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Enzyme Levels in Pea Seedlings Grown on Highly Salinized Media

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ABSTRACT

The levels of 18 enzymes were determined in leaves, stems, and roots of 11-day-old pea seedlings grown in a liquid medium or in the same medium containing, in addition, 5 atmospheres of either NaCl, KCl, Na_2SO_4, or K_2SO_4. Though the plants grown in saline media were stunted, the specific activities of the enzymes were the same in the given tissues of all plants. Also, the electrophoretic pattern of isozymes of malate dehydrogenase was not altered by growth of the plants in a saline medium. However, the isozyme pattern of peroxidase from roots of salt-grown plants was altered in that two of the five detectable isozymes migrated a little more slowly than those in extracts from nonsaline plant tissues.

Morphologically, the most typical symptom of saline injury to a plant is retarded growth, resulting in a stunted plant. For instance, in beans and radishes the surface area of a leaf from a plant growing in 3 atm of NaCl is approximately 50% less than that from a plant not subjected to salt insult (Ref. 18, and Nieman, R., personal communication). At the cellular level, even though the total surface area of a leaf from a salt-damaged bean or radish plant is about one-half that of a normal leaf, the number of cells per unit area is nearly the same under both conditions. Thus, one may conclude that salinity has somehow depressed cell division but has had little or no influence on cellular expansion in the plane parallel to the leaf surface (16).

Saline effects on the plant can be detected at the subcellular level also. The chlorophyll content of leaves is different (17), and the ratio of protein, DNA, and RNA synthesis is altered (Ref. 16, and Nieman, R., personal communication). It is possible, then, that other subcellular constituents might also be affected by salt injury to the plant. Therefore, a study of some enzyme patterns and levels in pea seedlings was undertaken to see if their specific activities are altered in any way by growth of the plant in a saline environment. The results are presented in this article, and the data indicate that the specific activities of the enzymes selected for this survey were not altered to any significant degree. It should be noted though, that Poljakoff-Mayber and her co-workers have studied enzyme levels in root tips from salt-damaged pea plants, and their results are not in agreement with those reported here (8, 12, 21, 22).

METHODS

Growth of Plants and Preparation of Extracts. Pea seeds (Alaska) were germinated in moist towels at approximately 25 C for 5 days. The seedlings were transferred to aerated one-half strength Hoagland's solution containing, if added, 1 atm of the salt under study (22.4 mM for NaCl and KCl, or 17.5 mM for Na_2SO_4 and K_2SO_4). The growth conditions were as follows: 16-hr photoperiod; daytime temperature of 27 to 28 C and night temperature of 25 C; and light intensities of 3.8×10^4 lux at plant height. Light was provided by a bank of fluorescent plus tungsten bulbs.

After 24 hr of growth in 1 atm of salt, salinity was increased to 2 atm. Subsequently, salt was added to the media in 1-atm increments at 12-hr intervals to a maximum of 5 atm. This concentration was selected as the maximal amount because plants growing in 5 atm of NaCl were roughly one-half the height of control plants. The salt concentrations were increased stepwise in order to minimize the effect of osmotic shock. In calculating the amount of salt needed to increase the osmotic pressure of each medium to the desired level, the contribution by the basic medium (one-half Hoagland's solution) of 0.4 atm is ignored.

After the seedlings had been growing for 3 days in liquid media, one-half of each medium was replaced with an equivalent volume of a fresh solution of the same composition and osmotic strength. In another 3 days the plants were harvested. The pH of the media throughout the entire 6-day period was maintained between 5 and 6. In the above media, the osmotic pressure refers to the concentration of the added salt. The osmotic pressure contributed by the basic medium (one-half Hoagland's solution) is ignored.

Both control plants and saline-grown plants had produced 3 to 4 nodes in the 11-day growth period; thus, the plants were assumed to be of nearly the same physiological age as well as chronological age. Except for differences in plant height and size of leaves, the plants grown under the several saline environments appeared healthy.

