
FIRST LEAF
SECOND LEAF
GROWING POINT
THIRD LEAF
BUD
EPIBLAST
PERICARP
SEED COAT
COLEORHIZA
PRIMARY ROOT
ROOT CAP

VII. OUTLINE OF EXPERIMENTS PERFORMED

1. Changes in Proteins

EXPTS. I and II.

(i) Rideau grains imbibed for 5 hours (partial) and 14 hours (complete); Marquis grains imbibed for 5 hours (complete), according to the method of Ku (1965).

(ii) Marquis grains vernalised for one week; Rideau grains vernalised for five weeks (Ku, 1965).

(iii) Dissected grains into endosperm-pericarp and embryo-scutellum.

(iv) Used imbibed and vernalised grain parts in protein determination, cf. Tables I and II.

2. Changes in Amino Acids

EXPTS. III and IV.

(i) Same imbibition, vernalisation and dissection procedure as in Expts. I and II.

(ii) Used imbibed and vernalised grain parts in amino acid determination, cf. Tables III-VIII and Figs. II - V.

3. Distribution of ^{32}P in Phosphate Compounds

(a) During Imbibition:

EXPT. V

(i) Allowed Rideau grains to imbibe ^{32}P-orthophosphate solution.

(ii) Followed uptake and incorporation into water-soluble organic phosphates and phospholipids with time - Table IX and Fig. VI.

(b) During Vernalisation:

EXPTS. VI and VII

(i) Allowed complete imbibition of ^{32}P-orthophosphate by Rideau and Marquis

(ii) Vernalized grains partially and completely

(iii) Dissected some imbibed and some vernalised grains.

(iv) Fractionated different phosphate compounds from grain parts and whole grains. Incorporation of ^{32}P into these phosphates shown in Table X, XII, XV and Figs. VII, IX-XI measured in duplicate 5 grain samples.

- organic phosphates chromatographed and autoradiographed obtained from (iv), cf. Table XII and Fig. XII.
- lipids used in chromatography and autoradiographed, cf. Tables XIV, XVI-XXII and Plates I-IV, and fatty acid analysis, cf. XXIV-XXVIII obtained from (iv).

EXPT. VIII

(i) Rideau grains allowed to imbibe ^{32}P-orthophosphate solution for 5 and 14 hours.

(ii) Vernalised both sets of imbibed grains for three and five weeks.

(iii) Assayed incorporation of ^{32}P into water soluble organic phosphates of whole grains, cf. Table XI and Fig. VIII.

VIII. EXPERIMENTAL

(A) Materials and Methods

(1) Imbibition Procedures

(a) Without ^{32}P-orthophosphate (amino acid studies) - Three millilitres of glass distilled water were added to 5 grams (approx. 120 grains) of spring or winter wheat, Triticum vulgare L. [varieties Marquis (M) or Rideau (R), respectively]. The grains were contained in blackened, sterilized Petri-dishes (9 cm. diameter) lined with filter paper. Two separate periods of imbibition were utilized for Rideau wheat grains, namely 5 (partial) or 14 (complete) hours (R_5 or R_{14}); for Marquis wheat, a 5-hour imbibition period (M_5) is necessary for complete imbibition. The grains were allowed to imbibe water in a growth chamber maintained at 24 $\pm$ 1°C (Ku, 1965). The above procedure was repeated once.

(b) With ^{32}P-orthophosphate - A solution of 2 mC of phosphorus-32 (^{32}P) as the orthophosphate ion (Atomic Energy of Canada Ltd.) in dilute HCl (8.4 ml) was neutralized with 1.0 N sodium hydroxide to the phenol red end point (pH 6.8) and made up to 10.0 ml with distilled water. This stock solution, containing 0.2 mC per ml, was used in the labelling experiments.

To study the effect of imbibition in Rideau wheat, 2.5 ml of the stock solution of ^{32}P-orthophosphate was added to 40 grains in a stoppered 25 ml Erlenmyer flask covered with aluminum foil to exclude light. Batches of ten grains were removed after 2, 5, 7, and 14 hours of imbibition; the grains were removed from the Erlenmyer flask, blotted

on moistened filter paper, washed one at a time in distilled water for about fifteen seconds and dried again on filter paper. This operation was followed to ensure the removal of excess ^{32}P-orthophosphate. The grains were then subjected to the Bligh and Dyer (1959) lipid extraction procedure described below (see page 20.).

To compare the imbibition of ^{32}P in both Marquis and Rideau wheats, forty-five grains of each variety were separately allowed to imbibe 2.5 ml of the stock ^{32}P solution in a stoppered 25 ml Erlenmyer flask covered with aluminum foil for five and fourteen hours respectively (Ku, 1965) at room temperature. At the end of the imbibition periods, the grains were removed from the Erlenmyer flask, blotted on moistened filter paper, washed one at a time in distilled water for about fifteen seconds and dried again on filter paper. This operation was followed to ensure the removal of excess ^{32}P-orthophosphate. The procedure was repeated once. In addition, Rideau grains were allowed to imbibe ^{32}P for 5 hours.

(2) Controls

These consisted of samples (15 grains each) taken immediately after completion of the imbibition procedures described for both the Rideau ($R_{14}K$) and Marquis (M_5K) grains respectively. The control grain parts were derived from the control grains.

(3) Vernalisation Procedures

(a) Without ^{32}P-orthophosphate - At the end of the imbibition periods, grains were placed in blackened, sterilized, Petri-dishes (9 cm. diameter) lined with filter paper and stored in a

refrigerator maintained at a temperature of 2 ± 1°C. For Marquis samples, seven days cold (Ku, 1965) exposure was necessary to complete vernalisation (M_5C_1); Rideau grains were exposed to the cold temperature for five weeks (R_5C_5 , $R_{14}C_5$), (Ku, 1965).

(b) With ^{32}P-orthophosphate - The chilling procedure followed was similar to that described under (a). However, Marquis grains were chilled for four days (M_5C_4) and seven days (M_5C_7) and Rideau grains for three weeks ($R_{14}C_3$) and five weeks ($R_{14}C_5$).

(4) Dissection of Grains

Protein and amino acid changes were assessed on grains of both varieties dissected into embryo-scutella and endosperm at the end of the imbibition and vernalisation periods.

Studies relating to phosphate metabolism and fatty acid changes in partially or fully vernalised grains of both varieties were undertaken on the separately dissected grain parts, i.e. plumule, radicle, scutellum and endosperm-pericarp.

Grains (maintained at 0 - 2°C) were dissected with a micro-dissecting needle and the dissections were aided by a dissecting microscope.

(5) Fractionation of ^{32}P-labeled Compounds

(a) Extraction of lipids - A modification of the Bligh and Dyer procedure was used to extract lipids and water soluble phosphates (Bligh and Dyer, 1959). For each treatment, five whole Marquis or Rideau grains were macerated in a mortar and then transferred to a

50 ml. glass stoppered Pyrex centrifuge tube (Scheme II); 8.0 ml of methanol, 4.0 ml of chloroform, and 3.2 ml of distilled water (2:1:0.8) were added to the macerated grains. The same procedure was followed with the macerated endosperm-pericarp samples (pooled from 5 grains). The other macerated grain part samples (pooled from 5 grains) were extracted with 4.0 ml of methanol, 2.0 ml of chloroform, and 1.6 ml of distilled water in a 15 ml glass stoppered pyrex centrifuge tube. All grain part extractions were done in duplicate.

The mixtures were agitated on a Vortex mixer for one minute, left at room temperature for one hour, and then centrifuged at 700 g for fifteen minutes; the supernatant was transferred to a stoppered pyrex centrifuge tube using a Pasteur pipette. The above extraction procedure was repeated once. Finally, the insoluble material residue (1) (Scheme II) was suspended in 1.0 ml of methanol-water (3:4, v/v) and the mixture centrifuged. The combined supernatants were then diluted with chloroform and water to give a final ratio of methanol, chloroform and water as 1:1:0.9; the resulting mixture was gently shaken briefly and centrifuged at 700 g for ten minutes.

The lower chloroform phase was separated from the upper methanol-water phase by Pasteur pipette. The chloroform solution was diluted with an equal volume of benzene and concentrated to dryness under a stream of nitrogen. The residue was immediately redissolved in 2.0 ml of chloroform, and the solution stored, if necessary, in the refrigerator. The methanol-water mixture was retained in order to determine its inorganic and organic phosphorus content.

(b) Extraction of acid soluble ^{32}P - A modification of the Moraczewski and Kelsey (1948) procedure was used. One millilitre of 15% TCA solution was added to residue (1) in each tube. The

contents were mixed intermittently on a Vortex mixer during a period of one hour; following centrifugation at 700 g for ten minutes, the supernatants were withdrawn and added to the methanol-water mixture obtained from the Bligh and Dyer procedure. Residue (1) was then finally washed with 1.0 ml of distilled water, centrifuged and the supernatant added to the TCA-methanol-water extract. This procedure was sufficient to remove all acid soluble phosphates. The TCA-extracted residue (2) (Scheme II) was used for the extraction of nucleic acids.

(c) Extraction of ^{32}P-labelled total nucleic acids - Schneider's (1945) method as modified by Moraczewski and Kelsey (1948) was used. Three millilitres of distilled water were added to residue (2) of all fractions. The resulting mixture was agitated in a mechanical shaker for fifteen minutes. The mixture in an unstoppered centrifuge tube was then immersed in boiling water for two minutes and 10.0 ml of a 10% TCA solution were added to it immediately. The tube was then stoppered and the heating continued for an additional ten minutes following this; the tube was cooled and centrifuged.

The supernatant, containing DNA and RNA nucleosides, was transferred to another 15 ml centrifuge tube. Residue (2) was then washed with 1.0 ml distilled water, and after centrifuging at 700 g for ten minutes, the washing was added to the TCA extract. The resulting residue - residue (3), (Scheme II) - was used in the extraction of phosphoproteins described below.

(d) Extraction of ^{32}P-labelled phosphoproteins - The method of Moraczewski and Kelsey (1948) was followed. To residue (3) was added 0.2 N sodium hydroxide (3.0 ml for samples of endosperm and whole grains and 1.0 ml for the other grain parts). The mixture was agitated on a Vortex mixer for one minute and left at room temperature (25°C) overnight. The mixture was then centrifuged for ten

minutes at 700 g and the supernatant removed by Pasteur pipette.

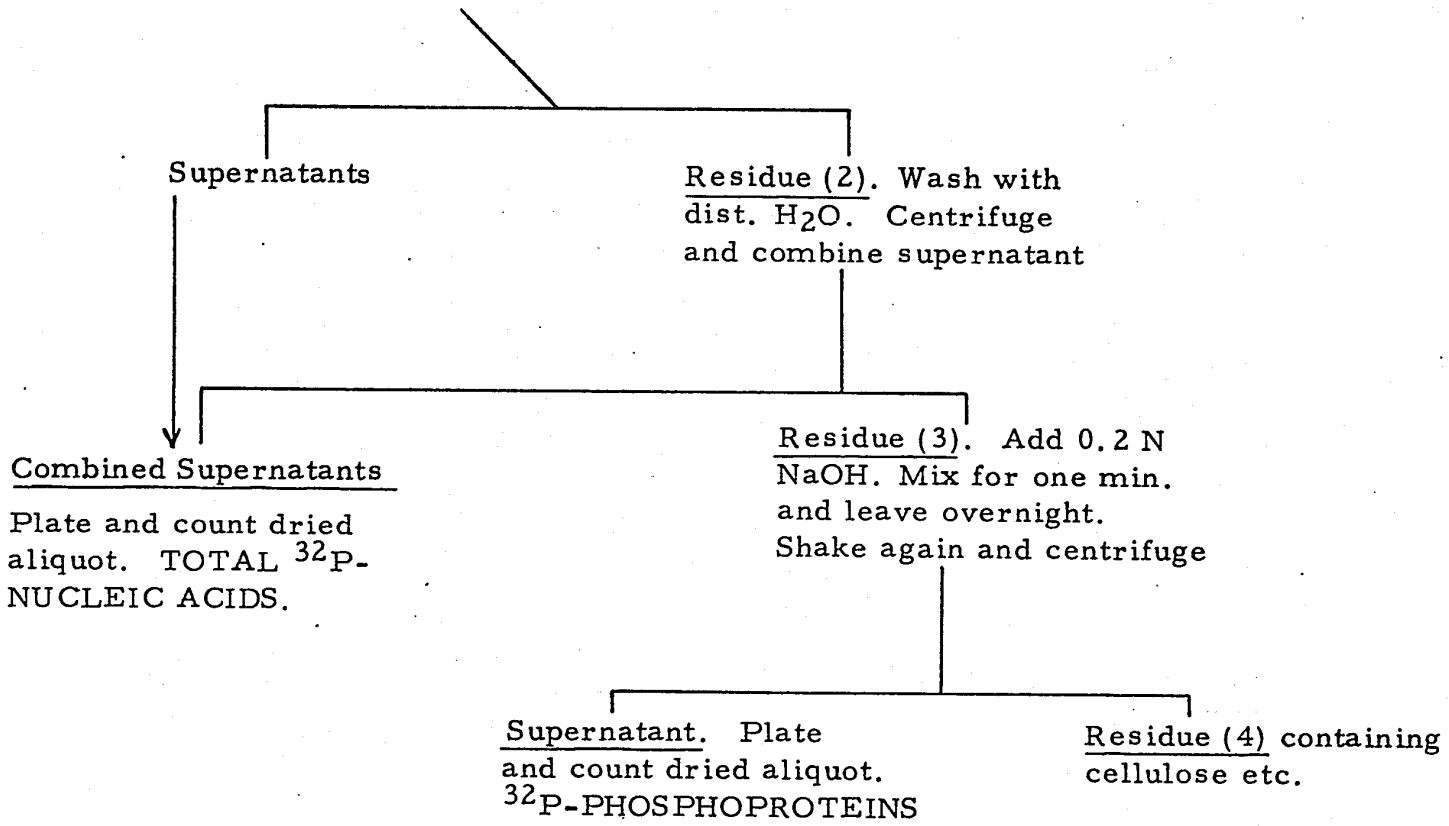
A summary of the fractionating procedure for the ^{32}P-labelled compounds is given in the flow sheet (Scheme II).

(e) Inorganic ^{32}P-determination - The ratio of P_{org} to P_i was determined by a modification of the method of Bernblum and Chain (1938). To 1.0 ml of the test solution was added 1.0 ml of a mixture of 2N H_2SO_4, 5% aqueous ammonium molybdate (1:1 v/v) and two millilitres of butanol. The contents were shaken and centrifuged for two minutes. Aliquots were removed from the upper alcoholic phase, plated, dried and counted to obtain inorganic ^{32}P. A similar procedure was followed with an aliquot from the lower aqueous phase to obtain organic ^{32}P.

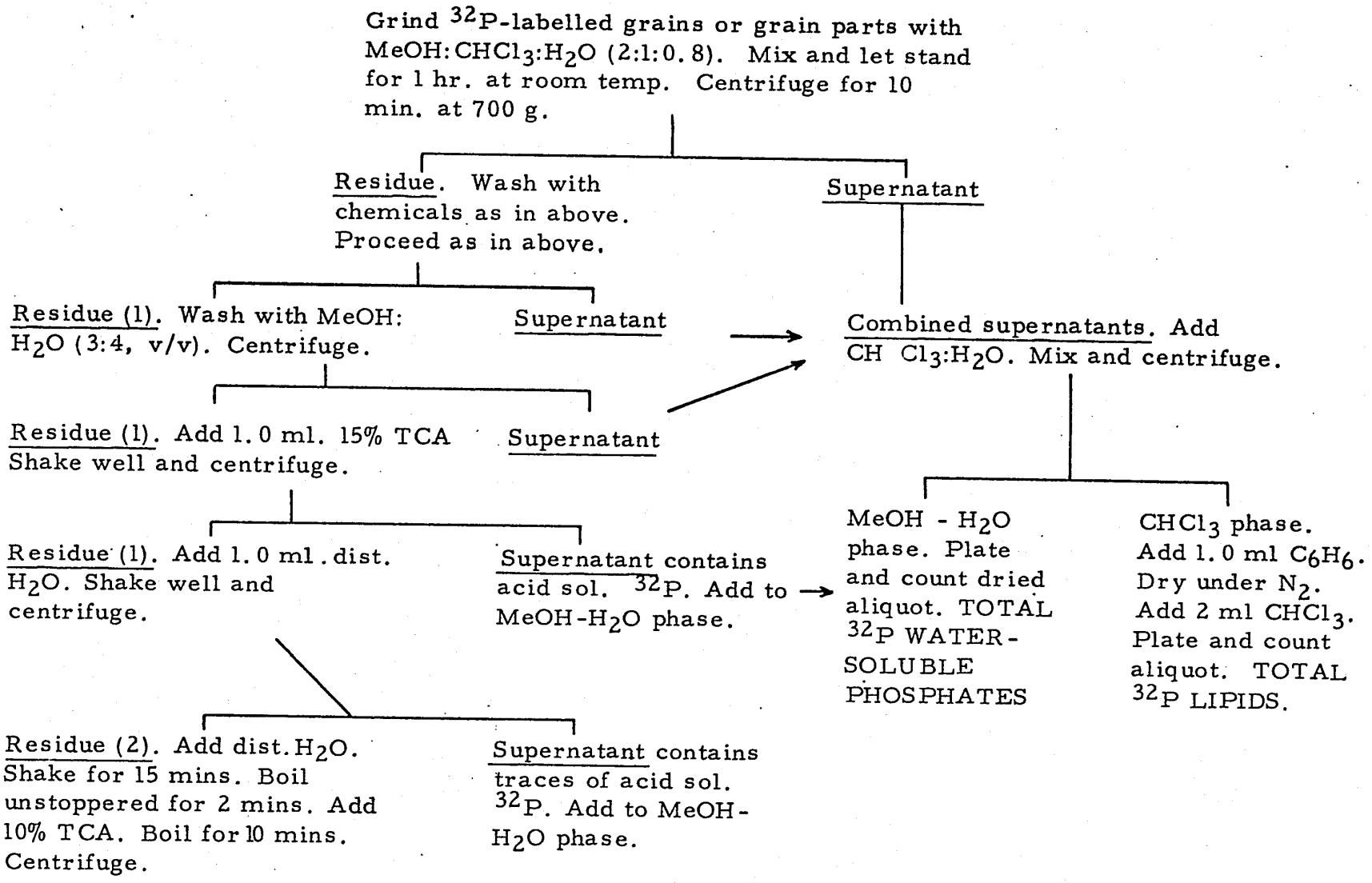
(6) Analytical Procedures

(a) Proteins - Total proteins were extracted with a sodium borate solution (Fowden, 1952) and determined quantitatively by the method of Lowry (Lowry et al, 1951).

(b) Amino acids and amides - Alcohol soluble amino acids and amides present in the various grain parts were extracted exhaustively with 60% ethanol (Folkes, 1959) at room temperature, until tests for the presence of amino acids (with ninhydrin) in the supernatant indicated complete extraction. The ethanol extract was concentrated in a stream of cold air and the residue resuspended in 1.0 ml of a 12% sucrose solution. Aliquot portions were then analysed with a Technicon Amino Acid Analyser (Spackman et al, 1958). With this procedure the amides, glutamine and asparagine, were eluted together with serine and threonine. In order to obtain resolution of these compounds, an



Scheme II. Procedure followed in the Fractionation of ^{32}P-labelled compounds.


Grind ^{32}P-labelled grains or grain parts with MeOH:$CHCl_3$:H_2O (2:1:0.8). Mix and let stand for 1 hr. at room temp. Centrifuge for 10 min. at 700 g.
Residue. Wash with chemicals as in above. Proceed as in above.
Supernatant
Residue (1). Wash with MeOH: H_2O (3:4, v/v). Centrifuge.
Supernatant
Combined supernatants. Add CH Cl_3:H_2O. Mix and centrifuge.
Residue (1). Add 1.0 ml. 15% TCA Shake well and centrifuge.
Supernatant
Residue (1). Add 1.0 ml. dist. H_2O. Shake well and centrifuge.
Supernatant contains acid sol. ^{32}P. Add to MeOH-H_2O phase.
MeOH - H_2O phase. Plate and count dried aliquot. TOTAL ^{32}P WATER-SOLUBLE PHOSPHATES
$CHCl_3$ phase. Add 1.0 ml C_6H_6. Dry under N_2. Add 2 ml $CHCl_3$. Plate and count aliquot. TOTAL ^{32}P LIPIDS.
Residue (2). Add dist. H_2O. Shake for 15 mins. Boil unstoppered for 2 mins. Add 10% TCA. Boil for 10 mins. Centrifuge.
Supernatant contains traces of acid sol. ^{32}P. Add to MeOH-H_2O phase.

aliquot was hydrolysed with 1 N HCl at 110°C for five hours (Borsook and Dubnoff, 1939). This hydrolysis resulted in the conversion of asparagine and glutamine into aspartic acid and glutamic acid respectively. The amide content was then taken as the difference between the glutamic acid and aspartic acid values obtained before and after hydrolysis.

(c) Paper chromatography of lipids - Aliquots of the chloroform extract of total lipids obtained by the Bligh and Dyer method were spotted on Whatman No. 3 MM filter paper impregnated with silica gel (Kates, 1967). A minimum of 1000 counts per minute, contained in the extract of ^{32}P-labelled phospholipids was applied to each spot. The chromatograms were developed in diisobutylketone-acetic acid-water (8:5:1, v/v) (Marinetti et al, 1957) by the ascending technique for fifteen to eighteen hours. The chromatograms were then removed from the tank, dried in the fume hood and stained by dipping the impregnated paper in a dilute Rhodamine 6G solution (Marinetti, et al, 1957) for three minutes. The excess stain was rinsed off with distilled water and the wet chromatogram was viewed immediately under ultraviolet light (Mineralight Lamp, 366 mμ), and the fluorescent spots with their various colours were marked with pencil (Marinetti et al, 1957).

To confirm the presence of phosphatidyl ethanolamine on the chromatograms, dry chromatograms after development were sprayed with a freshly prepared homogeneous solution of 0.25% ninhydrin in acetone and 2,6-lutidene (9:1) at room temperature (Marinetti and Stotz, 1955). The chromatograms were then air dried at room temperature.

To confirm the presence of galactolipids, chromatograms were dipped in 0.25% solution of sodium periodate and then dried for fifteen minutes. After this, the chromatograms were dipped in 1.0% solution of sodium metabisulphite until they became colourless (Sastry and Kates, 1964) and then finally dipped in a dilute solution of the Schiff reagent (Marinetti and Erbland, 1957).

(d Deacylation of phospholipids to obtain phosphate esters - Modifications of the Brockerhoff (1963) and Heubscher et al (1960) methods were followed. An aliquot consisting of one-twentieth of the chloroform solution of lipids obtained by the Bligh and Dyer method was brought to dryness in a 15 ml stoppered pyrex centrifuge tube under a stream of nitrogen. Chloroform (0.2 ml), methanol (0.3 ml), and 0.2 N methanolic sodium hydroxide (0.5 ml) were added in the above order to the residue in the centrifuge tube. The contents were well mixed and then left at room temperature for fifteen minutes. Methanol (0.2 ml), chloroform (0.8 ml), and distilled water (0.9 ml) were then added and the contents mixed and finally centrifuged.

The upper methanol-water phase was withdrawn completely and transferred to another 15 ml pyrex centrifuge tube. The lower chloroform phase was washed with 1.0 ml of a 10:9 methanol-water mixture which was removed after centrifugation and added to the methanol-water fraction.

The latter was shaken with Rexyn RG 50 cation exchange resin (approx. 100-200 mg) until neutral or slightly acid to litmus paper. The mixture was then centrifuged, and the supernatant removed by Pasteur pipette, transferred to another 15 ml pyrex centrifuge tube, and made alkaline by the addition of a few drops of 1.5 N ammonium hydroxide. The solution was evaporated to dryness under a stream of nitrogen and the residue taken up in 0.1 ml of the 10:9 methanol-water mixture, and spotted on Whatman No. 3 MM chromatography paper.

(e) Identification of phosphate esters - Aliquots of unknown (derived from deacylated phospholipids) and standard phosphate ester solutions were spotted on Whatman No. 1 paper. Each spot contained a minimum of ten micrograms of phosphorus. Chromatograms were run in saturated phenol-water (80%) solvent and in butanol-acetic

acid-water (5:3:1) solvent. The chromatograms developed in the butanol-acetic acid-water solvent were stained for phosphate following the method of Burrows et al (1952). The chromatograms developed in the phenol-water system were stained for vicinal hydroxyl groups by the procedure of White and Frerman (1967): the dry chromatograms were dipped in a solution containing 5 ml of a 0.25 M solution of sodium meta periodate and 95 ml of acetone. The wet chromatograms were then dried for fifteen minutes. After this, the chromatograms were then dipped in a solution made up of 2.12 gm of orthotolidine, 6.0 ml of glacial acetic acid, 49.0 ml of distilled water, and 950.0 ml of acetone. The chromatograms were then dried completely. The spots appeared yellow on a blue background.

Individual phosphate esters were identified by their colour reaction with the phosphate stain and by Rf value relative to known standards.

Aliquots of the methanol-water TCA phase were spotted on Whatman No. 1 filter paper and developed in tert.-butanol-water-picric acid (80:20:4, v/v/w). After autoradiography, the chromatograms were stained with sulfosalicylic acid-ferric chloride (Vorbeck and Marinetti, 1965) reagent to obtain phosphate esters which were identified by their Rf values relative to those of standard sugar phosphate esters.

