

Water Stress and Protein Synthesis

V. PROTEIN SYNTHESIS, PROTEIN STABILITY, AND MEMBRANE PERMEABILITY IN A DROUGHT-SENSITIVE AND A DROUGHT-TOLERANT MOSS¹

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ABSTRACT

The effects have been studied of water stress and desiccation on protein synthesis in the drought-tolerant moss *Tortula ruralis* and the drought-sensitive moss *Hygrohypnum luridum*. At any particular level of steady state water stress, the inhibition of protein synthesis was greater in *H. luridum* than in *T. ruralis*. Water stress-induced changes in the pattern of protein synthesis, as determined by the double label ratio technique, were minor in *T. ruralis*, but major in *H. luridum*. Proteins of both mosses were found to be stable during desiccation and subsequent rehydration. Changes in membrane permeability, as indicated by the leakage of amino acid, were observed during rehydration of desiccated moss and were dependent on the rate of desiccation. The leakage was small and reversible in *T. ruralis* but large and irreversible in *H. luridum*. Although *H. luridum* failed to recover from complete desiccation (80% loss in fresh weight), it was able to recover fully from steady state stress under conditions where a maximum loss of 55% in fresh weight was recorded.

distilled H₂O and used as experimental material. Procedures for collecting and preparing *T. ruralis* for experiments have been described previously (2). Gametophytes of *H. luridum* were collected from the banks of a stream near Banff, Alberta (4); they were kept in the glasshouse at 15 C during the day and 5 C during the night, and were profusely watered twice daily.

Administration of Desiccation. In order to administer slow desiccation, fresh moss of known weight was placed over a stirred saturated solution of ammonium nitrate (relative humidity 65%) for 24 hr. In both *T. ruralis* and *H. luridum*, a loss of 80% of original fresh weight was achieved. Rapid desiccation was administered by placing fresh moss of known weight on a single layer of cheesecloth which was in direct contact with activated silica gel particles contained in a closed dish. Within 20 to 30 min, a loss of 80% of original fresh weight occurred in both mosses.

Administration of Steady State Water Stress. Polyethylene glycol-6000² (Baker Chemical Co.) was used as osmoticum. Solutions with water potential (ψ_w) of -5, -10, -15, -20, -30, -40, -50, and -60 bars were prepared by using concentrations of 196, 289, 360, 420, 550, 680, 795, and 900 g/kg water, respectively. Solutions with ψ_w of -20 bars or higher were prepared according to Michel and Kaufmann (19); concentrations with ψ_w of less than -20 bars were obtained by extrapolation of their data, and therefore bear only an approximate relationship to the corresponding ψ_w . For this reason, reference to the actual concentrations of PEG used was considered to be more appropriate. Moss of known fresh weight was placed in 5 ml of PEG solution. In each experiment where steady state stress was used, moss was preincubated in the required PEG solution for at least 1.5 hr. We have determined that a steady state level of water stress, as indicated by a constant rate of amino acid incorporation into proteins, is achieved during this time.

Protein Synthesis. Leucine incorporation into proteins was used as a measure of protein synthesis. Duplicate samples of 250 mg fresh moss were preincubated in PEG solution for 1.5 hr and were then transferred to similar 5-ml solution containing 2 μ Ci/ml of [4,5-³H]leucine (43 Ci/m mol). When desiccated moss was used, dry weight of moss equivalent to 250 mg of fresh weight was rehydrated in distilled H₂O for 10 min before incubation with radioactive amino acid. It should be mentioned that desiccated *T. ruralis* regains more than 90% of its original fresh weight within 90 sec of rehydration, whereas *H. luridum* takes considerably longer to make a similar regain in fresh weight, but less than 10 min. Incorporation of amino acid was carried out for the time indicated in the respective experiments. After incorporation, the moss was washed with a cold (4 C) solution of 0.2 mg/ml carrier leucine and was extracted twice in 10 ml of 80% boiling ethanol containing 0.2 mg/ml leucine. Further steps in

The two mosses *Tortula ruralis* and *Hygrohypnum luridum* differ markedly in their responses to desiccation. For example; *T. ruralis* can be dried to 20% of its original fresh weight without impairing its ability to resume protein synthesis on rehydration (3). The aquatic moss *H. luridum*, on the other hand, loses the same percentage of original fresh weight during complete desiccation but does not resume protein synthesis on rehydration (4). Physiological and biochemical bases of this difference between these two mosses are not known.

Since air drying is an uncontrolled process, it is difficult to arrest and maintain it at an intermediate stage. We have made use of steady state water stress to administer a controlled loss of water from the moss tissue. We wished to compare the effects of water stress on the rate and pattern of protein synthesis in the two mosses. In view of their differential ability to recover from desiccation, it was considered useful to study the ability of *T. ruralis* and *H. luridum* to recover from steady state water stress. An attempt was also made to determine the stage at which *H. luridum* sustains irreversible damage. For this purpose, the stability of proteins and the distribution of protein and soluble radioactivities in the moss tissue were examined following desiccation and during rehydration.

MATERIALS AND METHODS

Plant Material. The apical 1 cm of the gametophytes of *T. ruralis* and *H. luridum* was cut and washed thoroughly with

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² Abbreviations: PEG: polyethylene glycol-6000; DOC, sodium deoxycholate.

the extraction of proteins were the same as described by Dhindsa and Cleland (10). Aliquots of the final NaOH extract were used to determine protein content by the method of Lowry *et al.* (18) and radioactivity by liquid scintillation spectrometry.