On the 11th day, all plants were harvested 4 hr after the lights were turned on. When harvested, the plants were separated into leaves, stems, and roots. The cotyledons and approximately 1 cm of the stem and root attached to them were discarded. Each tissue was weighed and placed in a chilled mortar along with a volume of cold buffer equal to the weight of tissue and a weight of acid-washed sand that was half that of the tissue. The composition of the buffer was: 0.1 M tris-chloride (pH 8.0), 1 M KCl, 0.01 M EDTA (sodium salt) (pH 8.0), and 0.4% β-mercaptoethanol. Tissues were macerated with a pestle until a relatively smooth suspension was obtained. The suspension was squeezed through two layers of cheesecloth, and the residue was discarded without further washing. The filtrate was centrifuged in the cold first at 750g for 15 min and then at 140,000g for 60 min. The pellets of each centrifugation were discarded while the final supernatant (soluble fraction) was used for the enzyme assays.

Enzyme Assays. All assays were done more or less as described in *Methods in Enzymology* (3) at 25 C. Because some enzymes were unstable in crude extracts, all assays were made

the same day as the extract was prepared, and the enzymes were always assayed in the same sequence. All reactions were followed by measuring the change in absorbance at 340 nm except for alkaline phosphatase and peroxidase, where the changes were measured at 410 and 470 nm, respectively. Extracts for each assay were diluted to a suitable concentration so that the rate of change in optical density did not exceed 0.020 optical density unit/min.

Glucose 6-Phosphate Dehydrogenase, 6-Phosphogluconate Dehydrogenase, Isocitrate Dehydrogenase, and Malate Dehydrogenase (Decarboxylating). One milliliter of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.05 M $MgCl_2$, 0.1 ml of 0.003 M TPN, and 1.5 ml of water were mixed with 0.2 ml of extract followed by 0.1 ml of either 0.02 M glucose 6-phosphate, 0.05 M 6-phosphogluconate, 0.01 M isocitrate, or 0.6 M malate. (Note the high concentration of malate needed to be sure the rate of the enzyme reaction was independent of substrate concentration.) All substrates were adjusted to approximately pH 7 before use.

Malate Dehydrogenase and Lactate Dehydrogenase. One milliliter of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.002 M DPNH, and 1.6 ml of H_2O plus 0.2 ml of extract and 0.1 ml of 0.001 M oxalacetic acid (not neutralized) or 0.1 M pyruvate (pH 7). The solutions of DPNH and oxalacetic acid were prepared fresh daily just before use.

Glutamate Dehydrogenase. One milliliter of 0.1 M tris-chloride (pH 8); 0.1 ml of 0.002 M DPNH, 0.1 ml of 1.0 M NH_4Cl, 1.5 ml of H_2O, 0.2 ml of extract, and 0.1 ml of 0.1 M α-ketoglutarate.

Glyoxylate Reductase. One milliliter of 0.1 M phosphate (pH 6.5), 0.1 ml of 0.002 M DPNH, 1.6 ml of H_2O, 0.2 ml of extract, and 0.1 ml of 0.3 M Na glyoxylate.

Pyruvate Kinase. 1.0 ml of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.002 M DPNH, 0.1 ml of 0.05 M $MgCl_2$, 0.1 ml of 0.1 M KCl, 0.1 ml of lactate dehydrogenase (approximately 1 enzyme unit/ml) 1.2 ml of H_2O, and 0.2 ml of extract. The rate of DPNH reduction was followed for 2 min after adding 0.1 ml of 0.05 M P-enolpyruvate, and the rate was measured again when 0.1 ml of 0.05 M ADP was added. The difference between the two rates was assumed to be the rate of the kinase reaction.