(f) Fatty acid analysis by GLC - The chloroform layer containing neutral and phospholipids which was obtained after the deacylation procedure contained the fatty acid components as methyl esters; it was washed with methanol-water (10:9) as described above until neutral to litmus paper. Two millilitres of benzene were added to the chloroform solution which was then evaporated under a stream of nitrogen at 30°C. The residue obtained was immediately taken up in 1.0 ml of chloroform and analysed by GLC on a column of 10% butane-

diol succinate on Celite (Gas Chrom A). The temperature of the column was maintained at 176°C and argon, maintained at a pressure of 12 pounds per square inch, was used as a carrier gas.

The component peaks were identified by their retention time relative to authentic standards of palmitic, stearic, oleic, linoleic and linolenic acids (obtained from the Hormel Institute, Minn.). Quantitative compositions were calculated from the areas under the peaks using a modified procedure of that described by Carrol (1961). The equation used is given as:

$$\% \text{ of component } X = \frac{(t_x)(h_x)}{(t_i)(h_i)} \times 100\%$$

where t_x = retention time of component x

h_x = peak height of component x

t_i = retention time of individual components

h_i = peak height of individual components

(7) Radioisotope techniques

(a) Plating and counting - The ^{32}P activity in phospholipids, water soluble phosphates, phosphoproteins and nucleic acids was determined by plating an aliquot (usually 0.1 ml), of each fraction, containing at least 1000 counts/min on aluminum planchets (3.1 cm diameter and 0.2 cm deep). The plated samples were air dried and counted using an end window Geiger-Mueller Counter (Nuclear Chicago Corporation). Counts were corrected for decay, starting from time of imbibition, and for efficiency of counting (efficiency of counting for ^{32}P, 28%).

(b) Autoradiography - Air dried chromatograms were stapled onto Kodak no-screen X-ray film and exposed for 24 hours per 1000 counts per spot. The developed autoradiogram was superimposed on the stained chromatogram in order to determine the position of the radioactive spots in relation to the unlabelled components. Tracings were then made of each chromatogram and photographs were also taken of the autoradiogram superimposed on the traced chromatogram.

(B) Results

(1) Changes in Proteins

Vernalisation of Rideau grains produced little or no change in the overall total protein content (Table 1). A slight increase was observed in the total proteins of the Marquis wheat grains after one week's cold treatment.

Proteins of the embryo-scutellum and endosperm-pericarp of both Rideau and Marquis grains were analysed in terms of proteins soluble at pH 4.5 and those insoluble at this pH. These data are shown in Table II. A slight increase in soluble proteins was noted in the partially imbibed (R_5C_5)[1] embryo-scutellum axis of the Rideau grain. However, the soluble protein content remained unaffected in the fully imbibed, vernalised Rideau ($R_{14}C_5$) and the Marquis (M_5C_1) embryo-scutellum axes.

In the endosperm-pericarp fraction, vernalisation effected a large decrease in the soluble proteins of the fully imbibed Rideau grains alone.

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[1] The following abbreviations will be used during the Results and Discussion sections:

R_5K	Rideau grains imbibed for 5 hours.
R_5C_5	Rideau grains imbibed for 5 hours and exposed to 2 ± 1°C for five weeks.
$R_{14}K$	Rideau grains imbibed for 14 hours.
$R_{14}C_3$	Rideau grains imbibed for 14 hours and exposed to 2 ± 1°C for three weeks.
$R_{14}C_5$	Rideau grains imbibed for 14 hours and exposed to 2 ± 1°C for five weeks.
M_5K	Marquis grains imbibed for 5 hours.
M_5C_4	Marquis grains imbibed for 5 hours and exposed to 2 ± 1°C for four days.
M_5C_7	Marquis grain imbibed for 5 hours and exposed to 2 ± 1°C for seven days.

TABLE I

Total Protein Content of Spring and Winter Wheat Grains following Imbibition and Vernalisation.

Imbibition Time - Hours	Chilling Time - Weeks	Treatment	Total protein mg per 20 grains
	Rideau		
5	0	R_5K*	105.4 ± 7.8
5	5	R_5C_5	99.5 ± 4.2
14	0	$R_{14}K$*	86.7 ± 3.8
14	5	$R_{14}C_5$	70.2 ± 9.3
	Marquis		
5	0	M_5K	99.2 ± 8.7
5	1	M_5C_1	130.0 ±16.5

Values are arithmetic means SE obtained from 20 grain sample analyses from two separate experiments (See P. 15)

TABLE II

Concentration of Soluble and Insoluble Proteins at pH 4.5 in the Embryo-Scutellum and Endosperm-Pericarp of Spring and Winter Wheat Grains following Imbibition and Vernalisation.
(µg. per 20 grain parts)

	EMBRYO-SCUTELLUM		ENDOSPERM-PERICARP	
Treatment	Soluble protein	Insoluble protein	Soluble protein	Insoluble protein
R_5K *	5.1 ± 0.9	8.6 ± 2.6	35.8 ± 1.9	55.9 ± 2.4
R_5C_5	9.7 ± 2.3	6.2 ± 0.2	32.2 ± 4.0	51.4 ± 1.6
$R_{14}K$ *	3.8 ± 1.7	4.9 ± 0.3	31.3 ± 0.9	46.7 ± 2.2
$R_{14}C_5$	3.7 ± 0.8	6.4 ± 2.2	10.5 ± 3.4	49.7 ± 3.0
M_5K	6.7 ± 1.2	6.2 ± 0.8	22.1 ± 4.1	64.2 ± 2.6
M_5C_1	7.3 ± 1.3	5.0 ± 3.0	28.2 ± 6.7	97.5 ± 5.5

Values are arithmetic means ± SE observed from 20 grain sample analyses, from two separate experiments (see page 15).
* Separate experiments

No significant changes in the proteins insoluble at pH 4.5 were noted in the embryo-scutellum axes of the partially and fully imbibed Rideau or Marquis grains following vernalisation. However, a marked increase in the content of insoluble protein was noted in the Marquis grains following vernalisation.

(2) Changes in Amino Acids

The concentrations of each of the free amino acids and amides soluble in 60% ethanol have been expressed in three different ways on a protein basis, dry weight per grain part basis, and per grain part (Tables III-V and VII-VIII).

(a) Total alcohol soluble amino acids - The distribution of these compounds in the grain parts varied with grain variety and the period of imbibition (Table III).

In the fully imbibed Rideau and Marquis control series, it was observed that, on a protein basis, the endosperm contained larger amounts of total alcohol soluble amino acid and amide compounds than their corresponding embryo-scutella.

In both the winter wheat series (Table III), vernalisation resulted in dramatic increases in the total alcohol soluble amino acid/amide compounds. This was true for both the embryo-scutellum and endosperm fractions. In marked contrast, a depletion of total alcohol soluble amino acids and amides was noted in the endosperm of Marquis wheat following vernalisation.

(b) Individual amino acids - Proline: Comparatively high levels of proline always characterised the vernalised embryo-scutella in the 5-hour imbibed spring and winter wheats (Table IV and Figs. II and III). In terms of protein, the actual increase over the

TABLE III

Total Alcohol Soluble Amino Acid/Amide Contents in the Various Grain Parts following Imbibition and Vernalisation

Treatment	Embryo-Scutellum			Endosperm		
	μ moles per 100 mg. protein	μ moles per 100 mg. dry wt. of grain part	μ moles per 100 grain parts	μ moles per 100 mg. protein	μ moles per 100 mg. dry wt. of grain part	μ moles per 100 grain parts
*R_5K	28.8 ± 2.9	1.5 ± 0.2	19.7 ± 0.1	18.7 ±	0.20 ± 0.01	77.7 ± 8.3
R_5C_5	559.2 ± 5.7	3.4 ± 0.3	46.5 ± 4.5	136.8 ± 6.6	1.5 ± 0.1	496.5 ± 26.0
$R_{14}K$	59.4 ± 5.9	1.9 ± 0.2	25.8 ± 2.5	90.0 ± 5.2	0.8 ± 0.1	320.3 ± 18.9
$R_{14}C_5$	194.4 ± 5.2	8.8 ± 2.3	98.1 ± 26.3	257.3 ± 18.0	1.7 ± 0.1	716.4 ± 50.2
M_5K	34.7 ± 1.6	2.6 ± 0.2	22.1 ± 1.1	144.6 ± 2.7	1.5 ± 0.1	535.5 ± 9.9
M_5C_1	110.5 ± 9.4	5.5 ± 0.6	68.1 ± 6.3	88.1 ± 3.1	1.4 ± 0.1	509.0 ± 17.6

* R_5K, R_5C_5; Rideau wheat 5 hr. imbibed. Control (K) and chilled for 5 weeks (C_5)

$R_{14}K$, $R_{14}C_5$; Rideau wheat 14 hr. imbibed. Control (K) and chilled for 5 weeks (C_5)

M_5K, M_5C_1; Marquis wheat 5 hr. imbibed. Control (K) and chilled for 1 week (C_1).

Values are arithmetic means ± S.E. obtained from duplicate 20 grain sample analyses.

	Threonine	0.73 ± 0.12	11.84 ± 0.74	18.23 ± 2.04	13.01 ± 0.64	4.03 ± 0.12	3.74 ± 0.22
	Proline	0.03 ± 0.01	0.65 ± 0.12	0.24 ± 0.03	0.34 ± 0.11	0.05 ± 0.01	1.74 ± 0.21
Embryo-scutellum	Serine	0.17 ± 0.01	0.30 ± 0.03	0.16 ± 0.02	1.14 ± 0.20	0.33 ± 0.02	0.63 ± 0.10
	Threonine	0.11 ± 0.01	0.23 ± 0.03	0.08 ± 0.01	0.54 ± 0.10	0.33 ± 0.01	0.29 ± 0.01
* *	Proline	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Endosperm	Serine	0.02 ± 0.01	0.25 ± 0.01	0.09 ± 0.01	0.21 ± 0.02	0.19 ± 0.02	0.19 ± 0.01
	Threonine	0.01 ± 0.01	0.13 ± 0.01	0.17 ± 0.02	0.09 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
	Proline	0.03 ± 0.01	0.75 ± 0.06	0.03 ± 0.01	0.31 ± 0.04	0.04 ± 0.01	2.14 ± 0.23
Embryo-scutellum	Serine	0.22 ± 0.02	0.04 ± 0.01	0.02 ± 0.01	1.14 ± 0.21	0.27 ± 0.01	0.60 ± 0.03
	Threonine	0.13 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.50 ± 0.01	0.33 ± 0.01	0.43 ± 0.07
* * *	Proline	0.06 ± 0.01	0.06 ± 0.01	0.36 ± 0.01	0.54 ± 0.21	0.70 ± 0.01	0.43 ± 0.07
Endosperm	Serine	0.75 ± 0.05	8.24 ± 0.11	3.51 ± 0.10	8.86 ± 0.14	6.85 ± 0.12	6.74 ± 0.32
	Threonine	0.29 ± 0.04	4.53 ± 0.10	6.34 ± 0.11	3.13 ± 0.61	1.54 ± 0.41	2.14 ± 0.11

\+ see Table III

* μ moles per 100 mg. protein

** μ moles per 100 mg. dry weight of grain parts

*** μ moles per 100 grain parts

Grain Part	Amino Acid						
	Proline	0.60 ± 0.01	9.94 ± 0.80	7.30 ± 1.01	6.23 ± 1.04	0.74 ± 0.11	34.74 ± 4.02
Embryo-scutellum	Serine	3.31 ± 0.20	4.51 ± 0.51	4.92 ± 0.50	23.10 ± 4.83	4.31 ± 0.20	10.73 ± 0.51
*	Threonine	1.93 ± 0.11	4.04 ± 0.51	2.54 ± 0.51	10.74 ± 0.72	5.2 ± 0.1	5.84 ± 0.21
	Proline	0.20 ± 0.03	1.73 ± 0.10	1.14 ± 0.52	2.03 ± 1.04	0.20 ± 0.04	0.80 ± 0.01
Endosperm	Serine	1.84 ± 0.10	22.72 ± 1.34	10.13 ± 0.51	31.74 ± 2.73	18.44 ± 2.01	11.83 ± 0.61
	Threonine	0.73 ± 0.12	11.84 ± 0.74	18.23 ± 2.04	13.01 ± 0.64	4.03 ± 0.12	3.74 ± 0.22
	Proline	0.03 ± 0.01	0.65 ± 0.12	0.24 ± 0.03	0.34 ± 0.11	0.05 ± 0.01	1.74 ± 0.21
Embryo-scutellum	Serine	0.17 ± 0.01	0.30 ± 0.03	0.16 ± 0.02	1.14 ± 0.20	0.33 ± 0.02	0.63 ± 0.10
	Threonine	0.11 ± 0.01	0.23 ± 0.03	0.08 ± 0.01	0.54 ± 0.10	0.33 ± 0.01	0.29 ± 0.01
* *	Proline	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Endosperm	Serine	0.02 ± 0.01	0.25 ± 0.01	0.09 ± 0.01	0.21 ± 0.02	0.19 ± 0.02	0.19 ± 0.01
	Threonine	0.01 ± 0.01	0.13 ± 0.01	0.17 ± 0.02	0.09 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
	Proline	0.03 ± 0.01	0.75 ± 0.06	0.03 ± 0.01	0.31 ± 0.04	0.04 ± 0.01	2.14 ± 0.23
Embryo-scutellum	Serine	0.22 ± 0.02	0.04 ± 0.01	0.02 ± 0.01	1.14 ± 0.21	0.27 ± 0.01	0.60 ± 0.03
* * *	Threonine	0.13 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.50 ± 0.01	0.33 ± 0.01	0.43 ± 0.07
	Proline						

TABLE IV

The Concentration of Some Selected Amino Acids in the Various Grain Parts following Imbibition and Vernalisation

Grain Part	Amino Acid	Grain Variety and Treatment: +R_5K	R_5C_5	$R_{14}K$	$R_{14}C_5$	M_5K	M_5C_1
	Proline	0.60 ± 0.01	9.94 ± 0.80	7.30 ± 1.01	6.23 ± 1.04	0.74 ± 0.11	34.74 ± 4.02
Embryo-scutellum	Serine	3.31 ± 0.20	4.51 ± 0.51	4.92 ± 0.50	23.10 ± 4.83	4.31 ± 0.20	10.73 ± 0.51
*	Threonine	1.93 ± 0.11	4.04 ± 0.51	2.54 ± 0.51	10.74 ± 0.72	5.2 ± 0.1	5.84 ± 0.21
	Proline	0.20 ± 0.03	1.73 ± 0.10	1.14 ± 0.52	2.03 ± 1.04	0.20 ± 0.04	0.80 ± 0.01
Endosperm	Serine	1.84 ± 0.10	22.72 ± 1.34	10.13 ± 0.51	31.74 ± 2.73	18.44 ± 2.01	11.83 ± 0.61
	Threonine	0.73 ± 0.12	11.84 ± 0.74	18.23 ± 2.04	13.01 ± 0.64	4.03 ± 0.12	3.74 ± 0.22
	Proline	0.03 ± 0.01	0.65 ± 0.12	0.24 ± 0.03	0.34 ± 0.11	0.05 ± 0.01	1.74 ± 0.21
Embryo-scutellum	Serine	0.17 ± 0.01	0.30 ± 0.03	0.16 ± 0.02	1.14 ± 0.20	0.33 ± 0.02	0.63 ± 0.10
* *	Threonine	0.11 ± 0.01	0.23 ± 0.03	0.08 ± 0.01	0.54 ± 0.10	0.33 ± 0.01	0.29 ± 0.01

control grains was as much as 50-fold in M_5 and 18-fold in R_5 grains. Little change was observed in the R_{14} series.

Changes in the endosperm level of proline in no way paralleled increased levels in the embryo-scutella; slight increases were observed when the data were computed on a protein basis (Figs. IV and V). In contrast, no large changes in the proline content of the endosperm were observed when the concentrations of proline were expressed in terms of dry weight or whole grains.

Serine: When calculated on a protein basis, the content of this amino acid in the Rideau grain parts increased with vernalisation (Table IV, and Figs. II - V). A varietal difference was noted in the endosperm-pericarp of vernalised Marquis grains, with serine content decreasing following vernalisation. In terms of changing amino acid content with protein, the greatest increase (5-fold) was observed in the R_{14} embryo-scutella fractions. Change with vernalisation was as $R_{14} > M_5 > R_5$. In the endosperm, the change was as $R_5 > R_{14} > M_5$ with a 14-fold increase being observed in the R_5 series.

Dry weight based data of the embryo-scutellum fractions also indicated a change in serine content with vernalisation, the order of change being $R_{14} > R_5 = M_5$. In the endosperm, the change was as $R_5 > R_{14} > M_5$ (Table IV, Figs. IV and V). A similar sequential change was noted in the endosperm fraction when the serine content was computed on a whole grain basis.

Threonine: Computed on a protein basis, 2- and 4-fold increases were observed in the content of this amino acid in the R_5 and R_{14} embryo-scutella fractions respectively following vernalisation (Table IV). However, the content of this acid remained more or less constant in the embryo-scutella fractions of the M_5 series, i.e. $R_{14} > R_5 > M_5$. In the endosperm, the threonine content increased

Embryo-scutellum	butyric						
	Histidine	0.15 ± 0.05	1.00 ± 0.10	0.25 ± 0.05	0.80 ± 0.25	0.10 ± 0.05	1.30 ± 0.10
	Arginine	1.15 ± 0.13	2.00 ± 0.05	1.45 ± 0.05	1.70 ± 0.40	0.80 ± 0.05	2.60 ± 0.30
	Lysine	0.30 ± 0.11	0.80 ± 0.05	0.30 ± 0.05	0.30 ± 0.05	0.15 ± 0.05	0.40 ± 0.05
	Ornithine	0.10 ± 0.01	0.30 ± 0.05	0.10 ± 0.01	0.20 ± 0.1	0.10 ± 0.05	0.45 ± 0.05
	Aspartic	1.64 ± 0.92	2.50 ± 0.70	3.15 ± 2.10	1.75 ± 0.90	2.00 ± 0.40	1.80 ± 0.30
	Glutamic	2.78 ± 0.43	3.30 ± 0.80	4.40 ± 0.85	5.90 ± 0.05	3.30 ± 0.15	5.05 ± 0.40
*** Endosperm	γ-amino-butyric	0.55 ± 0.12	0.10 ± 0.01	0.30 ± 0.05	0.35 ± 0.05	0.65 ± 0.05	0.25 ± 0.05
	Histidine	0.20 ± 0.01	0.20 ± 0.05	0.40 ± 0.05	0.65 ± 0.15	0.10 ± 0.02	0.85 ± 0.05
	Arginine	2.56 ± 0.12	0.80 ± 0.15	1.25 ± 0.25	2.65 ± 0.05	1.15 ± 0.10	1.95 ± 0.30
	Lysine	0.32 ± 0.10	0.10 ± 0.05	0.40 ± 0.10	0.35 ± 0.05	0.30 ± 0.05	0.70 ± 0.05
	Ornithine	0.10 ± 0.05	0.05 ± 0.02	0.10 ± 0.02	0.20 ± 0.05	0.35 ± 0.05	0.10 ± 0.02

\+ See Table III

* μ moles per 100 mg. protein

** μ moles per 100 mg dry weight of grain part

*** μ moles per 100 grain parts

	Histidine	0.13 ± 0.01	0.77 ± 0.08	0.21 ± 0.04	0.72 ± 0.02	0.10 ± 0.01	0.79 ± 0.08
	Arginine	0.90 ± 0.02	1.47 ± 0.05	1.09 ± 0.06	1.57 ± 0.36	0.90 ± 0.01	1.65 ± 0.26
	Lysine	0.25 ± 0.03	0.61 ± 0.01	0.22 ± 0.06	0.31 ± 0.08	0.23 ± 0.02	0.34 ± 0.06
	Ornithine	0.10 ± 0.01	0.22 ± 0.03	0.10 ± 0.02	0.21 ± 0.09	0.12 ± 0.01	0.36 ± 0.02
Endosperm	Aspartic	0.05 ± 0.02	0.07 ± 0.01	0.08 ± 0.05	0.04 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
	Glutamic	0.07 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.14 ± 0.01	0.09 ± 0.01	0.14 ± 0.01
	Y-amino-butyric	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	Histidine	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
	Arginine	0.06 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
	Lysine	0.08 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
	Ornithine	0.01 ± 0 .01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
**** Embryo-scutellum	Aspartic	0.66 ± 0.12	1.00 ± 0.05	1.15 ± 0.05	0.75 ± 0.10	0.60 ± 0.05	1.50 ± 0.30
	Glutamic	0.74 ± 0.11	6.51 ± 0.80	2.8 ± 0.25	2.60 ± 0.20	1.55 ± 0.05	6.70 ± 1.05
	Y-amino-butyric	0.33 ± 0.12	0.30 ± 0.05	0.15 ± 0.05	0.10 ± 0.05	0.50 ± 0.05	0.50 ± 0.05
	Histidine	0.15 ± 0.05	1.00 ± 0.10	0.25 ± 0.05	0.80 ± 0.25	0.10 ± 0.05	1.30 ± 0.10
	Arginine	1.15 ± 0.13	2.00 ± 0.05	1.45 ± 0.05	1.70 ± 0.40	0.80 ± 0.05	2.60 ± 0.30
	Lysine	0.30 ± 0.11	0.80 ± 0.05	0.30 ± 0.05	0.30 ± 0.05	0.15 ± 0.05	0.40 ± 0.05

	Arginine	1.75 ± 0.10	2.51 ± 0.09	0.34 ± 0.02	3.44 ± 0.74	1.20 ± 0.09	4.20 ± 0.43
	Lysine	0.56 ± 0.12	1.11 ± 0.03	0.71 ± 0.02	0.71 ± 0.17	0.30 ± 0.01	0.70 ± 0.12
	Ornithine	0.19 ± 0.02	0.41 ± 0.06	0.33 ± 0.01	0.50 ± 0.20	0.16 ± 0.01	0.73 ± 0.04
* Endosperm	Aspartic	0.54 ± 0.21	0.48 ± 0.02	0.90 ± 0.06	0.65 ± 0.32	0.54 ± 0.11	0.31 ± 0.06
	Glutamic	0.73 ± 0.12	0.91 ± 0.22	1.23 ± 0.30	2.11 ± 0.02	0.88 ± 0.05	0.90 ± 0.07
	γ-amino-butyric	0.11 ± 0.01	0.04 ± 0.01	0.10 ± 0.02	0.14 ± 0.03	0.18 ± 0.02	0.05 ± 0.01
	Histidine	0.05 ± 0.01	0.06 ± 0.01	0.10 ± 0.02	0.30 ± 0.06	0.03 ± 0.01	0.15 ± 0.01
	Arginine	0.65 ± 0.23	0.21 ± 0.04	0.40 ± 0.07	1.01 ± 0.02	0.31 ± 0.03	0.34 ± 0.05
	Lysine	0.08 ± 0.01	0.04 ± 0.01	0.11 ± 0.04	0.14 ± 0.03	0.08 ± 0.01	0.12 ± 0.01
	Ornithine	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.02 ± 0.01
	Aspartic*	0.44 ± 0.05	0.77 ± 0.06	0.88 ± 0.06	0.69 ± 0.23	0.68 ± 0.02	1.18 ± 0.25
	Glutamic	0.55 ± 0.08	4.77 ± 0.64	2.08 ± 0.19	2.33 ± 0.21	1.83 ± 0.05	5.48 ± 0.96
** Embryo-scutellum	γ-amino-butyric	0.20 ± 0.01	0.21 ± 0.03	0.14 ± 0.05	0.09 ± 0.02	0.61 ± 0.01	0.41 ± 0.04
	Histidine	0.13 ± 0.01	0.77 ± 0.08	0.21 ± 0.04	0.72 ± 0.02	0.10 ± 0.01	0.79 ± 0.08
	Arginine	0.90 ± 0.02	1.47 ± 0.05	1.09 ± 0.06	1.57 ± 0.36	0.90 ± 0.01	1.65 ± 0.26
	Lysine	0.25 ± 0.03	0.61 ± 0.01	0.22 ± 0.06	0.31 ± 0.08	0.23 ± 0.02	0.34 ± 0.06

TABLE V

The Concentration of Acidic and Basic Amino Acids in the Various Grain Parts following Imbibition and Vernalisation

	Amino Acid	R_5K +	R_5C_5	$R_{14}K$	$R_{14}C_5$	M_5K	M_5C_1
* Embryo-scutellum	Aspartic	0.84 ± 0.11	1.31 ± 0.11	2.80 ± 0.20	1.52 ± 0.30	0.91 ± 0.02	2.40 ± 0.50
	Glutamic	1.13 ± 0.22	8.11 ± 1.10	6.50 ± 0.60	5.10 ± 0.40	2.40 ± 0.07	10.90 ± 1.91
	γ-amino-butyric	0.44 ± 0.13	0.41 ± 0.05	0.50 ± 0.02	0.2 ± 0.05	0.80 ± 0.02	0.83 ± 0.07
	Histidine	0.25 ± 0.01	1.31 ± 0.13	0.70 ± 0.02	1.60 ± 0.60	0.14 ± 0.01	2.20 ± 0.27
	Arginine	1.75 ± 0.10	2.51 ± 0.09	0.34 ± 0.02	3.44 ± 0.74	1.20 ± 0.09	4.20 ± 0.43
	Lysine	0.56 ± 0.12	1.11 ± 0.03	0.71 ± 0.02	0.71 ± 0.17	0.30 ± 0.01	0.70 ± 0.12
	Ornithine	0.19 ± 0.02	0.41 ± 0.06	0.33 ± 0.01	0.50 ± 0.20	0.16 ± 0.01	0.73 ± 0.04
* Endosperm	Aspartic	0.54 ± 0.21	0.48 ± 0.02	0.90 ± 0.06	0.65 ± 0.32	0.54 ± 0.11	0.31 ± 0.06
	Glutamic	0.73 ± 0.12	0.91 ± 0.22	1.23 ± 0.30	2.11 ± 0.02	0.88 ± 0.05	0.90 ± 0.07
	γ-amino-butyric	0.11 ± 0.01	0.04 ± 0.01	0.10 ± 0.02	0.14 ± 0.03	0.18 ± 0.02	0.05 ± 0.01
	Histidine	0.05 ± 0.01	0.06 ± 0.01	0.10 ± 0.02	0.30 ± 0.06	0.03 ± 0.01	0.15 ± 0.01

20-fold in R_5 but decreased in the R_{14} series following vernalisation, ($R_5 > R_{14} > M_5$). Once again, a varietal difference was noted. Threonine content remained more or less unchanged in the Marquis grain endosperm when it was computed on a dry weight or grain basis (Table IV).

The mono amino dicarboxylic acids: In the protein based data, vernalisation resulted in a 2- to 8-fold increase in the content of aspartic and glutamic acids in the embryo-scutella fractions of both five hour imbibed spring and winter grains (Table V). However, in the R_{14} series, both amino acids decreased with vernalisation. In the endosperm, the pattern of change was also related to the imbibition period. No change was observed in the five hour imbibed spring and winter wheats, (Table V; Figs. II - V). Comparable parameters of change were not observed when the content of these amino acids were computed on a dry weight or grain basis.

The di-amino-monocarboxylic acids: Histidine - In terms of protein, changes in the concentrations of this amino acid (2-16-fold) were observed in all the embryo-scutella fractions following vernalisation (Table V and Figs. II - V). In all cases, low levels of histidine were observed in the endosperm before and after vernalisation. This observation held true for the three methods of computing the data (Table V).

Arginine - On a protein basis, an increase in the content of this amino acid was noted in all the embryo-scutella fractions following vernalisation (Table V and Figs. II and III). Computed on a dry weight basis, levels of change appeared to be very small. Arginine content was low in the endosperm and did not indicate a consistent pattern of change relating to grain variety or imbibition or vernalisation. This observation also held true for the dry weight computed data.

FIGURE II

Legend

Embryo-scutellum: - Concentrations of amino acids and amides in control grains.

Concentration $\mu \times 10^{-1}$ moles per 100 mg. protein.

1. Aspartic acid; 2. Threonine; 3. Serine; 4. Glutamic acid; 5. Proline; 6. Glycine; 7. Alanine; 8. Valine; 9. Cystine; 10. Methionine; 11. Isoleucine; 12. Leucine; 13. Tyrosine; 14. Phenylalanine; 15. γ-Aminobutyric acid; 16. Ornithine; 17. Lysine; 18. Histidine; 19. Arginine; 20. Asparagine; 21. Glutamine.

Control

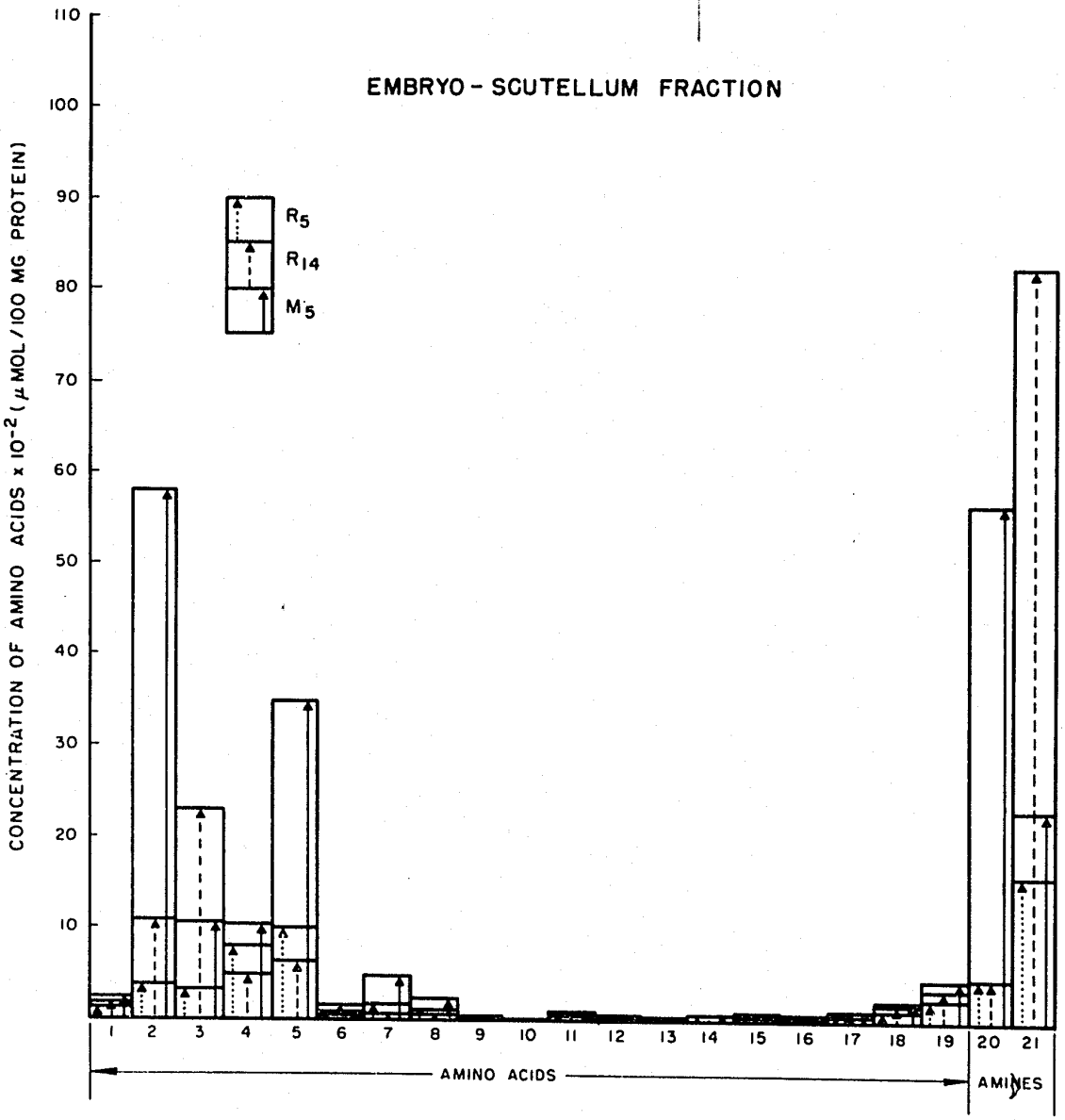

EMBRYO - SCUTELLUM FRACTION
CONCENTRATION OF AMINO ACIDS x 10⁻² (μMOL/100 MG PROTEIN)
R5
R14
M5
110
100
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
AMINO ACIDS
AMINES

FIGURE III

Legend

Embryo-scutellum: - Concentration of amino acids in vernalised grains.

Concentration $\mu \times 10^{-1}$ moles per 100 mg. protein.

1. Aspartic acid; 2. Threonine; 3. Serine; 4. Glutamic acid; 5. Proline; 6. Glycine; 7. Alanine; 8. Valine; 9. Cystine; 10. Methionine; 11. Isoleucine; 12. Leucine; 13. Tyrosine; 14. Phenylalanine; 15. γ-Aminobutyric acid; 16. Ornithine; 17. Lysine; 18. Histidine; 19. Arginine; 20. Asparagine; 21. Glutamine.

Vernalised

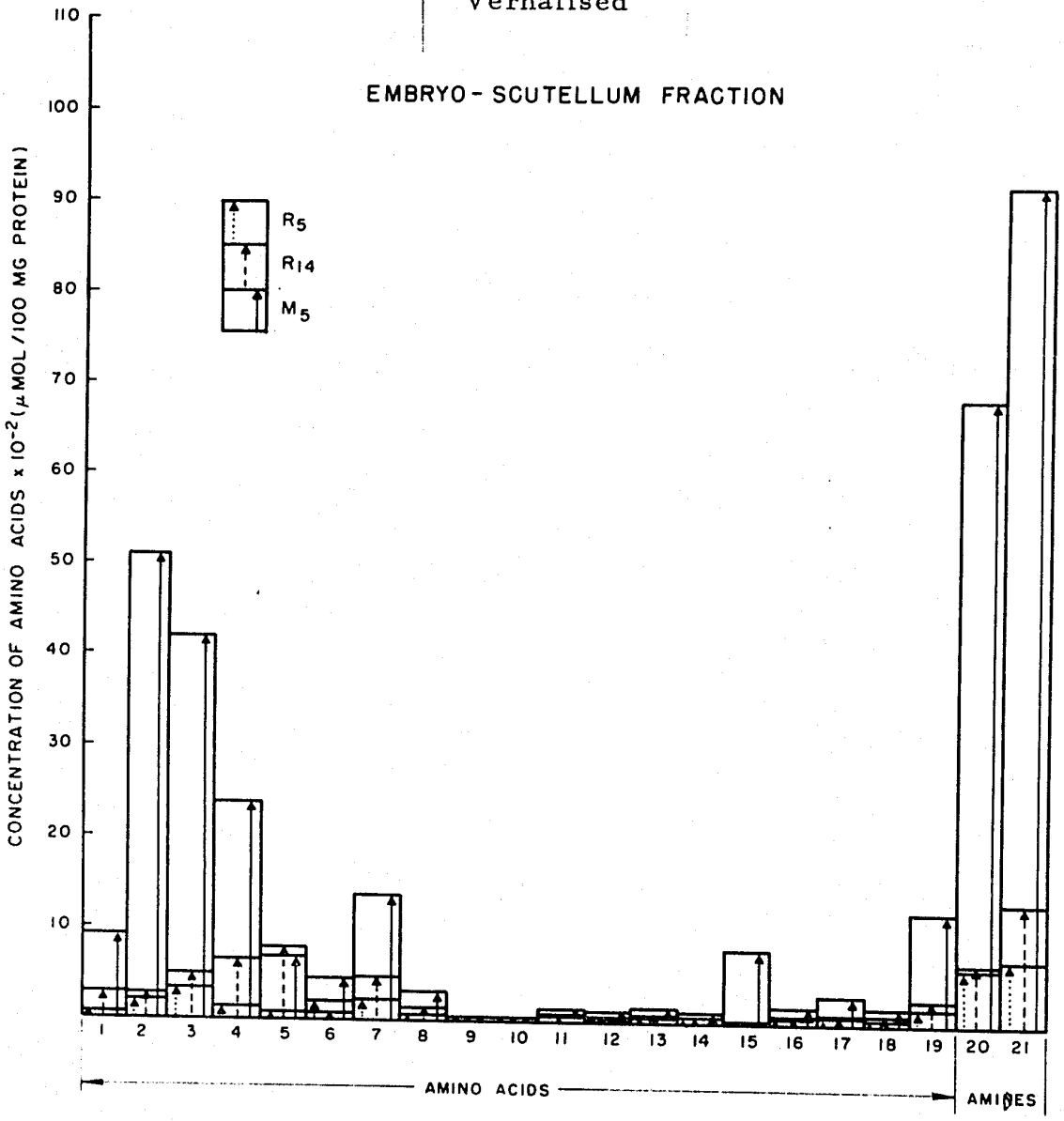

EMBRYO - SCUTELLUM FRACTION
CONCENTRATION OF AMINO ACIDS x 10^{-2} (μ MOL / 100 MG PROTEIN)
110
100
90
80
70
60
50
40
30
20
10
R_5
R_{14}
M_5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
AMINO ACIDS
AMIDES

FIGURE IV

Legend

Endosperm - Concentration of amino acids in control grains.

Concentration $\mu \times 10^{-1}$ moles per 100 mg. protein.

1. Aspartic acid; 2. Threonine; 3. Serine; 4. Glutamic acid; 5. Proline; 6. Glycine; 7. Alanine; 8. Valine; 9. Cystine; 10. Methionine; 11. Isoleucine; 12. Leucine; 13. Tyrosine; 14. Phenylalanine; 15. γ-Aminobutyric acid; 16. Ornithine; 17. Lysine; 18. Histidine; 19. Arginine; 20. Asparagine; 21. Glutamine.

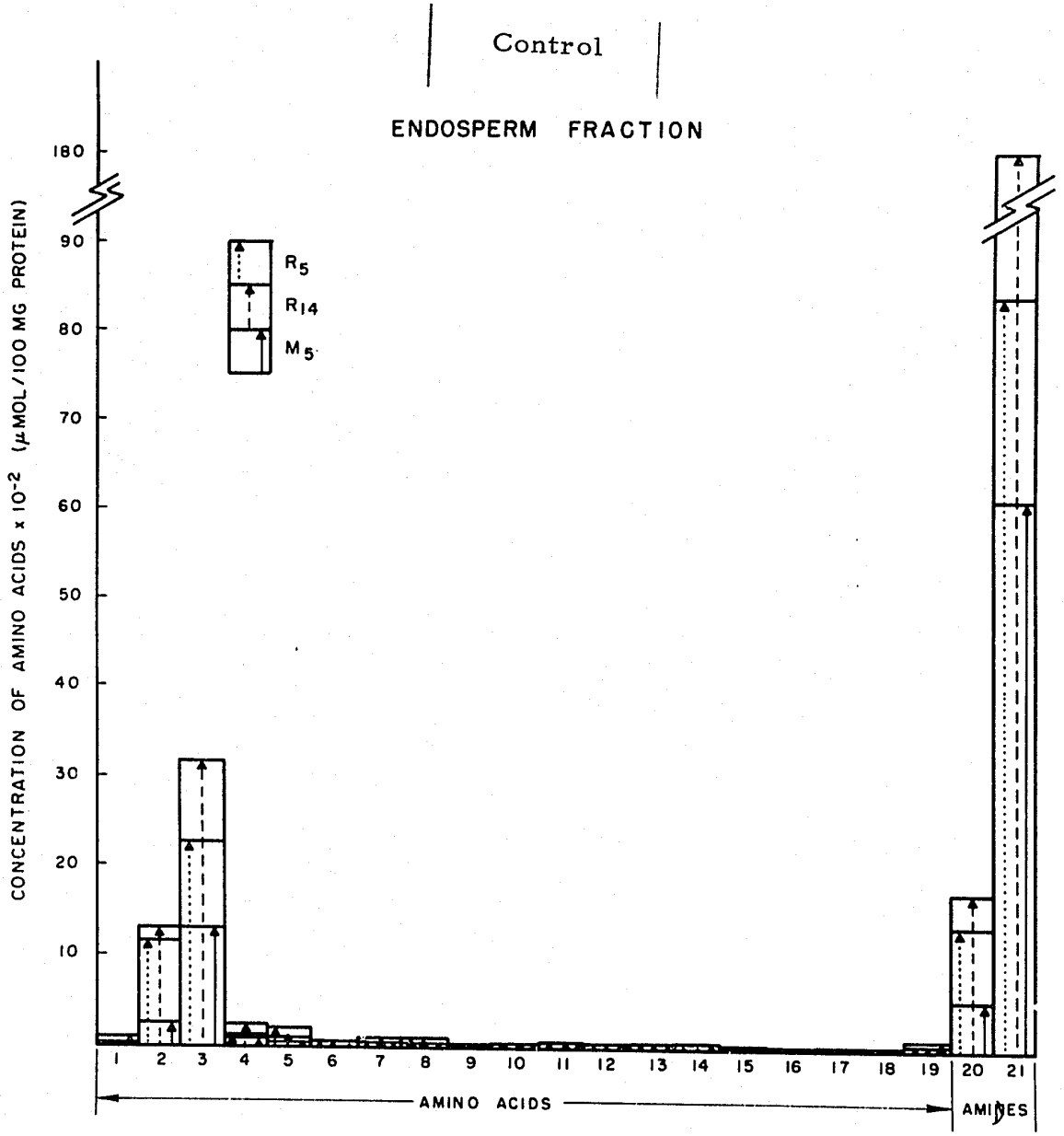

Control
ENDOSPERM FRACTION
CONCENTRATION OF AMINO ACIDS x 10⁻² (μMOL/100 MG PROTEIN)
180
90
80
70
60
50
40
30
20
10
R5
R14
M5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
AMINO ACIDS
AMIDES

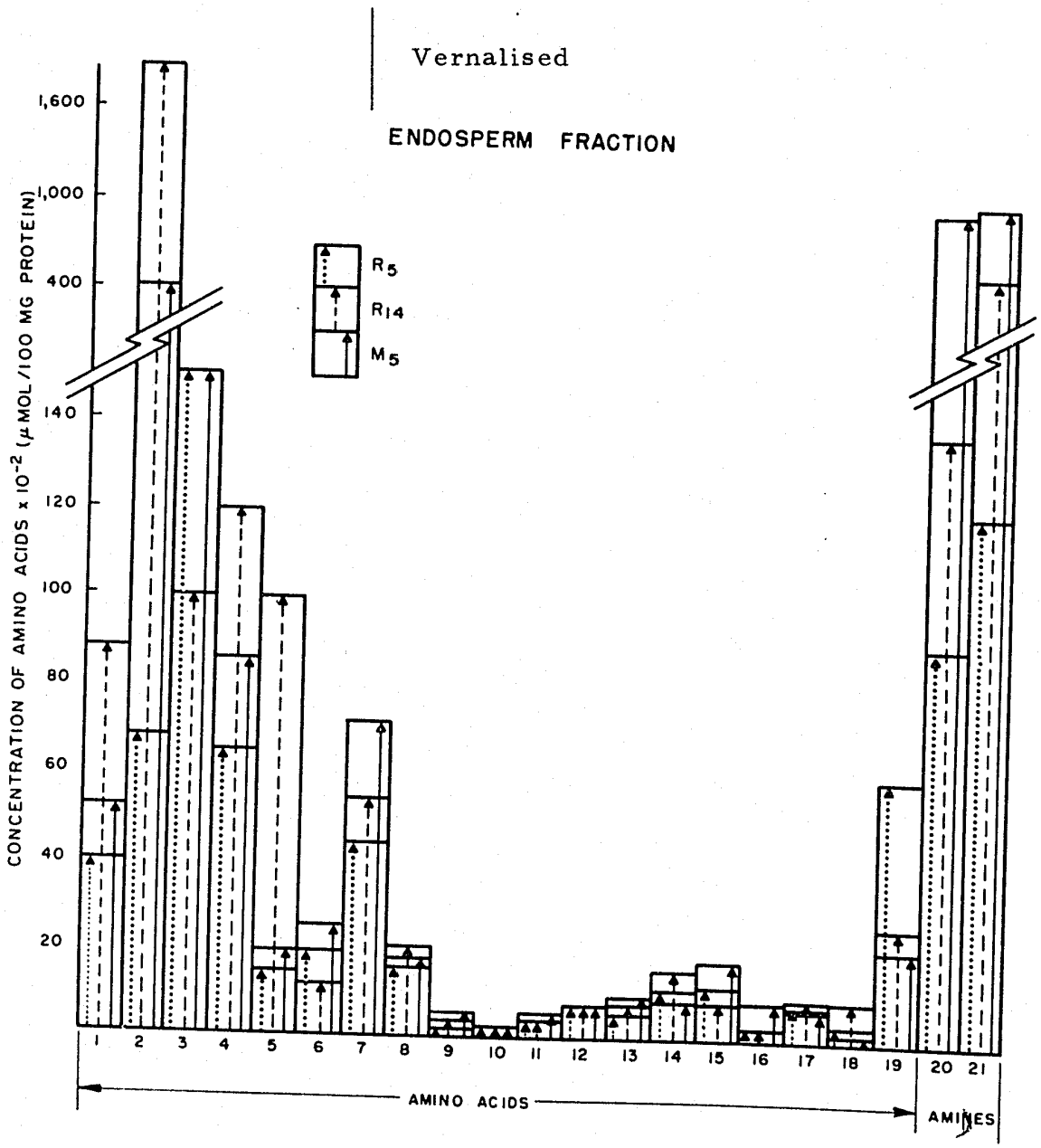
FIGURE V

Legend

Endosperm: - Concentration of amino acids in vernalised grains.

Concentration $\mu \times 10^{-1}$ moles per 100 mg. protein.

1. Aspartic acid; 2. Threonine; 3. Serine; 4. Glutamic acid; 5. Proline; 6. Glycine; 7. Alanine; 8. Valine; 9. Cystine; 10. Methionine; 11. Isoleucine; 12. Leucine; 13. Tyrosine; 14. Phenylalanine; 15. γ-Aminobutyric acid; 16. Ornithine; 17. Lysine; 18. Histidine; 19. Arginine; 20. Asparagine; 21. Glutamine.


Vernalised
ENDOSPERM FRACTION
CONCENTRATION OF AMINO ACIDS x 10⁻² (μMOL/100 MG PROTEIN)
1,600
1,000
400
140
120
100
80
60
40
20
R5
R14
M5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
AMINO ACIDS
AMINES

Lysine and Ornithine - Only the embryo-scutella of vernalised, 5-hour imbibed grains contained twice as much lysine and ornithine as unvernalised controls (protein based data). However, only slight changes were apparent when the data was computed on a dry weight or grain basis. Only low levels of lysine or ornithine were observed in the endosperm fractions.

(c) Ratio of acidic to basic amino acids - When ratios of total mono-amino dicarboxylic acids to total diamino-monocarboxylic acids were computed for the M_5 or R_{14} series, a decrease was noted to parallel vernalisation (Table VI). This decrease held true for all three methods of computing the data, apart from the exceptional case of M_5 embryo-scutella fraction based on dry weight.

(d) Amides -

(i) Total amide content. Large amounts of amide were present in the non-vernalised Rideau and Marquis grains. A consistent increase in the total amide content was observed to be co-incident with vernalisation in all cases (Table VII). This increase was as $R_{14} > M_5 > R_5$ utilizing the protein based data. In the endosperm fractions, the change in amide content with vernalisation varied with grain variety. Whereas total amides increased in the Rideau winter wheats, a large decrease was noted in the Marquis endosperm fractions (protein based data). Varietal and treatment difference were as $R_{14} > R_5 = M_5$ (Fig. IV and V, and Table VII). Notwithstanding the method of computing the amide content, this trend was noted in the endosperm fractions of all series (Table VII).

TABLE VI

Ratio of Acidic to Basic Amino Acids in the Grain Parts following Imbibition and Vernalization

Series	$R_{14}K$	$R_{14}C_5$	M_5K	M_5C_1	$R_{14}K$	$R_{14}C_5$	M_5K	M_5C_1	$R_{14}K$	$R_{14}C_5$	M_5K	M_5C_1
Grain part	Protein based				Dry weight based				Grain part based			
Embryo-scutellum	3.6	1.9	2.7	1.9	1.8	1.1	1.9	2.1	1.3	1.1	1.7	2.0
Endosperm	1.8	1.1	2.8	1.7	3.5	1.9	2.8	1.9	3.4	1.9	2.5	1.7

TABLE VII.