Hexokinase. One milliliter of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.003 M TPN, 0.1 ml of 0.05 M $MgCl_2$, 0.1 ml of glucose 6-phosphate dehydrogenase (approximately 1 enzyme unit/ml), 1.3 ml of H_2O, and 0.2 ml of extract. The rate of TPN reduction was measured first with 0.1 ml of 0.05 M ATP added to the reaction mixture and then also with 0.1 ml of 0.1 M glucose. The difference in rates was assumed to be the rate due to hexokinase.

Phosphoenolpyruvate Carboxylase. One milliliter of 0.1 M tris-chloride (pH 8), 0.1 ml of 0.2% β-mercaptoethanol, 0.1 ml of 0.002 M DPNH, 0.1 ml of 0.05 M $MgCl_2$, 0.1 ml of 0.5 M $NaHCO_3$, 1.3 ml of H_2O, 0.2 ml of extract, and 0.1 ml of 0.1 M P-enolpyruvate.

Phosphoenolpyruvate Carboxykinase. After the rate of DPNH oxidation was determined in the P-enolpyruvate carboxylase reaction mixture, 0.1 ml of 0.05 M ADP was added, and the rate of DPNH oxidation was again measured.

Aldolase. One milliliter of 0.1 M tris-chloride (pH 8.5), 0.1 ml of 0.003 M DPN, 0.3 ml of 0.17 M Na arsenate (pH 8.5), 0.1 ml of 0.2% β-mercaptoethanol, 0.1 ml of glyceraldehyde 3-phosphate dehydrogenase (approximately 1 enzyme unit/ml), 1.1 ml of H_2O, 0.2 ml of extract, and 0.1 ml of 0.3 M fructose-1,6-diP.

Glyceraldehyde 3-Phosphate Dehydrogenase. The same reaction mixture as described for aldolase was used except that the glyceraldehyde 3-phosphate dehydrogenase is replaced with aldolase (approximately 2 enzyme units/ml).

Glutamate-Oxalacetate Transaminase. One milliliter of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.002 M DPNH, 0.2 ml of 0.1 M α-ketoglutarate, 0.2 ml of 0.1 M L-aspartate, 1.3 ml of H_2O, and 0.2 ml of extract.

Glutamate-Pyruvate Transaminase. One milliliter of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.002 M DPNH, 0.2 ml of lactate dehydrogenase (approximately 1 enzyme unit/ml), 0.2 ml of 0.1 M α-ketoglutarate, 0.2 ml of 0.1 M alanine, 1.1 ml of H_2O, and 0.2 ml of extract.

Alkaline Phosphatase. One milliliter of 0.1 M tris-chloride (pH 7.5), 0.1 ml of 0.05 M $MgCl_2$, 1.7 ml of H_2O, 0.2 ml of extract, and 0.1 ml of 0.02 M *o*-nitrophenylphosphate.

Peroxidase. One milliliter of 0.1 M phosphate (pH 6.5), 0.1 ml of 0.05 M guaiacol and 0.1% H_2O_2 each, 1.7 ml of H_2O, and 0.1 ml of extract. The two substrates were prepared fresh just before use.

Protein Determination. Protein was measured by the Folin-Ciocalteau phenol method (15) after the crude extract had been treated to remove interfering substances (27).

Electrophoresis. Isozymes of malate dehydrogenase were separated by vertical starch gel electrophoresis and detected by staining as described previously (27). The isozymes of peroxidase were also separated by electrophoresis on vertical starch gel plates using the same buffer as for malate dehydrogenase separation. However, because peroxidase isozymes that move toward the cathode will migrate off the gel if the electrodes are connected to the gel in the usual manner, the connections were reversed. Therefore, for peroxidase isozyme electrophoresis the upper buffer reservoir was connected to the anode and the lower one to the cathode. Peroxidase was detected by flooding a gel slice with 30 ml of a freshly prepared solution of 0.2 M phosphate buffer (pH 5.5), 0.02 M guaiacol, and 0.02 M H_2O_2. Usually the stained isozyme bands were visible within 5 min and the stain persisted for several hours.