Total Amide Contents in the Various Grain Parts following Imbibition and Vernalisation

Treatment	Embryo-Scutellum			Endosperm		
	μ moles per 100 mg. protein	μ moles per 100 mg. dry wt. of grain part	μ moles per 100 grain parts	μ moles per 100 mg. protein	μ moles per 100 mg. dry wt. of grain part	μ moles per 100 grain parts
*R_5K	12.9 ± 1.8	0.7 ± 0.1	8.5 ± 0.5	12.9 ± 1.0	0.18 ± 0.01	53.5 ± 1.0
R_5C_5	20.6 ± 0.2	1.2 ± 0.1	16.3 ± 2.0	97.8 ± 4.6	1.1 ± 0.1	355.0 ± 17.0
$R_{14}K$	21.5 ± 1.6	0.7 ± 0.1	9.5 ± 0.5	56.4 ± 1.0	0.19 ± 0.01	200.0 ± 4.0
$R_{14}C_5$	138.3 ± 41.8	6.3 ± 2.0	69.5 ± 20.0	198.0 ± 12.8	1.3 ± 0.1	569.0 ± 36.0
M_5K	16.0 ± 0.7	1.2 ± 0.11	10.0 ± 1.0	110.3 ± 0.2	1.2 ± 0.1	440.0 ± 1.0
M_5C_1	26.2 ± 1.0	1.3 ± 0.1	6.0 ± 0.5	67.2 ± 1.5	1.1 ± 0.1	389 ± 9.0

* See Table III

TABLE VIII

The Concentration of Each Amide in the Various Grain Parts following Imbibition Vernalisation

Treatment	Amide	Embryo-Scutellum			Endosperm		
		μ moles per 100 mg. protein	μ moles per 100 mg. dry wt. of grain part	μ moles per 100 grain parts	μ moles per 100 mg. protein	μ moles per 100 mg. dry wt. of grain part	μ moles per 100 grain parts
*R_5K	Asparagine	5.9 ± 0.4	0.30 ± 0.02	4.0 ± 0.5	0.9 ± 0.1	0.01 ± 0.01	4.0 ± 0.5
	Glutamine	6.9 ± 1.3	0.4 ± 0.1	4.5 ± 0.5	13.7 ± 1.2	0.13 ± 0.01	49.5 ± 2.0
R_5C_5	Asparagine	4.9 ± 0.5	0.3 ± 0.1	4.0 ± 0.5	12.0 ± 0.5	0.15 ± 0.01	49.5 ± 4.5
	Glutamine	15.7 ± 1.5	0.9 ± 0.1	11.0 ± 1.0	84.1 ± 3.4	0.9 ± 0.1	305.5 ± 12.5
$R_{14}K$	Asparagine	6.2 ± 0.8	0.19 ± 0.02	2.5 ± 0.5	1.4 ± 0.1	0.01 ± 0.01	4.5 ± 1.0
	Glutamine	15.4 ± 0.8	0.5 ± 0.1	6.5 ± 0.5	55.0 ± 0.7	0.51 ± 0.01	196.0 ± 2.5
$R_{14}C_5$	Asparagine	56.1 ± 8.6	2.5 ± 0.4	28.5 ± 4.5	17.9 ± 7.9	0.12 ± 0.05	50.0 ± 20.0
	Glutamine	82.2 ± 33.3	3.8 ± 1.5	41.5 ± 17.0	180.0 ± 4.8	1.2 ± 0.1	518.5 ± 13.5
M_5K	Asparagine	6.9 ± 0.5	0.5 ± 0.1	4.0 ± 0.5	9.1 ± 0.1	0.01 ± 0.01	34.0 ± 0.5
	Glutamine	9.2 ± 0.2	0.3 ± 0.1	6.0 ± 0.5	109.2 ± 0.1	1.14 ± 0.01	406.0 ± 0.5
M_5C_1	Asparagine	4.9 ± 0.5	0.7 ± 0.1	3.0 ± 0.5	5.5 ± 0.3	0.09 ± 0.01	32.0 ± 1.5
	Glutamine	21.3 ± 0.5	1.1 ± 0.1	13.0 ± 0.5	61.8 ± 1.3	0.9 ± 0.1	357.0 ± 7.5

* See Table III

(ii) Individual amides. Asparagine and glutamine - A marked difference between grain variety and the imbibition period was noted with respect to the content of the individual amides.

In all three methods of assessing amide content, the concentrations of both asparagine and glutamine increased in both the embryo-scutellum and endosperm fractions of the R_{14} series following vernalisation (Table VIII and Figs. III - V). In the R_5 series, however, a decrease in the asparagine content was observed in the embryo-scutellum fraction to be associated with vernalisation. Both the amides increased with vernalisation in the endosperm fraction.

The spring variety of wheat, however, differed in its response to vernalisation with respect to the content of the individual amides. Whilst the glutamine concentration increased in the embryo-scutellum and endosperm fractions, the asparagine content decreased or remained more or less constant.

(3) Distribution of ^{32}P in Phosphate Compounds

(a) During imbibition: Water soluble inorganic ^{32}P rapidly accumulated during the early hours of imbibition to a maximum level at 7 hours and thereafter decreased linearly at a slower rate. At the same time organic ^{32}P increased linearly at a rate similar to the decrease in inorganic ^{32}P (Table IX, Fig. VI).

Thus the total water soluble ^{32}P imbibed by the Rideau grains showed an increase during the first five hours of imbibition and approached a maximum and constant level between 7 and 14 hours (Table IX, Fig. VI).

TABLE IX

^{32}P UPTAKE BY RIDEAU WHEAT WHOLE GRAINS WITH RESPECT TO THE IMBIBITION PERIOD (C.F. FIG. VI)

Duration of Imbibition in hours	^{32}P-Incorporation, cpm per whole grain ($x10^{-3}$)			
	Phospholipids	Water Soluble organic ^{32}P	Inorganic ^{32}P	Total water Soluble ^{32}P
2	4	115	439	554
5	18	92	769	860
7	26	148	807	955
14	40	366	607	973

FIGURE VI

(cf. TABLE IX)

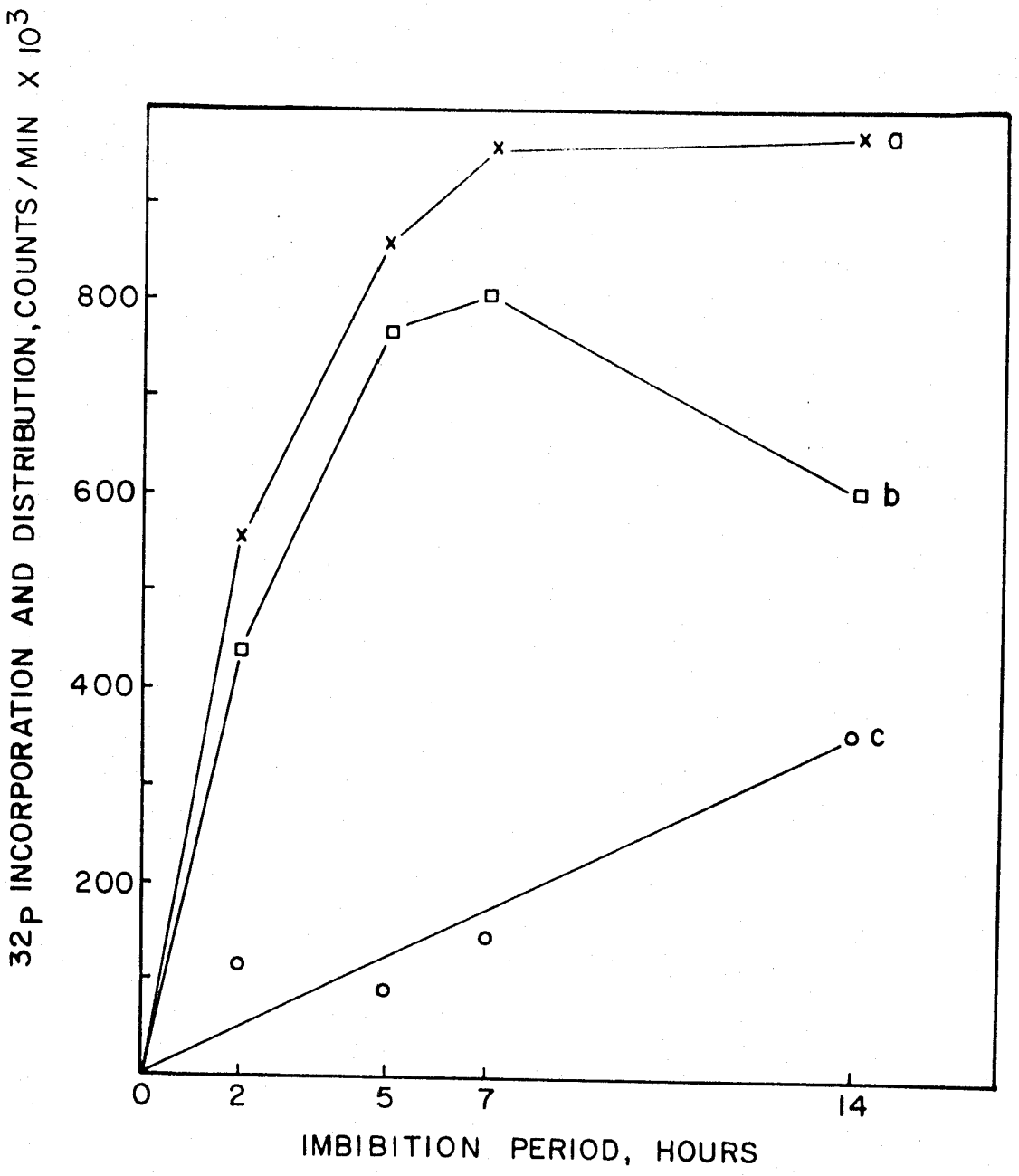
Legend

Incorporation of ^{32}P into water-soluble compounds, and distribution into organic and inorganic water soluble phosphates of Rideau wheat whole grains with respect to the imbibition periods.

a = Total water-soluble ^{32}P (total of b + c)

b = Water-soluble inorganic ^{32}P } separated by the method of Berenblum and Chain (1938)

c = Water-soluble organic ^{32}P }


32P INCORPORATION AND DISTRIBUTION, COUNTS / MIN X 10^3
800
600
400
200
0
2
5
7
14
IMBIBITION PERIOD, HOURS
a
b
c

Incorporation of ^{32}P into the phospholipids of Rideau wheat grains during the imbibition period was relatively very slow, and amounted to no more than about 10% of the incorporation of ^{32}P into water soluble organic phosphate (Table IX).

(b) During vernalisation: The effect of vernalisation following the imbibition period on the distribution of ^{32}P was investigated in whole grains as well as grain parts of both Rideau and Marquis wheats. The results obtained from these studies are expressed in Figs. VII - X and Tables X - XV.

(i) Whole grains. The total uptake of ^{32}P was always greater in the control (fully imbibed) Rideau grains than in the control Marquis grains (Table X, Fig. VII). Although these two varieties are at the same physiological state, with respect to the onset of germination, they exhibit different "metabolic states". During the course of vernalisation, the control Rideau grains (14 hours imbibed) showed a marked decrease in the total and water-soluble ^{32}P up to three weeks followed by a substantial increase in the level of ^{32}P when the chilling treatment was continued for another two weeks. In Marquis wheat, chilling led to an increase of the total and water-soluble ^{32}P to a maximum at four days, followed by a marked decrease after further chilling up to seven days. These marked decreases were probably due to a leaching out of inorganic ^{32}P since high ^{32}P activity was detected on the filter paper and the Petri dish.

In both Marquis and Rideau, the water soluble compounds accounted for a greater level of ^{32}P uptake than any other phosphate compound (Fig. VII). It was further observed that the organic ^{32}P water soluble compounds accumulated progressively with vernalisation in both partially and fully imbibed Rideau wheat grains (Table XI, Fig. VIII). The increase in water soluble organic phosphates occurred in both cases at the expense of the inorganic ^{32}P (Fig. VIII).

TABLE X

The Distribution of ^{32}P among Phosphate Compounds in Vernalised Marquis and Rideau Whole Grains (c.f. Fig. VII)

^{32}P-Incorporation, cpm per whole grain (x10-3)			
Phosphate Fraction	Control *	Four days Chilled	Seven days Chilled
MARQUIS			
^{32}P Water Soluble Compounds (inorganic and organic)	358	451	210
^{32}P Total Nucleic Acids	3	14	3
^{32}P-Phospholipids	5	5	5
^{32}P-Phosphoproteins	6	5	4
Total	372	475	222

Phosphate Fraction	Control **	Three weeks Chilled	Five weeks Chilled
RIDEAU			
^{32}P Water Soluble Compounds (inorganic and organic)	710	333	589
^{32}P Total Nucleic Acids	6	64	69
^{32}P-Phospholipids	6	40	25
^{32}P-Phosphoproteins	4	22	17
Total	726	459	700

* Grains allowed to imbibe ^{32}P-orthophosphate for five hours.

** Grains allowed to imbibe ^{32}P-orthophosphate for fourteen hours.

FIGURE VII

(cf. Table X)

Legend

^{32}P-incorporation into the water-soluble, nucleic acid, phospholipid and phosphoprotein fractions of Rideau and Marquis whole grains with respect to imbibition and vernalisation. Rideau grains were imbibed for fourteen hours, and Marquis grains for five hours, prior to chilling at 2 ± 1°C.

d = Total ^{32}P recovered

e = ^{32}P water-soluble compounds (inorganic and organic)

f = ^{32}P-labelled nucleic acids

g = ^{32}P-labelled phospholipids

h = ^{32}P-labelled phosphoproteins

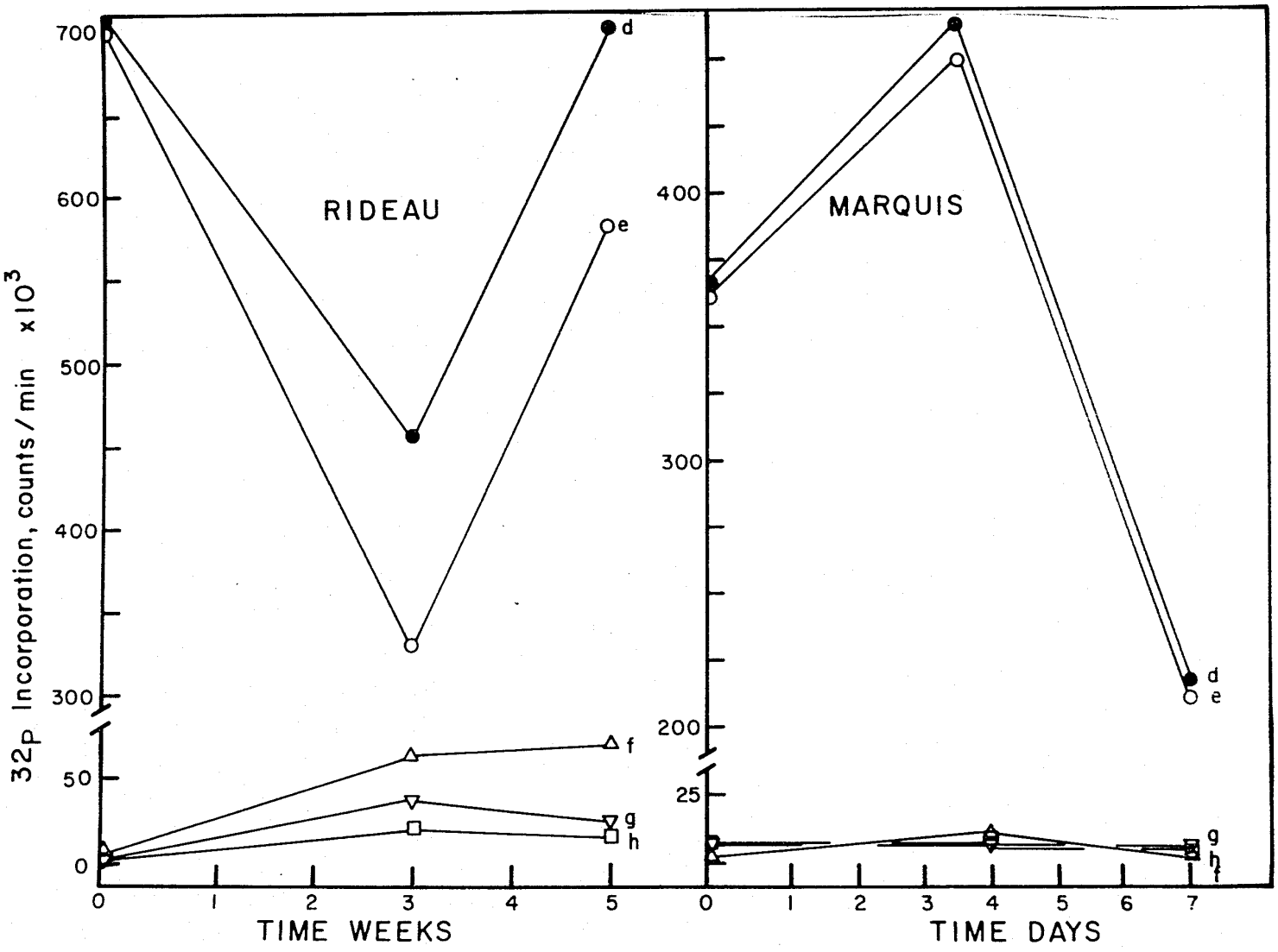

RIDEAU
MARQUIS
32P Incorporation, counts / min x10^3
700
600
500
400
300
50
0
400
300
200
25
d
e
f
g
h
0 1 2 3 4 5
TIME WEEKS
0 1 2 3 4 5 6 7
TIME DAYS

TABLE XI

(cf. Fig. VIII)

Incorporation of ^{32}P into Water Soluble Organic Phosphates of Rideau Wheat Whole Grains with Respect to Imbibition and Vernalization

(CPM per whole grain x 10^{-3})

Treatment	Water Soluble organic ^{32}P	Water Soluble inorganic ^{32}P	Total Water soluble ^{32}P
R_5K	92	769	870
R_5C_3	258	328	586
R_5C_5	368	368	736
$R_{14}K$	366	608	974
$R_{14}C_3$	361	218	579
$R_{14}C_5$	386	243	629

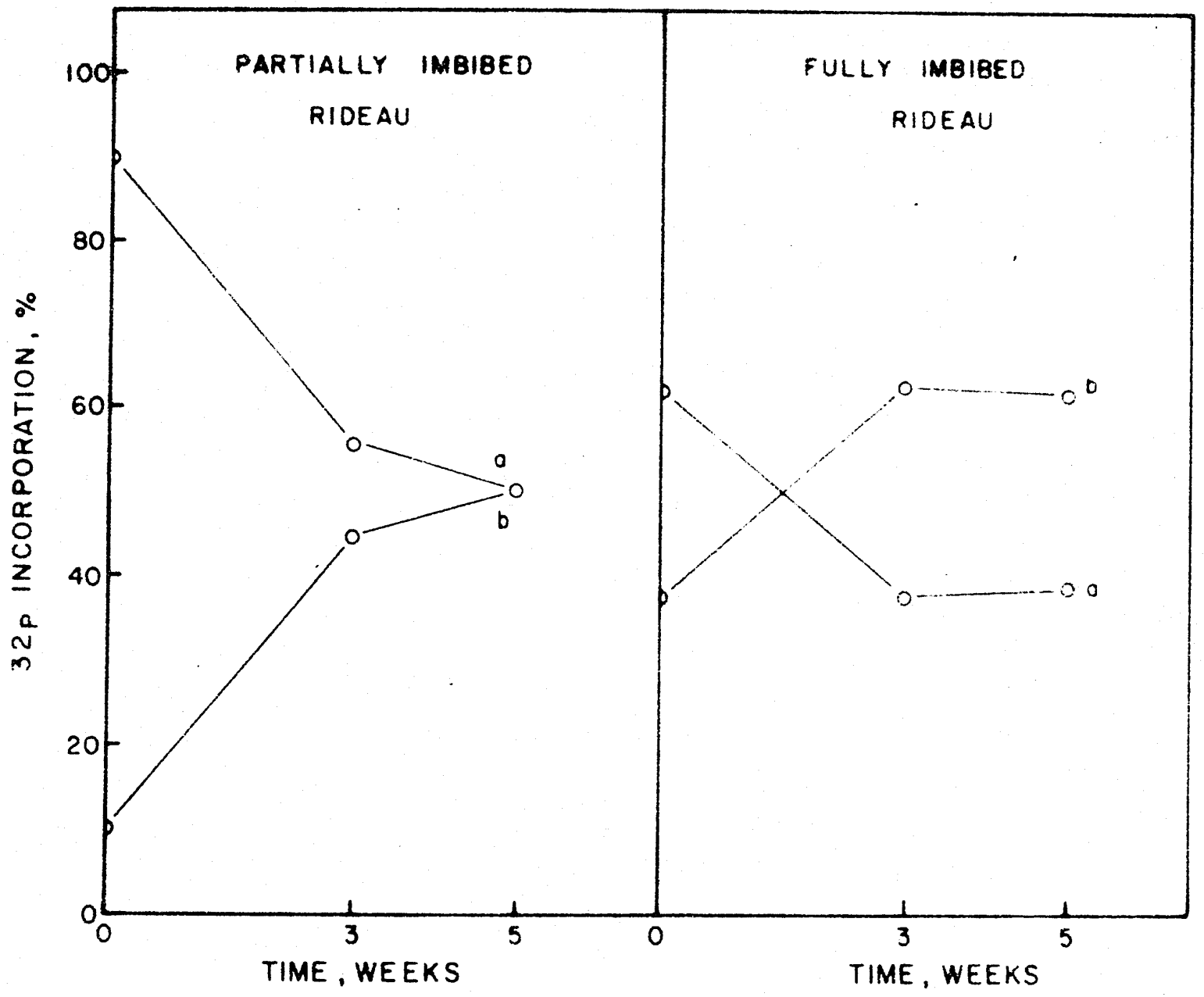
FIGURE VIII

Legend

Percentage incorporation of ^{32}P into the inorganic and organic water soluble phosphates of partially and fully imbibed Rideau whole grains with vernalisation.

a = inorganic ^{32}P-orthophosphate

b = water-soluble ^{32}P organic phosphates


PARTIALLY IMBIBED
RIDEAU
FULLY IMBIBED
RIDEAU
32P INCORPORATION, %
100
80
60
40
20
0
a
b
b
a
0
3
5
0
3
5
TIME, WEEKS
TIME, WEEKS

A relatively large increase of ^{32}P-labelled nucleic acids was observed in the Rideau whole grains during the first three weeks of chilling (Table X and Fig. VII). This same level was maintained thereafter. A five-fold increment in ^{32}P of nucleic acids of Marquis was observed in these compounds during the first four days of chilling. Further chilling resulted in a decrease to the control level (Table X and Fig. VII).

^{32}P-labelling of phospholipids increased seven-fold within the first three weeks of chilling the Rideau wheat whole grains and then showed a reduction on further cold treatment. No change was observed in labelling of phospholipids in Marquis grains (Table X and Fig. VII).

^{32}P-labelling of phosphoproteins in Rideau wheat whole grains increased five-fold within the first three weeks of vernalisation and remained fairly constant after further chilling. Little or no changes were observed in the labelled phosphoproteins of Marquis whole grains following vernalisation (Table X and Fig. VII).

(ii) Grain parts (within). In Figs. IX and X, ^{32}P-activity in the water soluble, phospholipid, nucleic acid and phosphoprotein fractions have been plotted as percentages of the total ^{32}P incorporated by the various grain parts (Tables XII-XV) as a function of the imbibition time and the duration of chilling.

Plumule: In both wheats the major ^{32}P-labelled fraction was the water-soluble phosphate fraction (Fig. IX). In Rideau the percentage of ^{32}P in this fraction decreased progressively on vernalisation while the percentages in the nucleic acid and phospholipid fractions increased correspondingly; a slight percentage increase occurred in the phosphoprotein fraction after 3 weeks' chilling. In

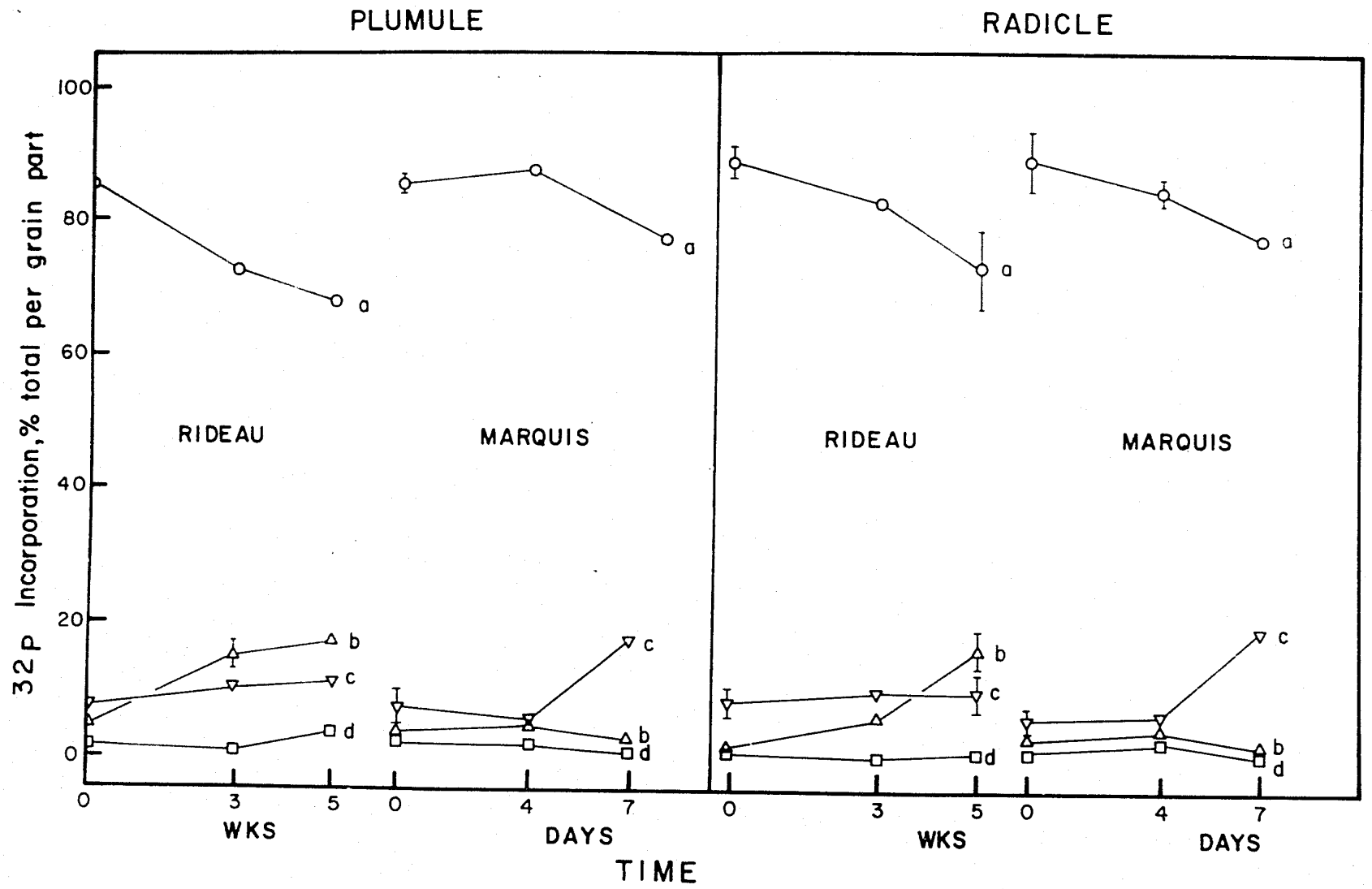
FIGURE IX

(cf. Table XII)

Legend

Percentage ^{32}P incorporation into the water soluble, nucleic acid, phospholipid and phosphoprotein fractions of the plumules and radicles of Rideau and Marquis wheat with respect to imbibition and vernalisation.

a = ^{32}P water-soluble compounds
b = ^{32}P nucleic acids
c = ^{32}P phospholipids
d = ^{32}P phosphoprotein


PLUMULE
RADICLE
32P Incorporation, % total per grain part
100
80
60
40
20
0
RIDEAU
MARQUIS
RIDEAU
MARQUIS
a
a
a
a
b
c
d
b
c
d
b
c
d
b
c
d
0
3
5
0
4
7
0
3
5
0
4
7
WKS
DAYS
WKS
DAYS
TIME

Marquis, however, the percentage of ^{32}P in the water-soluble fraction remained fairly constant up to four days, followed by a sharp decrease. The latter was reflected by a corresponding percentage increase in ^{32}P-labelled lipids but not in the other two fractions.

Radicle: In both varieties, water soluble compounds incorporated the largest percentage of ^{32}P-orthophosphate. Note that this percentage decreased with vernalisation (Fig. IX).

The loss of ^{32}P from water soluble compounds seemed to be reflected by the increased percentage incorporation in the nucleic acids of Rideau, and in the phospholipid of Marquis (Fig. IX).

It was observed that the percentage incorporation of ^{32}P into Rideau nucleic acids was greatest between the third and fifth weeks of vernalisation (Fig. IX and Table XIII). A similar observation held true for the percentage incorporation ^{32}P into Marquis phospholipids (Fig. IX and Table XIV).

Scutellum: In this organ also, the ^{32}P water soluble compounds accounted for the largest percentage incorporation of ^{32}P in both varieties (Fig. X). Whereas the loss of ^{32}P from water soluble compounds was great and almost linear with vernalisation in Rideau, in Marquis the loss of ^{32}P became evident only after four days' chilling.

In Rideau, the nucleic acid and phospholipid fractions accounted for the loss of ^{32}P from the water soluble compounds. The increases in the percentage incorporation of ^{32}P into these two compounds were not simultaneous. In Marquis, only the phospholipid fraction directly reflected the loss of ^{32}P from the water soluble compounds (Fig. X).

FIGURE X

Legend

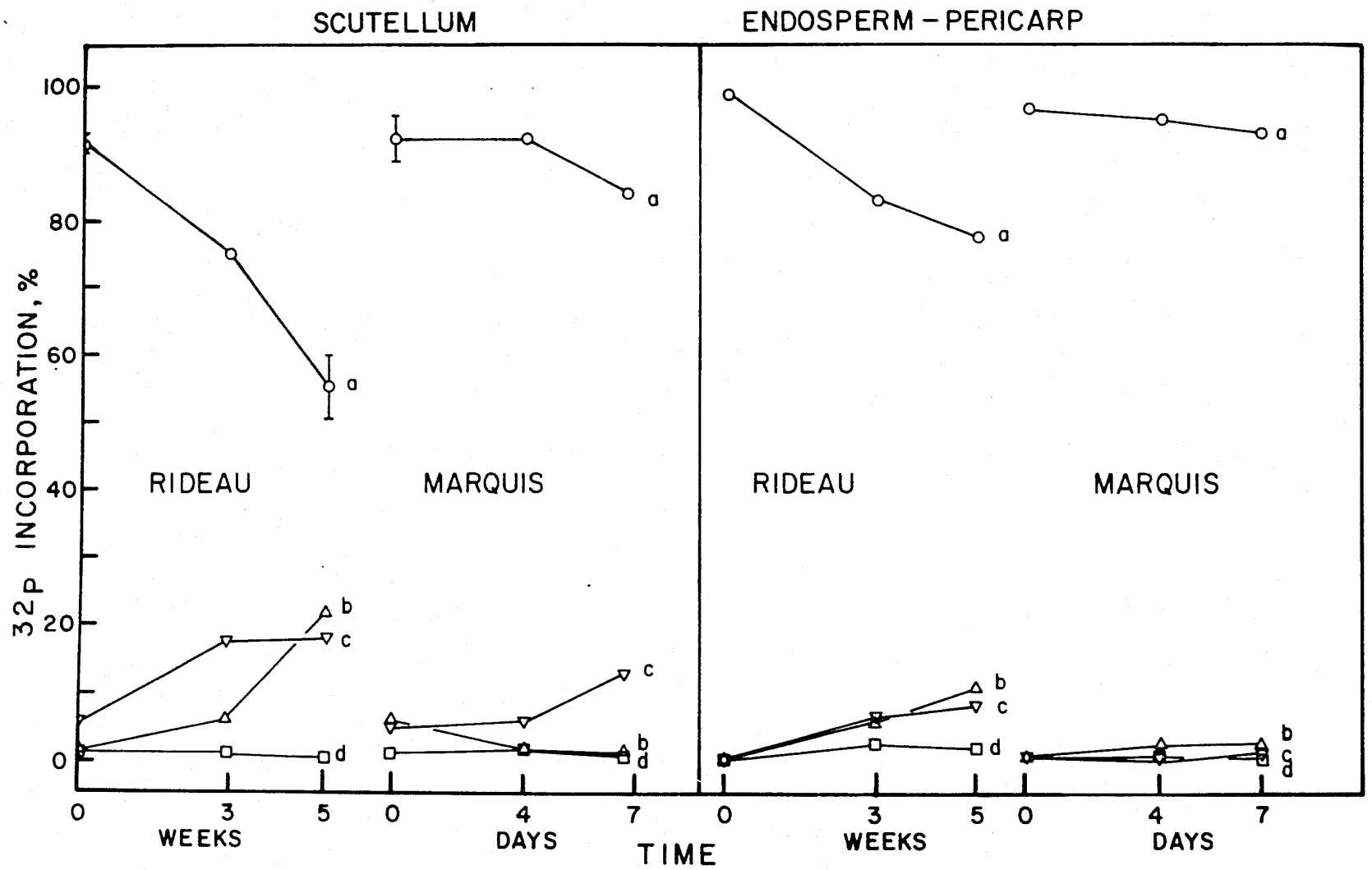
Percentage incorporation of ^{32}P into water soluble, nucleic acid, phospholipid and phosphoprotein fractions of the scutellum and endosperm-pericarp of Rideau and Marquis wheat following imbibition and vernalisation.

a = ^{32}P water soluble compounds

b = ^{32}P nucleic acids

c = ^{32}P phospholipids

d = ^{32}P phosphoproteins


SCUTELLUM
ENDOSPERM – PERICARP
100
80
60
40
20
0
32P INCORPORATION, %
RIDEAU
MARQUIS
RIDEAU
MARQUIS
a
a
a
a
b
c
d
c
b
d
b
c
d
b
c
d
0
3
5
0
4
7
0
3
5
0
4
7
WEEKS
DAYS
WEEKS
DAYS
TIME

The percentage incorporation of ^{32}P into the phosphoprotein fractions in both varieties was not affected by vernalisation.

Endosperm-pericarp: In both varieties, the water soluble compounds incorporated the largest percentage of ^{32}P (Fig. X). Whereas an appreciable loss of ^{32}P was noted in this fraction in Rideau, negligible changes were observed in Marquis concomitant with vernalisation. Little or no change was observed in the other three phosphate compounds in Marquis following chilling.

The loss of percentage ^{32}P activity from the water soluble compounds of Rideau was reflected in a percentage gain of ^{32}P incorporation by the nucleic acids and phospholipids.

(iii) Grain parts (between) - The percentage distribution of ^{32}P among the phosphorus containing compounds in each grain part as a function of the imbibition period and the length of cold treatment at $2^{o}C$ (Fig. XI) has also been calculated.

^{32}P water soluble compounds: These compounds were mainly associated with the Rideau and Marquis endosperm-pericarp fractions and showed a marked decrease in Rideau during the first three weeks of chilling. Concomitant net increases were noted in the other Rideau grain parts, the highest level being observed in the radicle (Fig. XI A).

Paper chromatography of the water soluble phosphates from Marquis, using the tert-butanol-water-picric acid solvent system, and autoradiography of these water-soluble phosphates (Fig. XII) confirmed the presence of large concentrations of inorganic ^{32}P (R_f 0.60) and lesser amounts of organic phosphates such as glucose phosphates (R_f 0.36). Four other ^{32}P-labelled unidentified phosphates were also noted, having R_f values of 0.69, 0.24, 0.44 and 0.77,

respectively in the tert.-butanol-water-picric acid solvent. However, it was not determined whether these organic phosphate compounds increased in concentration with vernalisation.

^{32}P nucleic acids: In both varieties the endosperm-pericarp incorporated the largest percentage of ^{32}P (Fig. XI B). A loss of the percentage ^{32}P was observed in the Rideau endosperm-pericarp following vernalisation; this loss was reflected in the Rideau scutellum which increased its percentage incorporation of ^{32}P.

In Marquis, the percentage incorporation of ^{32}P in the endosperm-pericarp increased slightly with vernalisation at the expense of the percentage ^{32}P incorporated into the scutellum and radicle.

The plumule of both varieties did not show any change in its percentage incorporation of ^{32}P as a result of vernalisation.

^{32}P phospholipids: In both varieties, the endosperm-pericarp accounted for the largest percentage incorporation of ^{32}P into phospholipids. Whereas a linear increase, with vernalisation, was observed in the percentage increase of ^{32}P into the Rideau endosperm-pericarp, a marked loss in ^{32}P percentage incorporation was observed within four days of chilling the Marquis endosperm-pericarp (Fig. XI C) after which no detectable loss in percentage incorporation was observed.

Although the percentage incorporation of ^{32}P remained constant in the Rideau scutellum and plumule, a marked loss was observed in the Rideau radicle. The percentage increase of ^{32}P into the Marquis scutellum, radicle and plumule reflected the percentage decrease of ^{32}P from the endosperm-pericarp (Fig. XI C).

^{32}P phosphoprotein: The endosperm-pericarp of both varieties incorporated the largest percentage of ^{32}P in their phosphoprotein fractions (Fig. XI D). Although vernalisation resulted in aloss

FIGURE XI

Legend

Percentage distribution of ^{32}P-phosphoprotein, ^{32}P-nucleic acids, ^{32}P-phospholipids and ^{32}P water-soluble compounds in the various grain parts of Rideau and Marquis wheat with respect to imbibition and vernalisation.

a = Endosperm-pericarp

b = Scutellum

c = Radicle

d = Plumule

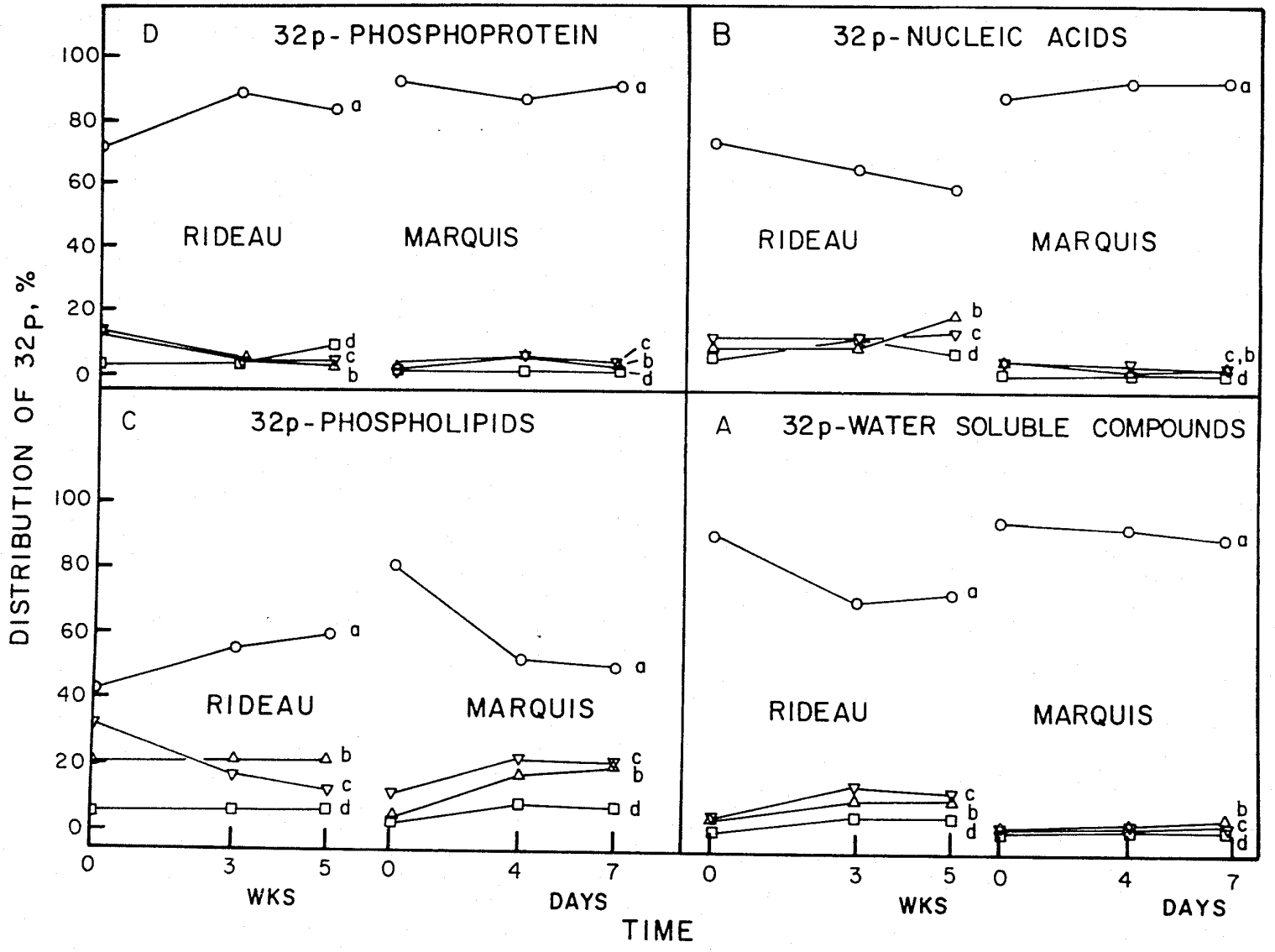
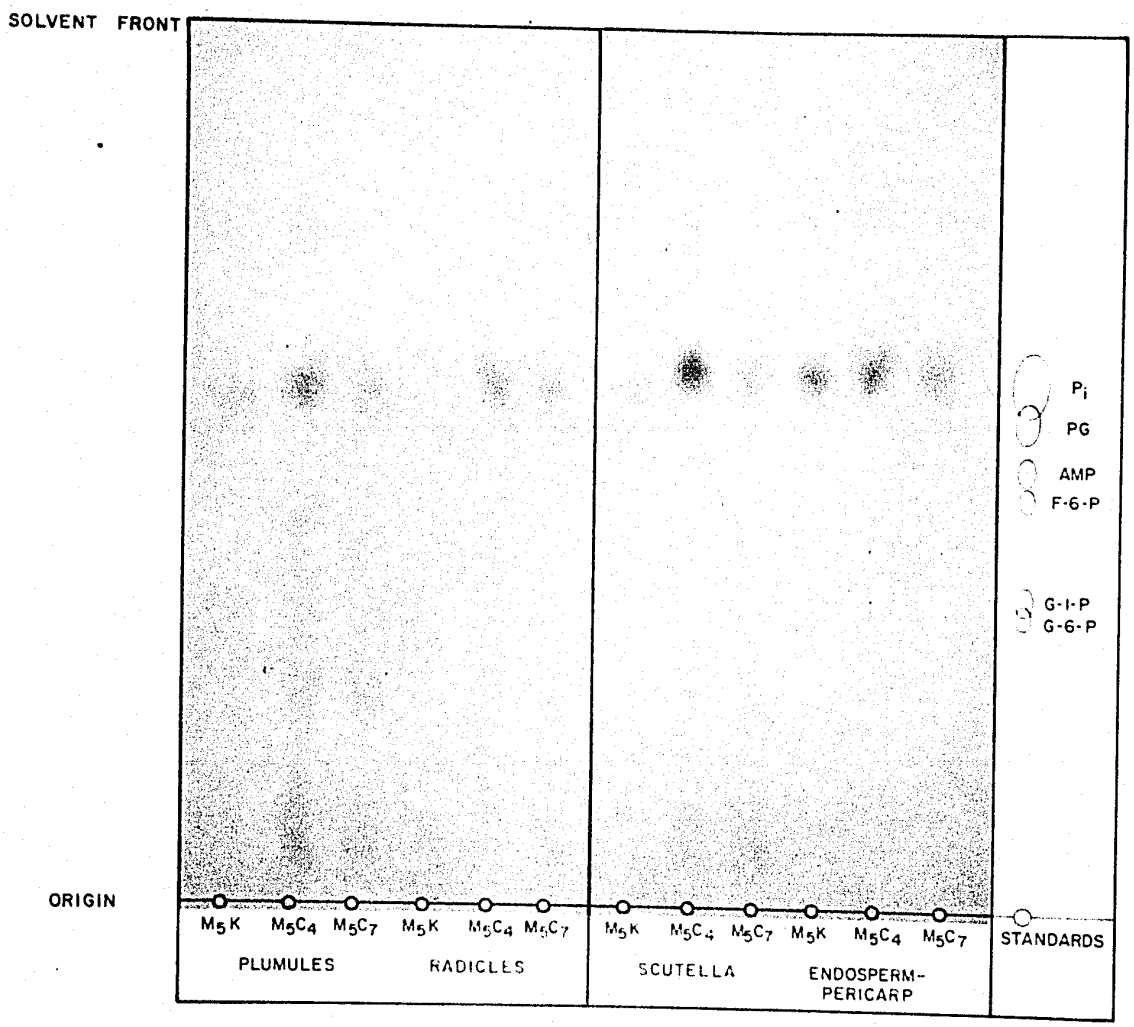

D
32p- PHOSPHOPROTEIN
B
32p- NUCLEIC ACIDS
C
32p- PHOSPHOLIPIDS
A
32p- WATER SOLUBLE COMPOUNDS
RIDEAU
MARQUIS
DISTRIBUTION OF 32P, %
100
80
60
40
20
0
a
b
c
d
0
3
5
WKS
4
7
DAYS
TIME

FIGURE XII

Legend

Autoradiographs of ^{32}P water-soluble organic phosphates in the grain parts of Marquis wheat following imbibition and vernalisation.

P_i	Inorganic ^{32}P-orthophosphate
GP	Glycerophosphate
AMP	Adenosine monophosphate
F-6-P	Fructose-6-phosphate
G-1-P	Glucose-1-phosphate
G-6-P	Glucose-6-phosphate


SOLVENT FRONT
ORIGIN
Pi
PG
AMP
F-6-P
G-1-P
G-6-P
M5K M5C4 M5C7 M5K M5C4 M5C7
PLUMULES
RADICLES
M5K M5C4 M5C7 M5K M5C4 M5C7
SCUTELLA
ENDOSPERM-PERICARP
STANDARDS

of percentage ^{32}P activity in the scutellum and radicle, it caused a slight increase in the percentage of ^{32}P incorporated into this fraction in the plumule. Negligible changes were observed in the percentage of ^{32}P incorporated into the phosphoproteins of the Marquis grain parts.

Actual incorporation: The actual incorporation of ^{32}P into the various phosphate compounds of Marquis and Rideau wheat grain parts is shown in Tables XII - XV. It was observed that the endosperm-pericarp of both varieties incorporated the largest quantity of ^{32}P. Conversely, the lowest ^{32}P incorporation was observed in the plumules of both varieties.

Whereas it was noted (on page 50) that the total ^{32}P from the various phosphate compounds recovered from Rideau whole grains decreased with vernalisation up to three weeks and thereafter increased (Table X and Fig. VII), it was observed that the summation of the various phosphate compounds from the dissected parts gave a picture similar to that observed for Marquis whole grains in Fig. VII.

	Control	3 weeks	5 weeks
Total ^{32}P recovered from Rideau whole grains	726×10^3	459×10^3	700×10^3
Total ^{32}P recovered from dissected Rideau grain	719×10^3	1011×10^3	932×10^3

These differences might be due to a leaching of ^{32}P-orthophosphate from the grains to the filter paper lining the Petri dish; leaching may be a reflection of different metabolism with different batches of grains.

TABLE XII

^{32}P Incorporation into Water Soluble Compounds ($P_{org.}$ + P_i) of Marquis and Rideau Wheats following Imbibition, Partial Vernalization, and Complete Vernalization

(CPM per dissected part x 10^{-3})

MARQUIS			
Grain part	Control (i.e. fully imbibed)	Four Days chilled	Seven Days chilled
Plumule	1.4 ± 0.4 *	3.9 ± 0.7	3.9 ± 0.3
Radicle	11.2 ± 6.5	9.1 ± 1.7	11.2 ± 2.3
Scutellum	9.7 ± 3.3	14.1 ± 1.9	15.5 ± 3.2
Endosperm-pericarp	409.1 ± 8.7	409.5 ± 6.3	208.5 ± 24.5
TOTAL	431.4	436.6	239.1
RIDEAU			
Grain part	Control (i.e. fully imbibed)	Three Weeks chilled	Seven Weeks chilled
Plumule	4.4 ± 0.8	41.5 ± 6.7	37.5 ± 0.8
Radicle	28.1 ± 1.8	121.1 ± 4.1	83.9 ± 21.6
Scutellum	27.1 ± 5.0	81.9 ± 6.0	66.2 ± 0.5
Endosperm-pericarp	644.9 ± 11.4	597.3 ± 1.2	511.6 ± 0.5
TOTAL	704.5	841.8	699.2

* Values are arithmetic means of duplicate experiments.

TABLE XIII

^{32}P Incorporation into Total Nucleic Acids (DNA + RNA) of Marquis and Rideau Wheats following Imbibition, Partial Vernalization, and Complete Vernalization

(CPM per dissected part x 10^{-2})

Grain part	Control (i.e. fully imbibed)	MARQUIS	Four Days chilled	Seven Days chilled
Plumule	0.7 ± 0.1 *		2.1 ± 0.1	1.7 ± 0.3
Radicle	3.0 ± 0.5		4.8 ± 1.2	3.0 ± 1.1
Scutellum	3.1 ± 1.0		2.8 ± 0.1	3.0 ± 0.1
Endosperm-pericarp	48.3 ± 6.4		143.8 ± 49.4	98.3 ± 21.0
TOTAL	55.1		153.5	106.0
Grain part	Control (i.e. fully imbibed)	RIDEAU	Three Weeks chilled	Seven Weeks chilled
Plumule	2.1 ± 0.2		90.8 ± 28.5	88.6 ± 1.6
Radicle	5.5 ± 0.7		91.6 ± 3.4	125.5 ± 11.8
Scutellum	4.3 ± 0.1		68.2 ± 12.2	239.8 ± 6.7
Endosperm-pericarp	33.3 ± 5.6		477.2 ± 25.9	729.6 ± 7.9
TOTAL	45.2		527.8	1183.5

* Values are arithmetic means of duplicate experiments

TABLE XIV

^{32}P Incorporation into Phospholipids of Marquis and Rideau Wheats following Imbibition, Partial Vernalisation and Complete Vernalisation

(CPM per dissected part x 10^{-2})

Grain part	Control (i.e. fully imbibed)	MARQUIS	Four Days chilled	Seven Days chilled
Plumule	1.2 ± 0.1 *		2.7 ± 0.8	8.9 ± 2.3
Radicle	5.9 ± 1.3		7.5 ± 2.4	27.2 ± 5.4
Scutellum	2.4 ± 0.1		5.5 ± 0.5	24.2 ± 5.3
Endosperm-pericarp	47.3 ± 10.0		17.1 ± 4.7	60.8 ± 2.1
TOTAL	56.8		32.8	121.1
Grain part	Control (i.e. fully imbibed	RIDEAU	Three Weeks chilled	Seven Weeks chilled
Plumule	3.9 ± 0.1		59.3 ± 1.2	61.0 ± 4.7
Radicle	26.1 ± 9.7		150.5 ± 18.0	114.2 ± 13.1
Scutellum	15.7 ± 0.3		190.0 ± 8.3	201.9 ± 3.7
Endosperm-pericarp	32.3 ± 2.7		498.9 ± 37.3	555.3 ± 1.0
TOTAL	78.0		898.7	932.4

* Values are arithmetic means of duplicate experiments

TABLE XV

^{32}P Incorporation into Phosphoproteins of Marquis and Rideau Wheats following Imbibition, Partial Vernalisation and Complete Vernalisation

(CPM per dissected part x 10^{-2})

Grain part	Control (i.e. fully imbibed	MARQUIS	Four Days chilled	Seven Days chilled
Plumule	0.4 ± 0.1 *		0.8 ± 0.1	0.6 ± 0.1
Radicle	1.4 ± 0.1		3.9 ± 0.7	2.1 ± 0.9
Scutellum	1.1 ± 0.3		3.5 ± 0.5	1.9 ± 0.1
Endosperm-pericarp	38.8 ± 6.2		51.8 ± 3.6	46.1 ± 0.8
TOTAL	41.7		60.0	50.7

Grain part	Control (i.e. fully imbibed	RIDEAU	Three Weeks chilled	Seven Weeks chilled
Plumule	1.1 ± 0.3		13.7 ± 1.9	19.9 ± 0.6
Radicle	3.9 ± 1.1		10.7 ± 0.1	9.2 ± 0.3
Scutellum	4.0 ± 0.6		10.5 ± 0.7	6.9 ± 0.6
Endosperm-pericarp	23.3 ± 3.4		249.5 ± 8.8	178.5 ± 4.1
TOTAL	32		284	214

* Values are arithmetic means of duplicate experiments

(4) Identification of Lipid Components

As shown in Tables XVI - XXIII and Plates I - IV, the lipids of the various grain parts have been identified by their Rhodamine staining behaviour, Rf values, ^{32}P-labelling, periodate-Schiff staining and by their deacylated products.