RESULTS AND DISCUSSION

Since the possibility exists that plant cell organelles and their associated enzymes might be affected by salinity in a manner different from that of "cytoplasmic" enzymes, it was decided to study enzyme patterns and levels in these two cell fractions

Table I. *Yield of Tissue from Pea Seedlings Grown in an Environment of 5 Atmospheres of Salt*

Measurements are the means of three different experiments and the deviations are standard deviations.

Salt	Leaves		Stems		Roots	
	Wet weight	Protein	Wet weight	Protein	Wet weight	Protein
	g/100 plants	*mg/g tissue*	*g/100 plants*	*mg/g tissue*	*g/100 plants*	*mg/g tissue*
Control	39 ± 4.1	9.8 ± 1.0	31 ± 5.6	2.6 ± 0.19	60 ± 15.3	1.7 ± 0.08
NaCl	20 ± 4.3	7.7 ± 0.37	15 ± 2.5	3.0 ± 0.26	41 ± 10.3	1.7 ± 0.26
KCl	29 ± 2.5	7.7 ± 1.0	21 ± 0.58	2.5 ± 0.33	57 ± 6.1	1.9 ± 0.08
Na_2SO_4	19 ± 4.1	9.4 ± 1.9	14 ± 4.2	3.5 ± 1.4	45 ± 8.3	1.7 ± 0.45
K_2SO_4	29 ± 6.6	9.4 ± 0.57	20 ± 5.3	2.7 ± 0.13	65 ± 20.9	1.7 ± 0.49

Table II. *Specific Activity of Enzymes from Pea Seedlings Grown in 5 Atmospheres of Salt*

All activities are in change in optical density units per minute. Activities are reported as averages of assays done in duplicate or triplicate. Each set of assays in the replicates was with extracts from plants grown at different times.