The main phospholipids, in all control and vernalised grain parts of both varieties, were phosphatidyl choline, phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl glycerol. All parts also contained another phospholipid which was tentatively identified as "lysophosphatidic acid" on the basis of ^{32}P incorporation, deacylated product, Rf value and Rhodamine staining behaviour (Tables XVI - XXIII).

The galactolipids, monogalactosyl and digalactosyl diglycerides were present only in the scutellum of Rideau (Table XXII) and in the endosperm-pericarp of both wheats (Tables XIX and XXIII). The proportion of these galactolipids appeared to increase after complete vernalisation (Plates II and IV).

No sulpholipid was observed in any of the grain parts.

In Marquis, the neutral lipids represented the major lipid fraction in the radicles and endosperm-pericarps; fairly high concentrations of neutral lipids were also detected in the plumule and in the scutellum (Plates I and II). By contrast, in Rideau, the neutral lipids constituted the major lipid fraction in the scutellum and the endosperm-pericarp, but only small concentrations of these lipids were present in the plumule and radicle (Plates III and IV).

(5) Changes in Lipid Components of Grain Parts

(a) Marquis

It was observed that the neutral lipids formed the highest proportion in the radicle and remained more or less constant with vernalisation (Plate I). The phospholipids most strongly labelled with ^{32}P were "lysophosphatidic acid" and lecithin. The labelling increased with vernalisation (Table XVI and Plate I).

"Lysophosphatidic acid" and the neutral lipids formed the major lipid components in the Marquis plumule (Plate I and Table XVII). Incorporation of ^{32}P increased in the "lysophosphatidic acid" and lecithin components with vernalisation. Neutrals also appeared to increase with chilling. A minor unidentified ^{32}P lipid (Spot 1, Plate I, Table XVII) was more heavily labelled with vernalisation.

As many as thirteen different lipids were identified in the scutellum and endosperm-pericarp of Marquis. In the scutellum the most prominent lipids were "lysophosphatidic acid", phosphatidyl glycerol, phosphatidyl inositol and an unknown phospholipid (Spot No. 4). "Lysophosphatidic acid" and lecithin were the two major phospholipids. It was also observed that while neutral lipids decreased in relative concentration with vernalisation, "lysophosphatidic acid" increased in both concentration and ^{32}P-labelling with vernalisation (Plate II and Table XVIII).

The most prominent lipids in the Marquis endosperm-pericarp were neutral lipids, lecithin, digalactosyl diglyceride and "lysophosphatidic acid". Phosphatidyl ethanolamine, phosphatidyl glycerol and monogalactosyl diglyceride were present in relatively minor concentrations. The remaining components were not identified (Plate II and Table XIX). One of these unidentified lipids (Spot No. 6)

increased tremendously with vernalisation. It was also observed that after vernalisation most of the labelling was confined to the lecithin component. Only traces of ^{32}P were observed in the "lysophosphatidic acid" component and in phosphatidyl glycerol, but an unidentified phospholipid (Spot No. 9) incorporated ^{32}P after vernalisation.

Digalactosyl diglyceride increased strikingly with vernalisation.

TABLE XVI

Lipid Components of Marquis Radicles[a] (see Plate I)

Spot Number	Rf Value	Rhodamin 6G Stain	^{32}P Labelling	Deacylated Product[b]	Tentative Identity of Components
1.	0.34	Gray (weak)	-	IMP	Phosphatidyl inositol
2.	0.41	Blue (weak)	-	-	Unidentified
3.	0.46	Yellow (strong)	+++	GPC	Phosphatidyl choline
4.	0.51	Blue (medium)	-	-	Unidentified
5.	0.55	Blue (medium)	Trace	GPG	Phosphatidyl glycerol
6.	0.59	Gray (weak)	+	GPE	Phosphatidyl ethanolamine
7.	0.64	Gray (weak)	-	N.D.	Diphosphatidyl glycerol
8.	0.71	Gray (weak)	trace	-	Unidentified
9.	0.77	Gray (weak)	-	-	Unidentified
10.	0.81	Blue (very strong)	++++	GPG	"Lysophosphatidic acid"
11.	0.86	Orange (weak)	-	-	)
12.	0.94	Yellow (very strong)	-	-	) Neutral Lipids

- 71 -

NOTES TO TABLE XVI:

a. No periodate - Schiff positive spots were found; Spot 6 was faintly ninhydrin positive.

b. Abbreviations:

IMP	Inositol monophosphate
GPC	Glycerylphosphoryl choline
GPG	Glycerylphosphoryl glycerol
GPE	Glycerylphosphoryl ethanolamine
N.D.	Not detected
+	Present
-	Absent

TABLE XVII

Lipid Components of Marquis Plumules[a] (see Plate I)

Spot Number	Rf Value	Rhodamin 6G Stain	^{32}P Labelling	Deacylated Product[b]	Tentative Identity of Components
1.	0.34	Gray (weak)	++	N.D.	Unidentified
2.	0.41	Blue (strong)	-	N.D.	Phosphatidyl glycerol
3.	0.47	Gray (weak)	-	N.D.	Phosphatidyl ethanolamine
4.	0.55	Gray (weak)	-	-	Unidentified
5.	0.61	Gray (weak)	-	-	Unidentified
6.	0.70	Gray (weak)	-	-	Unidentified
7.	0.77	Blue (strong)	+	GPG	"Lysophosphatidic acid"
8.	0.95	Yellow (strong)	-	-	Neutral lipids

Abbreviations:

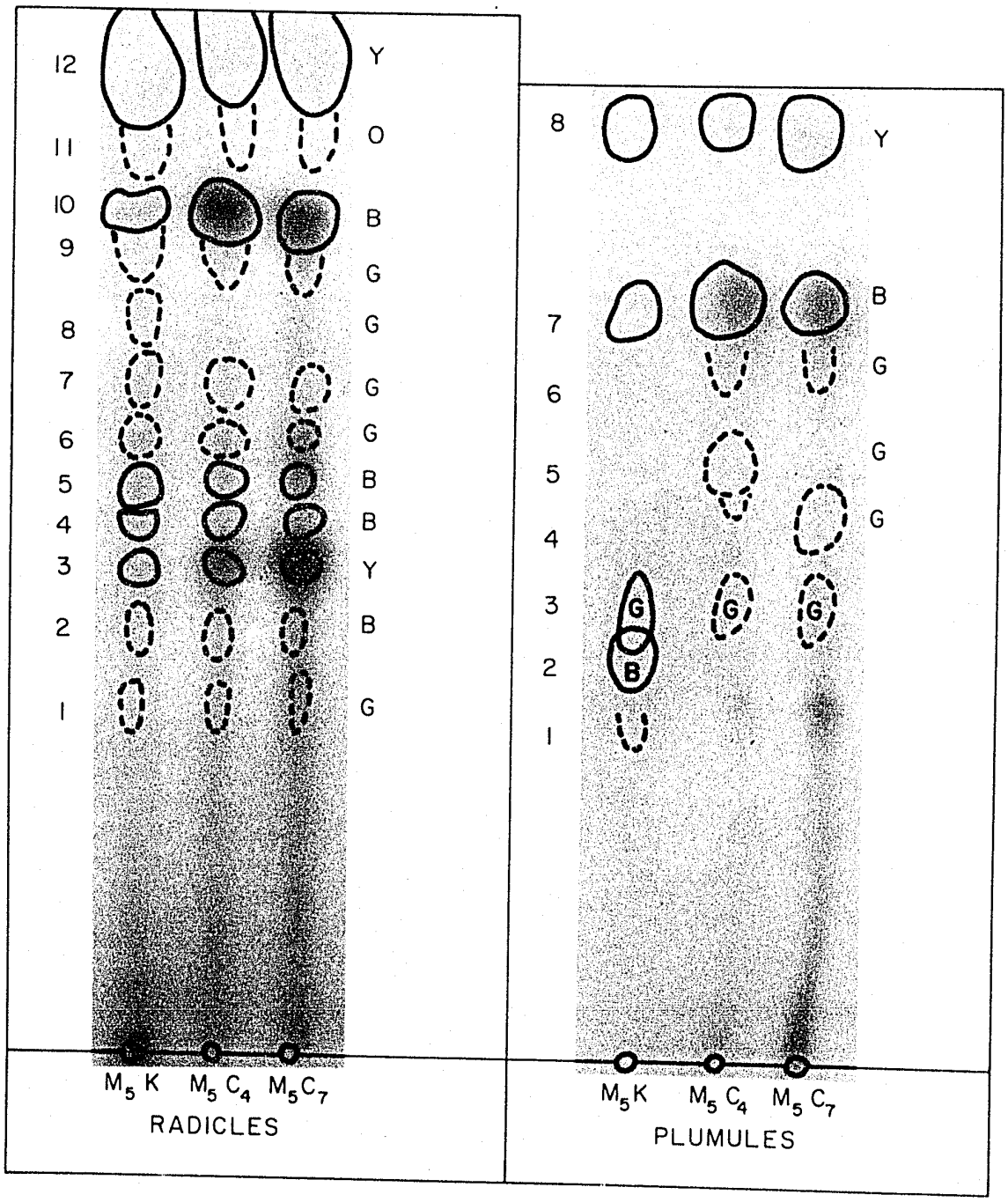
(a) No periodate - Schiff positive spots were found.
+ Present
- Absent
(b) N.D. - not detected
GPG Glycerylphosphoryl glycerol

PLATE I

(See Tables XVI and XVII)

Legend

Photograph of autoradiographs, of ^{32}P-labelled phospholipids of Marquis radicles and plumules, superimposed on the Rhodamine 6G stained chromatograms of these lipids following imbibition and vernalisation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M5 C7
PLUMULES

TABLE XVIII

Lipid Components of Marquis Scutella[a] (see Plate II).

Spot Number	Rf Value	Rhodamin 6G Stain	^{32}P Labelling	Ninhydrin Stain	Deacylated Product[b]	Tentative Identity of Components
1.	0.18	Blue (strong)	Trace	-	IMP	Phosphatidyl inositol
2.	0.34	Yellow (strong)	++	-	N.D.	Phosphatidyl choline
3.	0.41	Blue (weak)	-	-	-	Unidentified
4.	0.47	Blue (strong)	+	-	-	Unidentified Phospholipid
5.	0.54	Yellow (weak)	+	+	GPE	Phosphatidyl Ethanolamine
6.	0.59	Blue (strong)	-	-	GPG	Phosphatidyl glycerol
7.	0.72	Blue (strong)	-	-	-	Unidentified
8.	0.80	Blue (strong)	+++	-	-	"Lysophosphatidic acid"
9.	0.86	Yellow (strong)	-	-	-	Unidentified
10.	0.97	Yellow (strong)	-	-	-	)
11.	0.99	Blue (strong)	-	-	-	) Neutral Lipids

NOTES TO TABLE XVIII:

a. No periodate-Schiff positive spots were found.

b. Abbreviations:

IMP	Inositol monophosphate
GPG	Glycerylphosphoryl glycerol
GPE	Glycerylphosphoryl ethanolamine
N.D.	Not detected
+	Present
-	Negative

TABLE XIX

Lipid Components of Marquis Endosperm-Pericarp (see plate II)

Spot Number	R_f Value	Rhodamin 6G Stain	^{32}P Labelling	Periodate Schiff	Deacylated Product[a]	Tentative Identify of Components
1	0.07	Blue (weak)	+	-	-	Unidentified phospholipid
2	0.14	Blue (strong)	-	-	-	Unidentified
3	0.18	Blue (weak)	-	-	-	Unidentified
4	0.29	Yellow (strong)	-	+	G-Gal-Gal	Digalactosyl diglyceride
5	0.36	Blue (strong)	-	-	IMP	Phosphatidyl inositol
6	0.42	Yellow (strong)	-			Unidentified glycolipid
7	0.48	Yellow (strong)	++	-	GPC	Phosphatidyl choline
8	0.52	Blue (weak)	+	-	GPG	Phosphatidyl glycerol
9	0.56	Yellow (weak→strong)	+	-	-	Unidentified phospholipid
10	0.61	Blue (strong)	-	-	-	Unidentified
11	0.67	Yellow (strong)	-	+	G-Gal	Monogalactosyl diglyceride
12	0.73	Blue (strong)	+	-	GPG	"Lysophosphatidic" acid
13	0.88	Yellow (strong)	-	-	-	Neutral lipids

NOTES TO TABLE XIX:

Abbreviations:

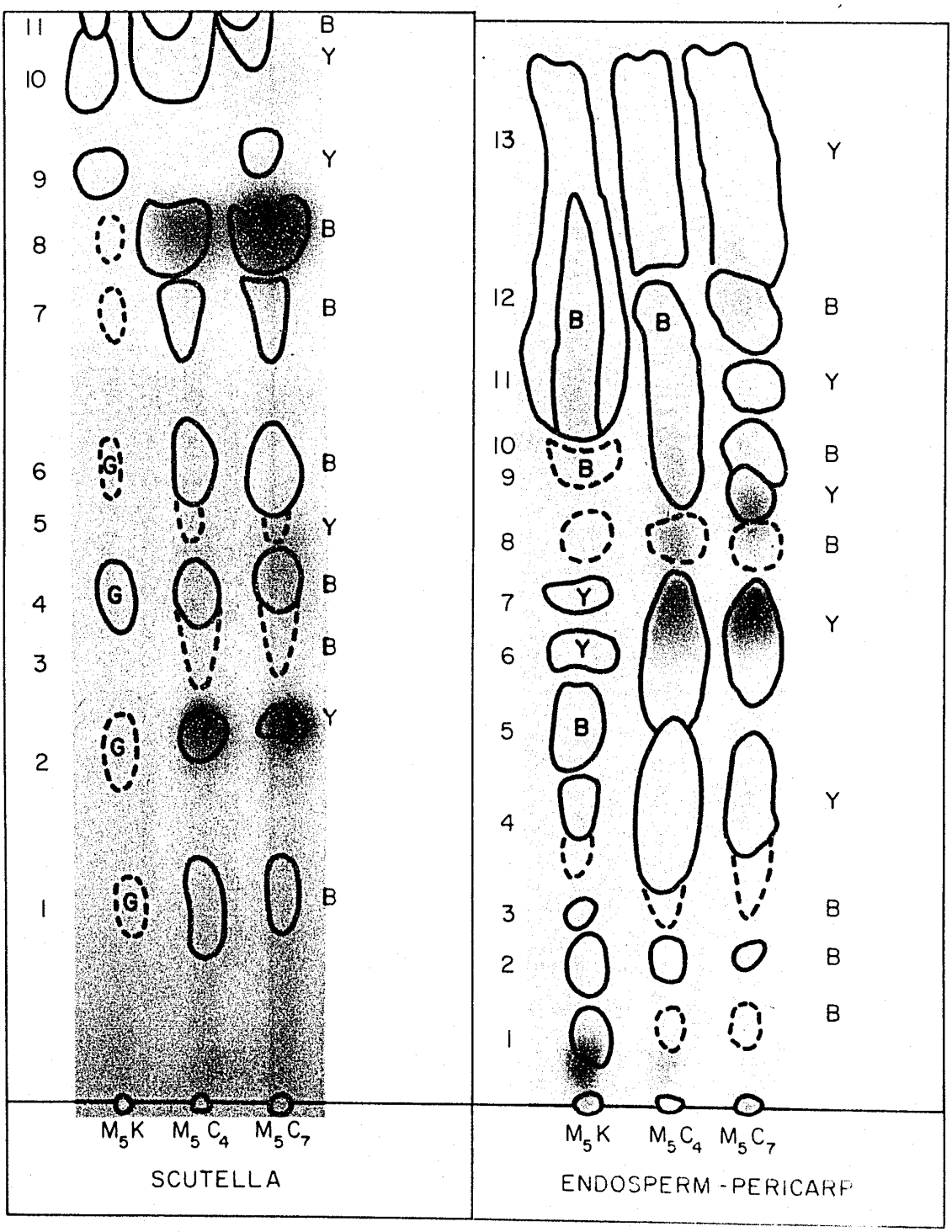
IMP	Inositol monophosphate
G-Gal-Gal	Glyceryl galactosyl galactose
G-Gal	Glyceryl galactose
GPC	Glycerylphosphoryl choline
GPG	Glycerylphosphoryl glycerol
+	Present
-	Absent

PLATE II

(See Tables XVIII & XIX)

Legend

Photograph of autoradiographs of ^{32}P-labelled phospholipids of Marquis scutella and endosperm-pericarps superimposed on the Rhodamine 6G stained chromatograms of these lipids following imbibition and vernalisation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ENDOSPERM - PERICARP

TABLE XX

Lipid Components of Rideau Radicles[a] (see plate III)

Spot Number	R_f Value	Rhodamin 6G Stain	^{32}P Labelling	Ninhydrin Stain	Deacylated Product[b]	Tentative Identity of Components
1	0.30	Gray	+	-	IMP	Phosphatidyl inositol
2	0.38	Blue	+	-	-	Unidentified
3	0.44	Yellow	++++	-	ND	Phosphatidyl choline
4	0.52	Blue	+	-	ND	Phosphatidyl glycerol
5	0.56	Yellow	++	+	GPE	Phosphatidyl ethanolamine
6	0.61	Blue	+	-	-	Unidentified phospholipid
7	0.75	Gray	+	-	-	Unidentified phospholipid
8	0.79	Blue	+++	-	GPG	"Lysophosphatidic acid"
9	0.84	Orange	-	-	-	Nonidentified
10	0.94	Yellow	-	-	-	) Neutral lipids
11	0.97	Yellow	-	-	-	) Neutral lipids

Abbreviations:

a) No periodate - Schiff positive spot detected

b) IMP = Inositol monophosphate
GPE = Glycerylphosphoryl ethanolamine
ND = not detected
\+ = present
\- = absent
GPG = Glycerylphosphoryl glycerol

(b) Rideau

The major lipids of the Rideau radicle were, in decreasing concentration, "Lysophosphatidic acid", neutral lipids, phosphatidyl glycerol and an unidentified phospholipid, (Spot 6, Table XXI and Plate III). Phosphatidyl choline, "Lysophosphatidic acid", phosphatidyl ethanolamine, and phosphatidyl glycerol showed the most ^{32}P labelling. It was observed that while the ^{32}P label decreased in "lysophosphatidic acid", it increased in the lecithin component with vernalisation.

In the Rideau plumules (Plate III and Table XXI), the major lipid was phosphatidyl glycerol. However, the most highly labelled phospholipids were lecithin, phosphatidyl ethanolamine and "lysophosphatidic acid". The labelling of these three components increased with vernalisation.

The neutral lipids, lysophosphatidic acid, digalactosyl diglyceride, lecithin and phosphatidyl were the major lipids in terms of decreasing concentration (Table XXII and Plate IV) in the Rideau scutellum. Once again, the phospholipid most labelled with ^{32}P was lecithin. This was followed by "lysophosphatidic acid", phosphatidyl ethanolamine and phosphatidyl inositol.

Fourteen different lipids were identified in the endosperm-pericarp of Rideau. Of these, the neutral lipids and lecithin were the most prominent. Very heavy ^{32}P labelling was observed in the lecithin, phosphatidyl ethanolamine and "lysophosphatidic acid" components. It was also observed that as the ^{32}P labelling decreased in the "lysophosphatidic acid" component, it increased in the lecithin component with vernalisation.

TABLE XXI

Lipid Components of Rideau Plumules[a] (see Plate III)

Spot Number	Rf Value	Rhodamin 6G Stain	^{32}P Labelling	Deacylated Product[b]	Tentative Identity of Components
1.	0.29	Yellow	+++	GPC	Phosphatidyl choline
2.	0.47	Blue	+	N.D.	Phosphatidyl glycerol
3.	0.57	Yellow	+	GPE	Phosphatidyl ethanolamine
4.	0.61	Gray	-	-	Unidentified
5.	0.68	Gray	-	-	Unidentified
6.	0.77	Blue	+	GPG	"Lysophosphatidic acid"
7.	0.98	Yellow	-	-	Neutral Lipids

Abbreviations:

(a) no periodate-Schiff positive spot detected

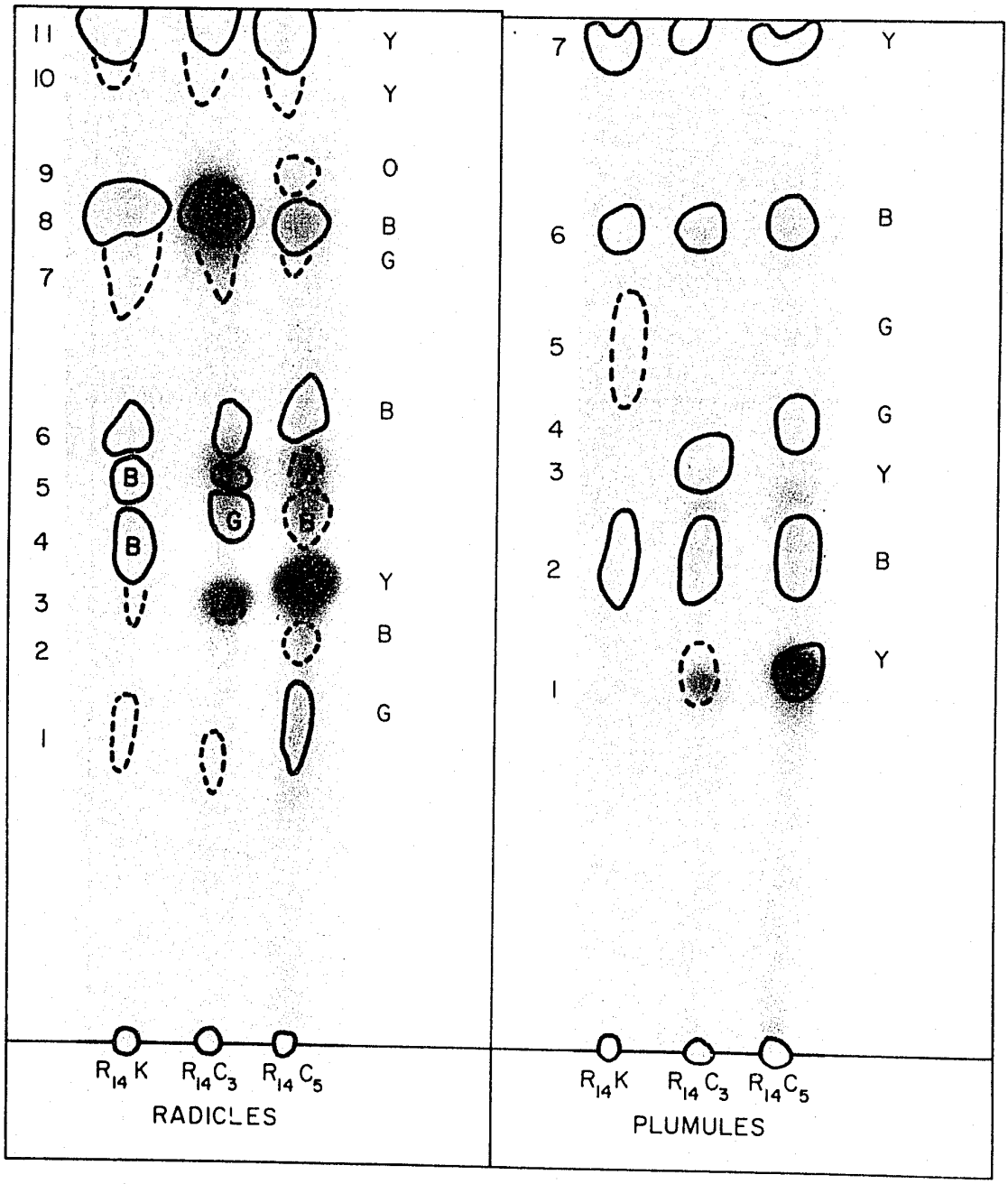
(b)
GPC = Glycerylphosphoryl choline
GPE = Glycerylphosphoryl ethanolamine
GPG = Glycerylphosphoryl glycerol
N.D. = Not detected
\+ = positive
\- = negative

PLATE III

(See Tables XX and XXI)

Legend

Photograph of autoradiographs of ^{32}P phospholipids of Rideau radicles and plumules superimposed on Rhodamine 6G stained chromatograms of these lipids following imbibition and vernalisation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R14K
R14C3
R14C5
RADICLES
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R14K
R14C3
R14C5
PLUMULES

TABLE XXII

Lipid Components of Rideau Scutellum (see Plate IV)

Spot Number	Rf Value	Rhodamin 6G Stain	^{32}P Labelling	Periodate Schiff	Ninhydrin Stain	Deacylated Product[a]	Tentative Identity of Components
1.	0.12	Blue	-	-	-	-	Unidentified
2.	0.21	Blue	+	-	-	IMP	Phosphatidyl inositol
3.	0.29	Blue	-	-	-	-	Unidentified
4.	0.36	Yellow	+++	-	-	GPC	Phosphatidyl choline
5.	0.44	Blue	-	-	-	-	Unidentified
6.	0.49	Blue	+	-	-	GPG	Phosphatidyl glycerol
7.	0.56	Yellow	++	-	+	GPE	Phosphatidyl ethanolamine
8.	0.64	Blue	-	-	-	N.D.	Diphosphatidyl glycerol
9.	0.72	Yellow	-	+	-	G-GAL	Monogalactoyl diglyceride
10.	0.78	Blue	++	-	-	-	"Lysophosphatidic acid"
11.	0.84	Yellow	-	-	-	-	)
12.	0.93	Yellow	-	-	-	-	) Neutral Lipids)

Abbreviations: (a)

IMP = Inositol monophosphate
GPC = Glycerylphosphoryl choline
GPG = Glycerylphosphoryl glycerol
GPE = Glycerylphosphoryl ethanolamine
G-GAL = Glyceryl galactose
N.D. = Not detected
\+ = present
\- = absent

It should be emphasized that in all the Marquis grain parts, ^{32}P incorporation with vernalisation was most marked in the "lysophosphatidic acid" component. By contrast, in all the grain parts of Rideau, ^{32}P incorporation with vernalisation was most marked in the lecithin component; this increased incorporation into lecithin apparently occurred at the expense of the "lysophosphatidic acid" component, even though the concentration of the latter changed only slightly with vernalisation.