Enzyme from Plant Grown in	Activity per mg of Protein			Activity per g of Tissue		
	Leaf	Stem	Root	Leaf	Stem	Root
Glucose 6-phosphate dehydrogenase[1] (EC 1.1.1.47)						
Control	0.16 ± 0.01	0.28 ± 0	0.145 ± 0.015	1.65 ± 0.05	0.75 ± 0.05	0.25 ± 0.025
NaCl	0.21 ± 0.02	0.21 ± 0.04	0.18 ± 0.02	1.7 ± 0.1	0.65 ± 0.05	0.34 ± 0.04
KCl	0.16 ± 0.02	0.19 ± 0.02	0.15 ± 0.01	1.1 ± 0.1	0.43 ± 0.03	0.29 ± 0.04
Na_2SO_4	0.15 ± 0.02	0.23 ± 0.03	0.15 ± 0.03	1.5 ± 0.3	0.75 ± 0.15	0.27 ± 0.03
K_2SO_4	0.14 ± 0.02	0.20 ± 0	0.20 ± 0.05	1.4 ± 0.2	0.53 ± 0.03	0.30 ± 0.13
Malate dehydrogenase[2] (NAD) (EC 1.1.1.37)						
Control	15.8 ± 2.9	27.8 ± 8.2	31.5 ± 7.8	155 ± 29	73 ± 20	54 ± 13
NaCl	22.7 ± 3.0	26.5 ± 5.2	42.5 ± 3.5	175 ± 23	79 ± 19	71 ± 9
KCl	19.7.± 4.8	24.3 ± 5.4	26.2 ± 4.2	147 ± 23	63 ± 4	49 ± 7
Na_2SO_4	15.4 ± 2.4	14.8 ± 1.1	24.3 ± 0.8	142 ± 21	60 ± 20	45 ± 5
K_2SO_4	14.6 ± 2.3	19.9 ± 5.1	32.7 ± 9.5	139 ± 28	53 ± 12	50 ± 6
Peroxidase[1] (EC 1.11.1.7)						
Control	11.8 ± 0.7	23.3 ± 3.3	484 ± 84	103 ± 7	56 ± 10	775 ± 125
NaCl	19.4 ± 0.2	25.7 ± 0.7	566 ± 11	141 ± 1	90 ± 6	785 ± 13
KCl	11.7 ± 1.0	16.7 ± 1.7	405 ± 13	109 ± 11	50 ± 3	810 ± 15
Na_2SO_4	14.6 ± 1.7	25 ± 3	483 ± 53	170 ± 20	135 ± 20	1101 ± 130
K_2SO_4	13.8 ± 1.0	27 ± 2	380 ± 13	126 ± 25	75 ± 4	875 ± 28
6-Phosphogluconate dehydrogenase[1] (EC 1.1.1.44)						
Control	0.15 ± 0	0.21 ± 0	0.33 ± 0.02	1.5 ± 0.2	0.55 ± 0.05	0.55 ± 0.05
Isocitrate dehydrogenase (NADP)[1] (EC 1.1.1.42)						
Control	0.29 ± 0.01	0.55 ± 0.05	1.1 ± 0	3.0 ± 0.4	1.5 ± 0.1	2.0 ± 0.1
Malate dehydrogenase (decarboxylating)[2] (EC 1.1.1.40)						
Control	0.02 ± 0.01	0.22 ± 0.05	0.40 ± 0.10	0.18 ± 0.06	0.57 ± 0.12	0.68 ± 0.14
Glyoxylate reductase[1] (EC 1.1.1.26)						
Control	0.29 ± 0.05	0.18 ± 0.01	0.06 ± 0.02	2.9 ± 0.2	0.49 ± 0.07	0.12 ± 0.04
Glutamate-oxalacetate transaminase[2] (EC 2.6.1.1)						
Control	0.61 ± 0.04	1.2 ± 0.25	1.6 ± 0.22	6.0 ± 0.66	3.2 ± 0.86	2.70 ± 0.24
Glutamate-pyruvate transaminase[2] (EC 2.6.1.2)						
Control	0.25 ± 0	0.24 ± 0.07	0	2.4 ± 0.28	0.64 ± 0.23	0
Glyceraldehyde 3-phosphate dehydrogenase[1] (EC 1.2.1.12)						
Control	0.35 ± 0.08	0.54 ± 0.10	0.81 ± 0.10	3.5 ± 0.5	1.5 ± 0.1	1.4 ± 0.1
Aldolase[2] (EC 4.1.2.7)						
Control	0.78 ± 0.03	0.42 ± 0.04	0	7.7 ± 0.56	1.1 ± 0.09	0
Phosphoenolpyruvate carboxylase[1] (EC 4.1.1.31)						
Control	0.22 ± 0.02	0.26 ± 0.02	0.02 ± 0.2	2.0 ± 0.2	0.6 ± 0.05	0.03 ± 0
Alkaline phosphatase[2] (EC 3.1.3.1)						
Control	0.48 ± 0.10	1.1 ± 0.11	0.77 ± 0.04	4.7 ± 0.98	2.8 ± 0.29	1.3 ± 0.10

[1] Assays done in duplicate and deviations are the range of the results.

[2] Assays done in triplicate and deviations are standard deviations.

separately. Therefore, pea seedling tissues were extracted in a buffer containing a high concentration of KCl, which presumably should protect the integrity of the organelles, and the particulate fraction was removed by centrifugation at 140,000*g*. This study, then, is restricted to those enzymes remaining in the supernatant fraction.

The specific activities of 18 enzymes in the leaves, stems, and roots of seedlings grown in media containing 5 atm of NaCl, Na_2SO_4, KCl, or K_2SO_4 were measured and were compared to the activities of these enzymes in plants not exposed to high saline environments. The yield of each tissue and the protein content of the soluble fraction from these tissues are listed in Table I. The specific activities of three of the enzymes in terms of protein and in terms of weight of tissue in plants grown under each of the five environmental conditions are shown in Table II. Though the values fluctuated somewhat for all enzymes assayed, in no instance was there a noteworthy change in the level of any particular enzyme under any of the growth conditions. Therefore, only the specific activities of the enzymes from plants grown in the absence of added salt (control plants) are listed in Table II for the remaining enzymes assayed. The variations in value observed were not proportionately larger than those shown for glucose 6-phosphate dehydrogenase or malate dehydrogenase. Even if these variations are real and not due to experimental error, they did not seem large enough to account for the injury suffered by plants due to salinity. Enzymes assayed but not listed in Table II were lactate dehydrogenase (EC 1.1.1.27), glutamate dehydrogenase (NAD) (1.4.1.3), pyruvate kinase (2.7.1.40), hexokinase (2.7.1.1), and phosphoenolpyruvate carboxykinase (4.1.1.32). They were omitted because their activities in the pea seedlings were either absent or too low to be accurately measured.

Some enzymes, like glucose 6-phosphate dehydrogenase or isocitrate dehydrogenase, are members of energy-yielding systems, and changes in their quantities in cells would probably severely disrupt the control processes of the cell. Therefore, one would not expect the specific activity of enzymes with this function to be affected by salinity. Other enzymes probably could vary in the plant cell without necessarily having a lethal effect. One such enzyme is peroxidase. It has been shown in other plants that this enzyme may increase by as much as 3-fold as a result of treating plants with growth retardants (Refs. 5, 6 for literature review). Since plants exposed to salinity are smaller than "normal" plants, salts in high concentrations may be considered as growth retardants also and, consequently, may have the same effect on peroxidase as reported for the other chemical growth retardants. However, as indicated in Table II, no such change in specific activity was observed.

The pattern of isozymes of two of the enzymes studied above, peroxidase and malate dehydrogenase, was examined electrophoretically. The molecular heterogeneity of these two enzymes in pea plants is well documented (19, 23, 27, 28). It is possible that, though salinity may not alter the total activity of a certain enzyme in a plant cell, the relative amounts of its isozymes might be shifted. There is evidence that not only are the amounts of known peroxidase isozymes in pea stem (19, 23) and tobacco pith cells (5) shifted by changes in or interference with plant hormone content, but new isozymes may be formed as a result of this treatment. Therefore, crude pea tissue extracts were examined electrophoretically for isozymes of peroxidase (Fig. 1). The results with leaf and stem tissue extracts were exactly the same; therefore, the patterns obtained with stems are omitted from Figure 1.

Peroxidase isozymes that moved toward the cathode migrated as distinct bands. However, in the direction of the anode, the pattern was badly streaked, and isozymes could be detected only

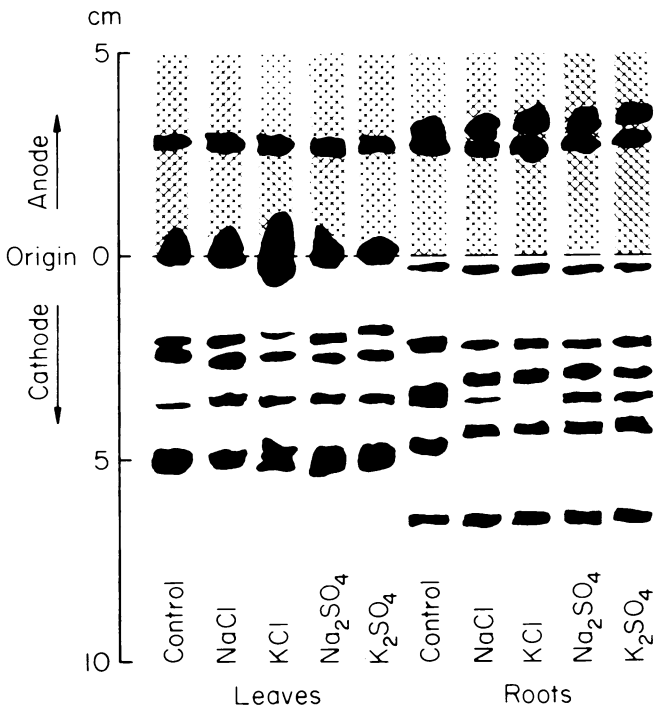

FIG. 1. Isozymes of the peroxidases of pea seedlings separated by vertical starch gel electrophoresis. Aliquots of extracts of leaves from pea seedlings grown under the various saline environments were placed in the first five slots as indicated; and extracts of roots from the same plants were placed in the next five slots.