(6) Changes in Fatty Acid Constituents of Lipids in Grain Parts

(a) Overall degree of unsaturation

Marquis - With regard to total saturated fatty acid components, net decreases were observed in the plumule, radicle and in the scutellum, while a slight net increase was detected in the endosperm-pericarp following vernalisation.

Cold treatment resulted in large increases in the proportions of total unsaturated fatty acids in all grain parts except the endosperm-pericarp where only slight changes were noted (Table XXIV). It should be noted that most of the lipids in the endosperm-pericarp were storage triglycerides whereas lipids in the other grain parts are largely membranes (Plates I and II).

It was also observed that the degree of unsaturation increased with vernalisation in all the grain parts except in the endosperm-pericarp (Table XXIV).

Rideau - Following vernalisation, net increases in the percent total saturated fatty acid components were noted in all the grain parts except in the plumule where a net decrease was observed. The greatest increases were noted in the radicle and scutellum.

Lipid Components of Rideau Endosperm-Pericarp (see Plate IV)

Spot Number	Rf Value	Rhodamin 6G Stain	^{32}P Labelling[a]	Periodate Schiff	Ninhydrin Stain	Deacylated Product[b]	Tentative Identity of Components
1.	0.10	Blue	+	-	-	IMP	Phosphatidyl inositol
2.	0.14	Blue	-	-	-	-	Unidentified
3.	0.21	Blue	-	-	-	-	Unidentified
4.	0.24	Yellow	+	-	-	G-GAL GAL	Unidentified Phospho-lipid (lyso PC ?)* + Digalactosyl diglyceride
5.	0.30	Blue	-	-	-	-	Unidentified
6.	0.36	Blue	-	-	-	-	Unidentified
7.	0.41	Yellow	+++	+	-	GPC	Phosphatidyl choline + Digalactosyl diglyceride
8.	0.49	Blue	+	-	-	GPG	Phosphatidyl glycerol
9.	0.56	Yellow	++	-	+	GPE	Phosphatidyl ethanol-amine
10.	0.60	Blue	-	-	-	-	Unidentified
11.	0.65	Yellow	-	+	-	G-GAL	Monogalactosyl di-glyceride
12.	0.72	Blue	-	-	-	-	Unidentified
13.	0.81	Blue	++	-	-	GPG	"Lysophosphatidic acid"
14.	0.87	Yellow	-	-	-	-	Neutral Lipids

Abbreviations: (a) + = present
- = absent

(b) IMP = Inositol monophosphate
G-GAL.GAL = Glyceryl galactosyl galactose
G-GAL = Glyceryl galactose. GPC = Glycerylphosphoryl choline
GPG = Glycerylphosphoryl glycerol
GPE = Glycerylphosphoryl ethanolamine
GPG = Glycerylphosphoryl glycerol.

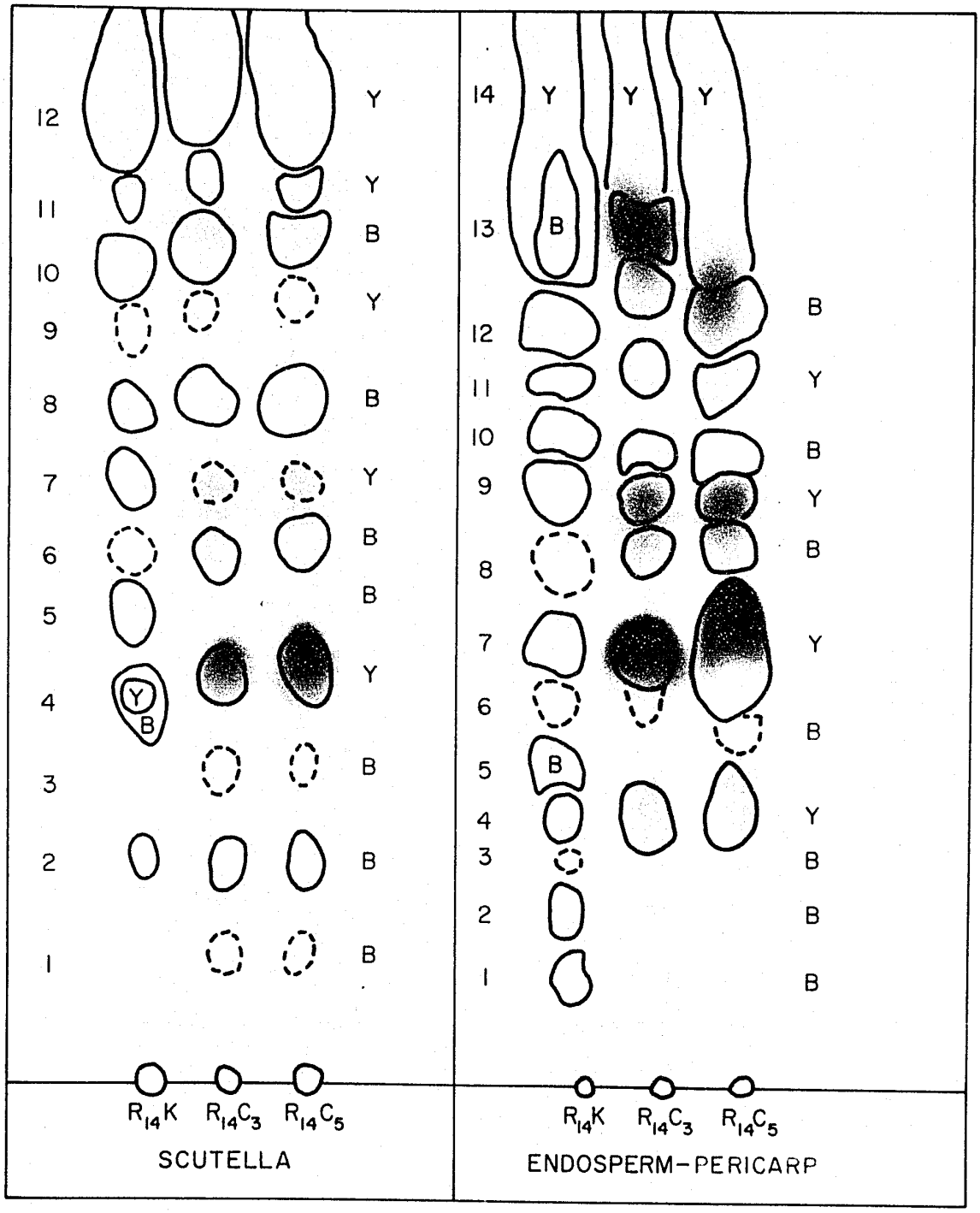
* Lyso P.C. = Lysophosphatidyl choline.

PLATE IV

(See Tables XXII and XXIII)

Legend

Photograph of autoradiographs of ^{32}P labelled phospholipids of Rideau scutella and endosperm-pericarps superimposed on Rhodamine 6G stained chromatograms of these lipids following imbibition and vernalisation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R14C3
R14C5
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R14C5
ENDOSPERM–PERICARP

The total percent unsaturated fatty acid components increased only in the plumule but decreased in the radicle and scutellum. These changes were reflected in the degree of unsaturation (Table XXIV). No changes were noted in the endosperm-pericarp.

(b) Individual fatty acids (Tables XXV-XXVII)

Palmitic acid (16:0) - Palmitic acid was the major saturated fatty acid component in the Marquis control plumule (Table XXV), radicle (Table XXVI) and scutellum (Table XXVII). Vernalisation apparently resulted in a decrease in the proportion of this acid in these three grain parts, but did not affect the proportion in the endosperm-pericarp (Table XXVIII).

This acid was a major component in all the fully imbibed Rideau grain parts. A net increase of this fatty acid with vernalisation was observed in all grain parts except in the plumule where a net decrease was noted (Table XXV).

Whereas the increases of this fatty acid in the scutellum (Table XXVII) and endosperm-pericarp (Table XXVIII) were gradual with chilling, it was observed that in the radicles (Table XXVI), cold treatment resulted in a decrease during the first three weeks of chilling. Thereafter, an increase was observed (Table XXVI).

Oleic acid (18:1) - This fatty acid increased in percentage with vernalisation in the radicle (Table XXVI) and scutellum (Table XXVII). In the Marquis plumule (Table XXV), a large increase was observed within the first four days of chilling (Table XXV) and this was followed by a very large percentage decrease during the next three days in the cold. A slight percentage decrease with vernalisation was noted in the Marquis endosperm-pericarp (Table XXVIII).

TABLE XXIV

Proportions of Total Saturated and Unsaturated Fatty Acids in the Grain Parts of Marquis and Rideau Wheats Following Imbibition and Vernalisation

M_5K	Plumule	Radicle	Scutellum	Endosperm
Total Sat. %	85.0	88.8	88.5	21.3
Total Unsat. %	11.8	11.1	11.4	78.6
Δ/mole	0.35	0.14	0.15	1.35
M_5C_4				
Total Sat. %	36.3	58.9	55.9	19.1
Total Unsat. %	54.5	40.6	43.9	80.9
Δ/mole	0.66	0.47	0.48	1.47
M_5C_7				
Total Sat. %	52.9	52.0	55.8	24.9
Total Unsat. %	46.5	47.4	44.0	74.5
Δ/mole	0.80	0.62	0.54	1.31
$R_{14}K$				
Total Sat. %	37.0	20.3	17.7	15.5
Total Unsat. %	62.8	79.5	82.1	84.3
Δ/mole	0.97	1.53	1.63	1.58
$R_{14}C_3$				
Total Sat. %	26.4	17.1	44.0	15.2
Total Unsat. %	73.5	82.8	55.9	83.6
Δ/mole	1.14	1.25	0.77	1.59
$R_{14}C_5$				
Total Sat. %	29.8	37.6	33.6	16.9
Total Unsat. %	70.1	62.3	66.3	82.9
Δ/mole	0.76	1.04	0.97	1.59

Total Sat. % = Total % of saturated fatty acid components.
Total Unsat. % = Total % of unsaturated fatty acid components.
Δ/mole = (degree of unsaturation) calculated thus:

1.0 x (% of monoene/100) + 2.0 x (% diene/100) + 3.0 x (% triene/100) obtained from GLC analyses [Tables XXV - XXVIII].

TABLE XXV

Fatty Acid Composition of the Plumules of Marquis and Rideau Wheats following Imbibition, Partial Vernalisation and Vernalisation (percentages)

Treatment	M_5K	M_5C_4	M_5C_7	$R_{14}K$	$R_{14}C_3$	$R_{14}C_5$
Fatty Acid [1]						
12:0				0.1		
13:0				0.6		
14:0				0.3		1.4
14:1		0.5				
15:0	2.4		6.2	0.4	2.3	3.1
15:1		0.3	1.8	0.5		
Unknown	3.1					
16:0	60.9	16.9	46.7	33.7	15.7	25.3
16:1					5.6	
16:2		4.5	28.3			
16:3		0.4	1.9			
18:0		19.4				
18:1	8.4	52.8	13.0	24.4	27.2	64.0
18:2	3.4		1.5	35.0	39.3	6.1
18:3					1.4	
Unknown		5.0	0.5			
19:0 *					8.4	
19:1 *				2.9		
20:0 *				1.9		
24:1 *	21.7					

[1] Abbreviation for fatty acids is n:x, where n is number of carbon atoms and x is number of double bonds.

* Tentative assignments based only on relative retention times on butanediol succinate polyester at 176°

TABLE XXVI

Fatty Acid Composition of the Radicles of Marquis and Rideau Wheats following Imbibition, Partial Vernalisation and Vernalisation (percentages)

Treatment	M_5K	M_5C_4	M_5C_7	$R_{14}K$	$R_{14}C_3$	$R_{14}C_5$
Fatty Acid[1]						
14:0		0.6	0.9	0.1	1.8	
14:1	1.2		0.4			
15:0		3.7	7.9	0.2	7.5	0.7
15:1	1.6	2.0	1.1		2.5	
16:0	76.1	52.3	39.4	19.7	7.8	22.5
16:1	1.8	3.7	4.8	0.2	16.1	8.9
16:2	3.3			0.5		
17:0		0.7				
17:1		0.1	0.7			
18:0	12.7					
18:1	3.2	27.3	32.6	12.2	35.7	11.7
18:2		4.2	7.8	60.7	14.6	41.7
18:3		1.1			13.9	
19:0 *				0.3		14.4
19:1 *				4.2		
20:0 *		1.6	2.1			
20:1 *		2.2		1.7		
22:0 *			1.7			

[1] Abbreviation for fatty acids is n:x, where n is number of carbon atoms and x is number of double bonds.

* Tentative assignments based only on relative retention times on butanediol succinate polyester at 176°

Cold treatment increased the proportion of this acid in the Rideau plumule and the scutellum, a very large percentage increase being noted in the plumule within three weeks of chilling, after which a decrease was observed (Table XXV).

Although a three-fold percentage increment was observed during the first three weeks of chilling, a net decrease was noted in the proportion of this fatty acid in the radicle (Table XXVI).

Linoleic acid (18:2) - In Marquis, linoleic acid formed the major fatty acid component in the endosperm-pericarp (Table XXVIII), but was a minor component in the other grain parts. Little change was observed in the concentration of this fatty acid in the endosperm-pericarp with vernalisation; a seven-fold increase in the proportion of this fatty acid with chilling was noted in the scutellum (Table XXVII).

In Rideau, linoleic acid was the major fatty acid constituent of all grain parts. Whereas chilling caused little or no change in the concentration of this fatty acid in the endosperm-pericarp, it apparently caused a decrease in the other grain parts, the most spectacular decrease being observed in the plumule (Table XXV).

Linolenic acid (18:3) - Trace amounts of this fatty acid were detected only in the partially and fully vernalised plumules of Marquis (Table XXV).

TABLE XXVII

Fatty Acid Composition of the Scutella of Marquis and Rideau Wheats Following Imbibition, Partial Vernalisation and Vernalisation (Percentages)

TREATMENT	M_5K	M_5C_4	M_5C_7	$R_{14}K$	$R_{14}C_3$	$R_{14}C_5$
FATTY ACID[1]						
14:0		0.7	0.3			
15:0	1.4	4.0	2.2		7.2	
15:1	2.6	3.7				
16:0	87.1	51.2	11.3	15.1	36.8	33.6
16:1		6.3		0.8	2.1	
16:2	1.8			0.6		
18:0			6.2			
18:1	4.8	30.2	21.4	16.6	32.3	35.2
18:2	2.2	3.7	15.6	61.7	21.5	31.1
19:0 *				2.6		
19:1 *				2.4		
20:0 *			27.5			
22:2 *			7.0			
23:0 *			8.3			

[1] Abbreviation for fatty acids is n:x, where n is number of carbon atoms and x is number of double bonds. * Tentative assignments based only on relative retention times on butanediol succinate polyester at 176°.

TABLE XXVIII

Fatty Acid Composition of the Endosperm-pericarp of Marquis and Rideau Wheats Following Imbibition, Partial Vernalisation and Vernalisation (Percentages)

TREATMENT	M_5K	M_5C_4	M_5C_7	$R_{14}K$	$R_{14}C_3$	$R_{14}C_5$
FATTY ACID[1]						
16:0	17.8	16.7	22.8	14.3	15.2	16.9
16:1	1.1	0.9	1.1			1.8
16:2					0.6	
18:1	18.5	13.7	16.3	10.9	9.5	8.5
18:2	56.8	66.2	57.1	71.0	71.7	69.5
18:3					1.8	3.1
19:0 *	3.5	2.4	2.1	1.2		
19:1 *	2.2			2.4		

[1] Abbreviation for fatty acids is n:x, where n is number of carbon atoms and x is number of double bonds.

* Tentative assignments based only on relative retention times on butanediol-succinate polyester at 176°.

IX. DISCUSSION

The present study highlights the fact that it is not valid to compare or contrast indiscriminately metabolic changes in one plant with another, even when morphologically similar tissues or plant parts are discussed. The practice of homologous comparison is of strictly limited use even when different varieties of the same species are discussed. Clear varietal differences have been noted between Marquis (spring) and Rideau (winter) wheat in all phases of my investigation, namely protein, amino acid, phosphate metabolism and fatty acid metabolism.

While these varietal differences might have been expected, it was surprising to find large variations in metabolism of the same grain variety, depending on the duration of the initial imbibition period, e.g. soluble protein and amino acid changes (cf. Tables III-VIII and Figs. II-V). Even after five weeks' chilling, during which period the grains were exposed to similar amounts of water, the initial degree of hydration of cellular constituents imposed a specific "stamp" upon subsequent metabolic reactions - even upon generations of cells which were not, themselves, exposed to the limited amount of water. That this pattern is even continued after germination has been shown by the recent studies of Ste. Marie (1969) investigating metabolic changes with root growth. The implication of these findings may give a deeper insight into the frequently observed differences in "growth potential" of crops derived from the same seed or grain source. My experimental work strongly indicates that the amount of water available during the very early stages of germination might be just as important in determining patterns of growth, as the availability of water subsequent to actual germination (i.e. when the root pierces the seed or grain coat).

(A) Changes in Proteins and Amino Acids

Vernalisation had little effect on the total concentration of wheat proteins (Table I). This study thus enforces the observations of Markowski et al (1962). The effects of vernalisation on spring and winter wheat grain proteins have to be looked for on more subtle bases. An indication of these has been presumably demonstrated by the change in solubility of grain proteins with vernalisation, most marked by the decrease in the endosperm of fully imbibed vernalised Rideau wheat (note that a similar change was not observed in the five-hour imbibed Rideau grain, nor in the Marquis spring wheat). The lesser amount of soluble proteins in the case of fully imbibed vernalised Rideau endosperm (Table II) is, most likely, due to the hydrolysis and utilisation of these proteins; this is suggested by the concomitant large increase in alcohol soluble free amino acids after vernalisation (Table III).

Changes in the insoluble protein fraction were also noted with vernalisation. The large increase in this fraction was strictly varietal and was observed only in the endosperm of Marquis wheat (Table II). This increase was paralleled by a marked decrease of alcohol soluble free amino acids with vernalisation (protein based data, Table III). The increase in insoluble proteins together with the decrease in alcohol soluble free amino acids suggest that vernalisation stimulates a synthesis of insoluble proteins in the Marquis endosperm.

The varietal differences in response to vernalisation are clearly demonstrated in these changes. Pauli and Mitchell (1960) reported an increase in total free amino nitrogen and total amide in the leaves of Pawnee winter wheat plants hardened under controlled conditions. Similar observations have been reported by Weinberger (1962) and Trione (1966) working with the leaves of spring and winter

wheat following vernalisation. The data obtained in the present study indicate that the concentration of some specific free amino acids also increased in the grains of Marquis and Rideau wheats with vernalisation. The amount of change with vernalisation was affected far more by the initial imbibition period, however, than by varietal differences (Tables IV and V).

Changes in the content of individual free alcohol-soluble amino acids in the non-vernalised and vernalised wheat grains were followed by Pavlov and Tyankova (1962) using chromatographic techniques. Weinberger (1962) noted that the first three leaves of Triticum vulgare L. variety Rideau, both vernalised and non-vernalised contain very little proline. The data of all these workers indicate that proline decreased with vernalisation. The present study, on the grain parts of both spring and winter wheats, using an amino acid analyser to identify the constituent alcohol soluble amino acids, are not in accord with these findings. Relatively large concentrations of proline were present in the vernalised embryo-scutella of all series (protein based data, Fig. IV). The results support and amplify those of Markowski et al (1962), Trione et al (1966) working with spring and winter wheat plants, and Kinbacher (1960) working with oat plants; these workers also observed a large increase in proline content with vernalisation.

Evidence accumulated using ^{14}C isotopes suggests that the mono-amino dicarboxylic acids are particularly important during germination (Kretovitch, 1966). The data obtained in the present study indicate that aspartic acid accumulated in the embryo-scutellum to a lesser degree than glutamic acid and showed very little change in the endosperm with vernalisation. Although the totalled acidic amino acid levels increased in both the embryo-scutellum and endosperm in all treatments, the greatest increase was noted in the embryo-scutellum.

The relatively high levels of glutamic acid and proline obtained in these studies are in agreement with the conclusion made by McConnell (1959) with ^{14}C marker studies on the maturing wheat grain. He suggested that a close metabolic relationship exists between glutamic acid, proline and arginine in the wheat plant. Transfer of nitrogenous constituents of the endosperm had been previously demonstrated during the germination of oats (Mer et al, 1963).

Working with ^{15}N labelled ammonium sulphate in different plants, Kretovitch et al (1966) noted that the highest concentration of ^{15}N was in glutamic acid. They also found that glutamic acid had a higher content of ^{15}N than aspartic acid and that the former underwent deamination and transamination more readily than the latter. Strict extrapolation of these results to the present study is obviously invalid; however, they may give broad indications of intermetabolic relationships. The data obtained in my own experiments are not decisive. They may be interpreted as evidence for the greater utilization of aspartic acid (hence its lower level) or as evidence of the more central utilization of glutamic acid (hence its higher content).

Steiner (1959) working with Endomycopsis vernalis observed that γ-aminobutyric acid ranked with alanine, glutamic acid and aspartic acid as one of the "key" amino acids which was most readily metabolised. In the Rideau and Marquis wheat grains, however, although the aspartic and glutamic acid levels increased after vernalisation in the embryo-scutellum of all series, γ-aminobutyric acid and alanine did not show any marked increases. As noted previously, differences in accumulation of metabolites is to be expected when different biological materials are used.

Respiratory proteins have been shown to contain a relatively high percentage of histidine (Haurowitz and Hardin, 1954). In plants, Chibnall (1939) has shown that the chloroplastic proteins of spinach contain a high histidine content. Since large amounts of these two types of protein must be synthesized to maintain the increasing respirtaory and photosynthetic apparatus of the developing seedling, the high proportion of histidine, noted only in the embryonic fraction of all the vernalised series, may be similarly related to its later utilization in photosynthesis.

It should be noted that although arginine accounted for the highest absolute concentration, with regard to basic amino acids, the percentage change of this amino acid is not as great as that of the other three basic amino acids (Table V).

Changes overridingly related to the varietal response to vernalisation are indicated only in the threonine fraction of Rideau grains (Table IV). Naylor and Tolbert (1958) observed that in most of the excised organs of sixteen species of higher plants studied, ^{14}C labelled aspartic acid was transformed to threonine. Billinski and McConnell (1957) found that in wheat plants metabolising labelled acetate, the distribution of ^{14}C was very similar in the carbon skeletons of aspartic acid and threonine. Perhaps the relatively low levels of aspartic acid noted here may be linked with the accumulation of threonine.

From the data presented, it can be seen that vernalisation leads to an increase in the concentration of both the total and some individual amino acids and amide contents (Tables III - V, VII and Figs. II - V).

It has been suggested that basic amino acids play leading roles in dormancy and sensecence, at least, being sensitive indicators of these stages of growth (Naylor, 1959). However, the point emphasized here is that it may not only be the actual concentrations of these amino acids which have morphogenetic significance, but also the ratio of the acidic to basic amino acids which may be important in the breaking of dormancy, and accelerated onset of flowering, in wheat plants.

On indication of the completion of vernalisation is given by Bassarskaya's test, cf. p. 3 (1934, 1936). This involves a shift in the iso-electric point of the proteins in the embryo. The decrease in the ratio of acidic to basic amino acids (Table VI) after vernalisation throws some light on this empirical test.

The greater availability of readily utilisable amino acids (and sugar residues) may produce the secondary factors which permit vernalised grains to grow and develop more rapidly than those which have not been vernalised.

The three methods used in this section to assess the changes in amino acid content, namely per microgram protein, mg. dry weight and grain part, clearly indicate that the base to which data are referred is of great importance. Similar parameters of change were noted when the data were referred to micrograms protein or grain part. As most grains and seeds have reserves of storage materials (mainly carbohydrates), the amount of which is directly dependent upon the nutritional level of the parent plant during grain (seed) maturation, in this case the dry weight of the grain or grain parts cannot be expected to be regarded as a constant. At best, in these circumstances, dry weight based data provide an arbitrary measure of comparison and should never be the only base to which data are referred.

(B) Changes in Phosphate Compounds

(1) Whole Grains - ^{32}P-orthophosphate is rapidly taken up by the Rideau whole grains during imbibition and is converted into organic water-soluble compounds at a linear rate (Fig. VI) between five and fourteen hours. ^{32}P-orthophosphate uptake apparently ceases after seven hours and the level of total ^{32}P remains constant up to 14 hours. Subsequent vernalisation of partially or fully imbibed Rideau grains resulted in an increase in percentage of water-soluble organic phosphates at the expense of the inorganic ^{32}P (Table XI and Fig. VIII). Of the several labelled organic phosphate compounds detected chromatographically, at least one was a glucose phosphate. It is of interest to note that Albaum and Umbreit (1943) have demonstrated a steady rise in the hexose content of oat embryos during germination.

The rather low incorporation of ^{32}P into the phospholipids of the Rideau wheat grains during the imbibition period (Table IX), relative to the high incorporation into organic water-soluble phosphates shows that, during imbibition, phospholipids are synthesized or turned over at a very slow rate, suggesting that little or no membrane synthesis occurs during this process. Vernalisation did not affect this slow synthesis or turnover of the phospholipids of Rideau whole grains (Fig. VII). David (1947), using histochemical procedures, reported no change in the lipids of corn with vernalisation. However, radioactive incorporation gives a more intrinsic picture of membrane changes. Thus, it would appear that for the grain as a whole, there is an overall net synthesis of membranes although at a slow rate, during vernalisation; this does not necessarily apply to each of the individual grain parts (see Discussion on p.102 (2) Grain Parts).

The nucleic acid data (Fig. VIII) suggest that, in the Rideau whole grain, there is a turnover or synthesis of total nucleic acids during vernalisation. This finding agrees with that of Konarev (1954) who noted that chilling produced marked increases in the RNA content ot winter rye and winter wheat. Markowski and Madej (1962) noted an increase in nucleic acids of spring and winter wheat germinated at 1.5°C.

Changes occurring in Marquis grains were on a much lower level of ^{32}P activity, primarily because of the lower initial uptake of ^{32}P-orthophosphate: this lower initial uptake level may be due to a difference in the metabolic state of the Marquis grain.

(2) Grain Parts - The loss of ^{32}P water soluble compounds (organic and inorganic) by all the grain parts of both wheat varieties may be partly due to conversion to non-water-soluble compounds, e.g. nucleic acids, phospholipids. Most of the loss, however, was due to a leaching of inorganic ^{32}P from the grains during the chilling period (Figs. IX and X).

The increase in ^{32}P labelled nucleic acids in all grain parts suggests a synthesis or turnover of these acids (Figs. IX and X). The data obtained in these studies support those of Siminovitch (1963) and Ku (1968) who reported increases in nucleic acid content of frost hardened black locust bark cells, and vernalised winter wheat roots, respectively. Finch and Carr (1956) have reported that chilling did not affect the RNA content of rye embryos. This result is at variance with subsequent studies including the present one, and particularly those of Wellensiek (1964, 1965) who demonstrated that it was the mitotic cells which respond to the cold treatment.

An increase in nucleic acid content of the embryo (both plumule and radicle) is not unexpected and can be directly related to the increased mitotic activity at the apices of the primary menistems. However, the increase in turnover of nucleic acids in the endosperm indicates a higher level of catabolic activity than is generally recognised and may be related to increased levels of cytokinins (purine derivatives) which in turn stimulate mitosis in the embryo.

With the exception of the Rideau plumule and endosperm-pericarp which showed ten-fold absolute increases in ^{32}P activity (Table XV), the uptake of ^{32}P by the phosphoprotein content of the various grain parts by both varieties did not change with vernalisation (Figs. IX and X).

(C) Changes in Lipids

(1) Phospholipids - In all the grain parts of both varieties, the major labelled lipid components were found to be phosphatidyl choline and the unknown phospholipid tentatively identified as "lysophosphatidic acid". Traces of ^{32}P were also detected in phosphatidyl ethanolamine in all vernalised grain parts (Plates I - IV) and in phosphatidyl inositol in the vernalised scutella of both wheats (Plates II and IV). In non-photosynthetic tissues such as seeds, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol and phosphatidyl inositol are the major phospholipids of membranes (Nichols and James (1964).

It would thus appear that vernalisation stimulates turnover or synthesis of membrane phospholipids.

Since rather low levels of ^{32}P were noted in phosphatidyl glycerol, phosphatidyl ethanolamine and phosphatidyl inositol, it would appear that these three phospholipids, relative to lecithin and "lysophosphatidic acid", may be more rapidly broken down or more slowly turned over in the vernalised grain parts of both wheat varieties. Since "lysophosphatidic acid" is only a trace component of membrane phospholipids, the question arises as to the high degree of labelling of "lysophosphatidic acid" and its role in the vernalisation process.

The most likely role it might play is that of an intermediate in lipid biosynthesis. Hajra and Agranoff (1968) (Scheme III[1]), have isolated a new phospholipid from guinea pig liver mitochondria which they have characterised as acyl dihydroxyacetone phosphate. They suggested that this new phospholipid was an intermediate in lipid biosynthesis, since if reduced enzymatically it gives lysophosphatidic acid which on acylation would form phosphatidic acid, the key intermediate in the biosynthesis of phosphatides and glycerides (Kennedy, 1961).

Three other pathways for the formation of phosphatidic acid are known. One (see Scheme III[2]) was elucidated by Kornberg and Pricer (1953). It involves the stepwise acylation of L, α-glycerophosphate with acyl-CoA. Another (Hokin and Hokin, 1959a) (Scheme III[4]) consists of the phosphorylation of a diglyceride with ATP, the reaction being catalysed by phosphokinase. The third pathway (Scheme III[3]) involves the phosphorylation of a monoglyceride to lysophosphatidic acid, the reaction being catalysed by monoglyceride phosphokinase (Pieringer and Hokin, 1962, a, b). This step is followed by the acylation of lysophosphatidic acid to phosphatidic acid with fatty acyl CoA catalysed by lysophosphatidase acyl-CoA transferase.

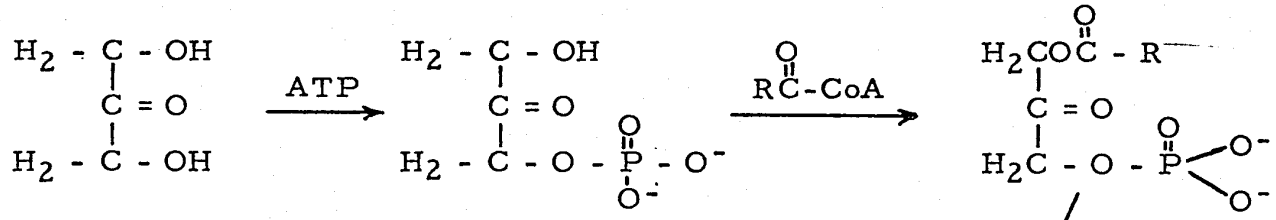
On the basis of its high degree of ^{32}P labelling, "lysophosphatidic acid" appears to be an important intermediate in synthesis of membrane phospholipids during vernalisation. In spinach leaves, the Kornberg-Pricer pathway (Scheme III [2]) has been demonstrated by Sastry and Kates (1966). Most likely this is the pathway that is operating in seeds and grain.

Since the incorporation of ^{32}P into phosphatidyl choline (lecithin) in all the grain parts of Rideau apparently occurred at the expense of ^{32}P labelling of "lysophosphatidic acid" (Plates III and IV), it is probable that lecithin biosynthesis in the Rideau grain takes place according to the pathway suggested by Bremer and Greenberg (1961) - see Scheme I, p. 10.

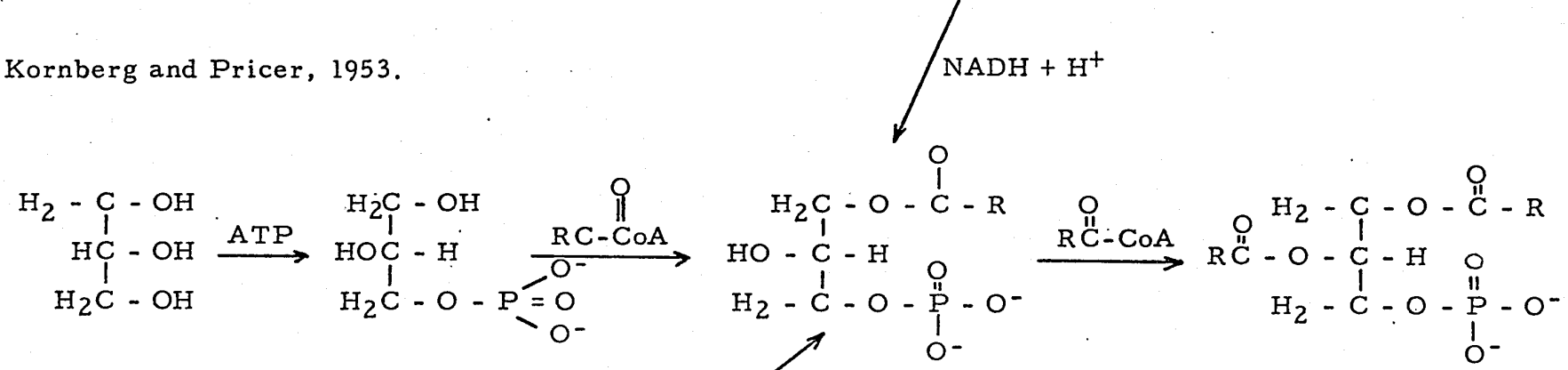
(2) Fatty Acids - Whereas palmitic and oleic acids are the major fatty acid components of the Rideau and Marquis embryo-scutellum axis lipids, oleic and linoleic acids are the major fatty acid components of lipids in the endosperm-pericarp of both varieties (Tables XXIV - XXVII). The data show that in both parts the relative proportions of these acids increase with the chilling treatment. Nelson et al (1963) noted that linoleic and oleic acids comprised the two major fatty acids of the bran, germ, endosperm and whole grain of Selkirk wheat. In other grain oil, the major fatty acids are linoleic, linolenic and palmitic acids (James and Nichols, 1966).

It is interesting to note that linolenic acid comprises a very small fraction of the lipids in the partially and fully vernalised Marquis plumules (Table XXV). This observation agrees with previous findings that linolenic acid is high in the photosynthetic tissues but low in non-photosynthetic tissues (Nichols and James, 1964; Chu and Tso, 1968).

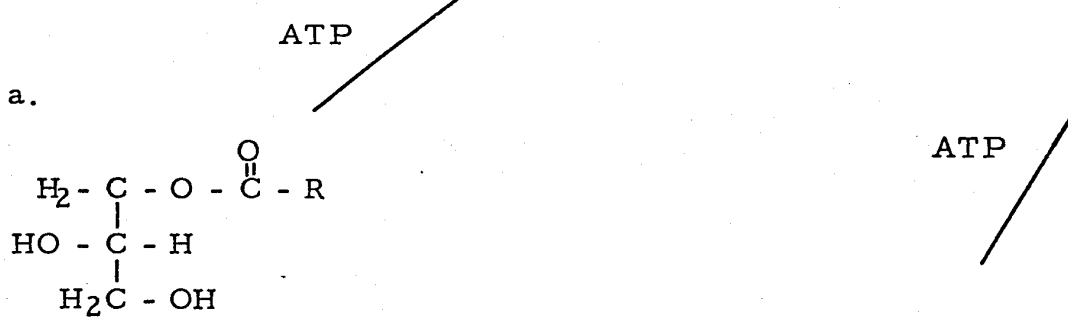
1) Hajra and Agranoff (1968)



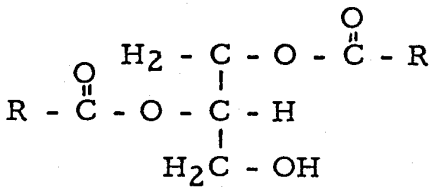
2) Kornberg and Pricer, 1953.



3) Pieringer and Hokin, 1962 a.



4) Hokin and Hokin, 1959



R = alkyl group

Scheme III. Four pathways of phosphatidic acid Biosynthesis.

Although linolenic acid was found in low concentrations, it was noted that the degree of unsaturation of the lipid fatty acid components increased with vernalisation in all the grain parts except in the endosperm-pericarp (Table XXIV). This observation supports the earlier finding that, in general, organisms growing at low environmental temperatures have more highly unsaturated fatty acids than those organisms growing at higher environmental temperatures (Gaughran, 1947).

(D) Summary of Discussion

It was observed that ^{32}P-orthophosphate incorporation into the various phosphate compounds - water soluble phosphates, phospholipids, phosphoprotein and nucleic acids - in the scutellum (Figs. X and XI) was more or less intermediate between the ^{32}P incorporation into these compounds in the embryonic axis (Figs. IX and XI) and the incorporation of ^{32}P into these phosphates of the endosperm-pericarp (Figs. X and XI). This observation clearly indicates that these morphologically distinct grain parts are also physiologically different. The scutellum is ontogenetically derived from the embryo. Its physiological role would seem to be related to a selective control of diffusion of metabolites between the endosperm and the embryo. Edelman et al (1959), using ^{14}C labelled glucose observed that the scutellum of wheat and barley was actually involved in the synthesis and selective transport of sugars derived from starch stored in the endosperm.

Classically, the endosperm is considered to contain "inert" reserve materials such as carbohydrates, fats and proteins which are hydrolysed by enzymes, the hydrolysates being utilised by the embryo during germination. However, the results obtained with ^{32}P labelling

suggest that these reserve materials are not metabolically inert since there is a marked increase in ^{32}P labelling of the phospholipid, nucleic acid and phosphoprotein fractions of the Rideau endosperm (Figs. IX and X) during vernalisation. Phosphoproteins such as casein from milk, ovo-vitellin from egg yolk and schthalin from fish sperm are known to be important in the nutrition of animal embryos and young growing animals. This role in animal metabolism is suggestive of a parallel role in young plant growth. It is therefore proposed that cold treatment evokes a physiologically important response in the endosperm; this response affects the development of the embryo. Thus, during vernalisation, the endosperm is a site of synthesis for some of the food reserves transported to the embryo.

The graphs in Fig. XI show that there is a mobilization of phosphate compounds from one grain part to another. In most cases, the changes in the scutellum tend to parallel those in the radicle which is the first organ to emerge during germination. It is therefore suggested that most of the metabolites which may have been transported from the endosperm through the scutellum find their way into the radicle and vice versa. This is particularly true for the ^{32}P water soluble compounds (Fig. XI C) of the Rideau grain. In effect, the earlier onset of mitosis in the radicle provides the metabolic sink along which a preferential diffusion gradient is established.

Differences noted between metabolic changes in the two varieties after complete imbibition are obviously varietal in origin. It is interesting to note that the vernalisation requirement of Rideau wheat is due to a recessive gene (Plessers, 1969). Therefore, the different metabolic patterns observed in these two varieties, during vernalisation, could be traced to this recessive gene. Thus, most likely, the sequence of metabolic events is as follows: during

imbibition, a rapid uptake of ^{32}P and a concomitant conversion into water-soluble organic phosphates occur; this conversion continues during vernalisation (Fig. VIII). In Rideau, during vernalisation, nucleic acids are turned over or synthesized; the latter is followed by a turnover or synthesis of phospholipids which are membrane components. In Marquis, by contrast, nucleic acid synthesis is not affected by vernalisation; phospholipid synthesis or turnover occurs during cold treatment.

X POSSIBLE MODE OF ACTION OF VERNALISATION TEMPERATURE ON WINTER GRAINS - A HYPOTHESIS

Numerous workers have concluded that metabolic changes observed in vernalised grains are not the direct response to cold treatment but rather are the by-products of the vernalisation process in the grains (Zech and Pauli, 1960; Trione et al, 1966). From the present results it would appear that the metabolic changes noted have been brought about directly by the vernalisation process.

It is proposed that cold acts initially at the gene level, the low temperature stimulating a rapid rate of nucleic acid synthesis. The data in Table XII and the observation by Wellensiek (1964, 1965), that mitotic cells "perceive" the chilling stimulus give strong backing to this proposal. In addition, Konarev (1954) observed that the RNA content increased in the wheat plumule as a result of cold treatment. This increase in nucleic acid synthesis would result in the formation of new proteins, e.g. soluble proteins (Table II) and enzymes. Teraoka (1968) detected new protein formation in wheat plumule after vernalisation. Increased protein degradation by the action of newly synthesised proteinases would account for the increase of alcohol-soluble free amino acids (Table III).

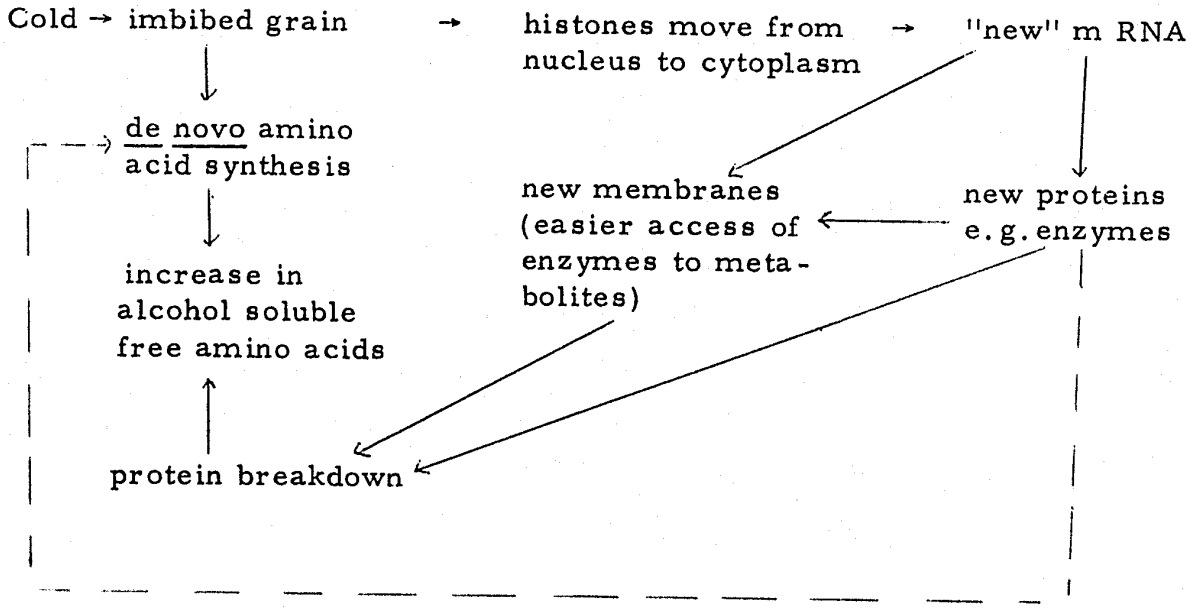
Wake et al (1968) and Teraoka (1968) respectively noted that the relative concentrations of some histone fractions of Trillium (lily) ovules and wheat embryos changed with cold treatment.

In addition, Ku (1968) observed that roots derived from vernalised Rideau and Marquis wheats contained increased amounts of cytoplasmic RNA and decreased amounts of histones bound in the nucleus. The removal of the histones may derepress metabolic pathways which are inoperative in control, non-vernalised tissues. In this

way one may envisage new metabolic reactions and different levels of metabolites. Changes in the amounts of one or more metabolites may serve as secondary factors in inducing further changes as a result of vernalisation.

The data in Tables XIII and XX-XXVII, as well as Plates I - IV show that vernalisation accelerates the turnover or synthesis of membrane lipids. These membrane changes might be related to the cell permeability changes after vernalisation noted by Filippenko (1936) and Chestakov and Sergeev (1937); they may also be related to the mitotic process which requires formation of new membranes.

Schematic Summary of the Metabolic Changes Resulting from the Action of Vernalisation:



XI. CLAIMS TO ORIGINALITY

1) Metabolic changes were observed in the individual grain parts - plumule, radicle, scutellum, and endosperm-pericarp - of both spring and winter wheats during imbibition and vernalisation.

2) Water uptake during the period of imbibition has been shown to affect the subsequent metabolism of amino acids or phosphates, in grain tissues, during the vernalisation treatment.

3) Demonstration that inorganic ^{32}P is rapidly taken up by Rideau wheat grains and converted into water-soluble organic phosphates during imbibition (Fig. VII); this conversion continues during vernalisation (Fig. VIII).

4) Demonstration that ^{32}P incorporation by nucleic acids was much greater, during vernalisation, in Rideau winter wheat than in Marquis spring wheat (Figs. VII, IX, X and XI B).

5) Demonstration that the rate of phospholipid synthesis or turnover, as evidenced by ^{32}P uptake, was much greater during the vernalisation of Marquis spring wheat than of Rideau winter wheat.

6) The chromatographic identification of labelled and unlabelled lipids before, during and after vernalisation in all the grain parts of spring and winter wheats (Plates I - IV).

7) Demonstration that phosphatidyl choline and "lysophosphatidic acid" were turned over or synthesized rapidly in all the grain parts of spring and winter wheats during chilling (Plates I - IV).

8) Demonstration that the high proportions of unsaturated fatty acid components (oleic and linoleic) in the Marquis and Rideau endosperm-pericarp did not, essentially, change during vernalisation. In the Rideau embryo-scutellum axis, the proportions of unsaturated fatty acid decreased during vernalisation while in the Marquis embryo-scutellum axis the proportions of unsaturated fatty acid increased.

XII. GENERAL CONCLUSIONS

1) The amount of water taken up by the grain affects the subsequent metabolism of amino acids (Table III) and water-soluble organic phosphates (Fig. VIII) in winter grains during vernalisation.

2) Vernalisation does not affect the total protein content of spring and winter wheats (Table I).

3) The total amino acid content of the spring and winter wheat grains increases as a result of vernalisation (Table III).

4) High proline values may be used as an index to denote the completeness of vernalisation of winter wheat grains (Table IV).

5) Inorganic ^{32}P is rapidly taken up by Rideau wheat grains and converted into water-soluble organic phosphates during imbibition (Fig. VI); this conversion continues during vernalisation (Fig. VIII). A slower incorporation of ^{32}P was observed in the phospholipid fraction (Table IX); this suggests that phospholipids are turned over during imbibition.

6) During vernalisation, a rapid synthesis or turnover of nucleic acids occurs in the grains and grain parts of Rideau wheat (Figs. VII, IX, X and XI B); by contrast, in the grains and grain parts of Marquis wheat turnover or synthesis of phospholipids is rapid (Figs. VII, IX, X and XI C).

7) Galactolipids are laregly confined to the endosperm-pericarp of wheat grains (Plates II and IV) but are also present in the Rideau scutellum as minor components.

8) "Lysophosphatidic acid" and phosphatidyl choline are turned over or synthesized rapidly in all the grain parts of both wheat varieties.

9) In general, the lipids of the endosperm-pericarp in both Marquis and Rideau wheats have very high levels of linoleic acid, oleic and palmitic acids being being relatively minor components; the proportions of these components showed little change with vernalisation. Whereas the fatty acid composition of the lipids in the fully imbibed Rideau embryo scutellum is similar to that of the Rideau endosperm-pericarp, the lipids of the fully imbibed Marquis embryo-scutellum contain very high levels of palmitic acid, and relatively low concentrations of linoleic and oleic acids.

XIII. LIST OF TABLES

Page no.

XIV. LIST OF PLATES

XV. LIST OF FIGURES

XVI. THESIS PUBLICATIONS

I. "Metabolic Changes associated with Imbibition and Vernalisation of Wheat Grains. I. Amino Acid-Amide Content". Can.J.Biochem., in press.

II. "The Effect of Imbibition and Vernalisation on the Uptake and Distribution of ^{32}P-orthophosphate into the Grains and Grain Parts of Spring and Winter Wheat", in preparation.

III. "Lipid Changes in the Grains and Grain Parts of Spring and Winter Wheat following Imbibition and Vernalisation", in preparation.

XVII. APPENDIX I.

Oven Dry Weights of the Various Grain Parts of Rideau and Marquis Wheats following Imbibition and Vernalisation (mg. per grain part).

	R_5K	R_5C_5	$R_{14}K$	$R_{14}C_5$	M_5K	M_5C_1
Wt. of embryo	1.2	1.3	1.3	1.1	0.8	1.1
Wt. of endosperm pericarp	36.9	33.9	37.0	40.5	34.7	37.0

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