as localized areas of more intense stain along the path of migration. Therefore, the extracts in the starch gel were subjected to electrophoresis in the reverse direction of that usually employed for vertical electrophoresis in that the bottom reservoir of buffer was connected to the cathode and the upper one to the anode. As shown in Figure 1, the isozyme pattern in leaves was the same whether or not the plants had been grown in salinized media. However, a small but definite variation in pattern was observable in peroxidase isozymes in roots. Most significantly, the changes that were detected in plants grown in saline environments were the same regardless of the salt used to establish that salinity. Five bands were detected in root extracts of nonsaline-grown plants that migrated in a cathodal direction. The isozyme that migrated 4.8 cm in control plant root extracts under these conditions was absent and replaced by one migrating slightly more slowly at 4.3 cm. Also the isozyme found at 3.7 cm in control extracts was greatly diminished or absent in the saline-grown plants, but an additional form was detected migrating 3.3 cm. All other isozyme bands in the saline-grown plants corresponded to bands present in nonsaline-grown controls. Since these shifts are so small, it is doubtful that they represent new catalytic proteins synthesized in response to the growth of the plants in a harmful environment. A more likely interpretation is that the new bands represent slight conformational changes that are unimportant enzymatically but do change their ability to migrate through the pores of the starch gel.

Another enzyme present in the pea plant in several separable isozymic forms is malate dehydrogenase (27, 28). The isozyme patterns of this enzyme in the extracts of the plants grown under the five environmental conditions were determined and compared. All isozymes migrated toward the anode in each tissue, and the patterns were exactly the same. Also, visual observation did not reveal any detectable differences in the intensity of any of

the isozyme bands. Unlike the results of Hason-Porath and Poljakoff-Mayber (8), no evidence of a band migrating toward the cathode could be detected.

The conclusion can be drawn from these results that the control mechanisms of the pea plant can keep intracellular reactions in balance even though the plant is growing in 5 atm of salt which, morphologically at least, is having some deleterious effect on the plant. Since it is known that in some species of plants enzyme content in growing tissue does fluctuate according to physiological age (4, 25), care was exercised to make sure that only young seedlings were used and at a time when all plants had produced the same number of nodes. Certain environmental parameters can control the level of a restricted number of enzymes. For instance, light can increase the level of enzymes as measured by specific activity (see Refs. 1, 2, 7, 9, 14, 20 for very recent work on this phenomenon), and perhaps even water stress, caused either by desiccation or by osmoticum changes, can influence enzymatic levels (11, 26). To the degree it has been studied here, however, salinity does not seem to be an external influence on the level of enzymes in pea plants, at least not for the enzymes reported in this article.

As mentioned before, Porath and Poljakoff-Mayber (21, 22) have reported fluctuations in cellular content of glucose 6-phosphate dehydrogenase, malate dehydrogenase, glucose 6-phosphate isomerase, phosphofructokinase, triosephosphate isomerase, and pyruvate kinase as a result of growing plants in environments of either NaCl or Na_2SO_4. Their results are completely at variance with the data presented here. They used a different strain of pea, grew the plant in vermiculite moistened with growth media, and limited their experimental material to root tips. Whether or not these differences in technique and methods account for the differences of results remains to be determined. Also, Hason-Porath and Poljakoff-Mayber do not find the stimulation of malate dehydrogenase *in vitro* by NaCl or other salts that has been reported previously (10, 13, 24, 27, 28, 29).

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