Leakage of Free Amino Acid. Fresh moss (7.5 g) was labeled with 200 μCi of [4,5- ^3H]leucine (38 Ci/m mol) contained in 40 ml solution for 1 hr. It was then washed with a 0.2 mg/ml solution of leucine at 0 to 4 C for 5 min before division into 30 samples of 250 mg each. Ten samples were subjected to slow desiccation and 10 to rapid desiccation. Two of the remaining 10 samples were immediately used to determine total protein content, protein radioactivity, and soluble radioactivity. The remaining fresh moss was placed in 0.2 mg/ml carrier leucine and duplicate samples were removed after 30, 60, 90, and 120 min. Total protein content, protein radioactivity, soluble radioactivity of the sample, and radioactivity that leaked into the bathing solution were determined. Similarly, samples which had been labeled and then desiccated were rehydrated for the same lengths of time as above. Total protein content, protein radioactivity, soluble radioactivity of the moss, and radioactivity that leaked into the bathing solution were determined here also.

Subcellular Distribution of Amino Acid Incorporation. Fresh moss (0.5 g) or desiccated moss with dry weight equivalent to 0.5 g fresh weight was used. After allowing amino acid incorporation to proceed for 1 hr, the moss was washed with distilled H_2O and its subcellular fractions were prepared as described elsewhere (7). A cell wall fraction was prepared by pelleting cell walls by low speed centrifugation and washing the pellet with grinding buffer. The cell wall pellet was extracted in sodium deoxycholate to prepare the DOC-soluble protein fraction (*i.e.* proteins dispersed by deoxycholate). Proteins which remained in the pellet constituted the DOC-insoluble fraction. The supernatant from the low speed centrifugation was centrifuged at 40,000g for 1 hr. The resulting supernatant constituted the soluble protein fraction (*i.e.* buffer-soluble). From the 40,000g pellet, the DOC-soluble and DOC-insoluble fractions were prepared as described for cell wall fractions. Aliquots of each of the two cell wall fractions and three cytoplasmic fractions were used to determine protein content and protein radioactivity.

Electrophoretic Separation of Proteins. One-half g of fresh moss or an equivalent weight of dry moss was used for all experiments where proteins were separated by polyacrylamide disc gel electrophoresis. After amino acid incorporation for 75 min, the moss was washed and homogenized in 3 ml of 0.1 M tris-Cl (pH 8) containing 0.5 M sucrose, 6 mM ascorbic acid, and 5 mM each of dithiothreitol and cysteine. The homogenate was centrifuged at 30,000g for 1 hr. The supernatant was used as the soluble protein extract, and a 100- μl aliquot of this was separated on polyacrylamide gels. Preparation of the gels and conditions of electrophoresis were according to Dhindsa and Cleland (10) except that a 1-cm stacking gel and a current of 2 mamp/gel were used. Electrophoresis was completed in 1 hr and 40 min for *T. ruralis* proteins and 3 hr 10 min for *H. luridum* proteins. Approximately the same amount of protein was loaded in the two cases.

Double Label Ratio Experiments. These experiments were conducted to determine the differential effects of water stress on the synthesis of soluble proteins. The rationale underlying the technique and procedures involved have been described elsewhere (10).

Fresh moss (0.5 g) was subjected to a steady state stress of -10 bars or -20 bars and was labeled with 100 μCi of [4,5- ^3H]leucine (38 Ci/mmol) in 5 ml solution for 75 min. Unstressed control moss (0.5 g) was labeled with 50 μCi of [U- ^{14}C]leucine (348 mCi/mmol). Another sample of control moss was labeled with [4,5- ^3H]leucine. In order to determine the effect of protein degradation on the protein pattern, one sample of ^3H -labeled moss was chased with carrier leucine (0.2 mg/ml) contained in

PEG solution. At the end of incorporation, a soluble protein extract was prepared from each sample.

Double label mixtures of protein extracts were prepared by mixing equal volumes of ^{14}C -labeled extract of control moss and ^3H -labeled extract from stressed moss. A control double labeled mixture was prepared by mixing equal volumes of ^{14}C -labeled and ^3H -labeled extracts, both from control moss. A 100- μl aliquot of each double labeled mixture was separated on polyacrylamide gel as described before.

A water potential lower than -20 bars was not used during double label experiments because of low incorporation into the protein bands separated on the gels.

Determination of Radioactivity in the Gels. After electrophoresis, gels were fixed in 12.5% trichloroacetic acid for at least 1 hr and then stained in 0.25% Coomassie brilliant blue in 7.5% acetic acid for 6 hr. Gels were destained in 7.5% acetic acid containing 5% methanol, scanned at 560 nm, and then cut into equal sized slices approximately 1 mm in thickness. Each slice was incubated in 0.5 ml of NCS (Amersham/Searle) for 2 hr at 50 C and 10 ml of scintillation liquid (Omnifluor, New England Nuclear) was added. Radioactivity in each slice was determined using a Nuclear-Chicago liquid scintillation spectrometer. In the case of double-labeled gels, the $^3\text{H}/^{14}\text{C}$ ratio for each gel slice was recorded.

RESULTS

Effects of Water Stress on Protein Synthesis. Inhibition of protein synthesis by different levels of steady state water stress in *T. ruralis* and *H. luridum* is shown in Figure 1. At each level of stress, the inhibition is considerably more in *H. luridum* than in *T. ruralis*. A mild water stress (-5 bars) does not appear to inhibit protein synthesis in *T. ruralis* whereas it causes nearly 40% inhibition in *H. luridum*. The difference between the two mosses is more pronounced at low water stress and lessens as the stress is increased. Protein synthesis in unstressed moss was considerably higher in *T. ruralis* (680 cpm/ μg protein) than in *H. luridum* (255 cpm/ μg protein). Thus, *H. luridum* has a lower rate of protein synthesis in the unstressed state and is also more sensitive to water stress than *T. ruralis*.

The effects of water stress (-10 and -30 bars) on protein

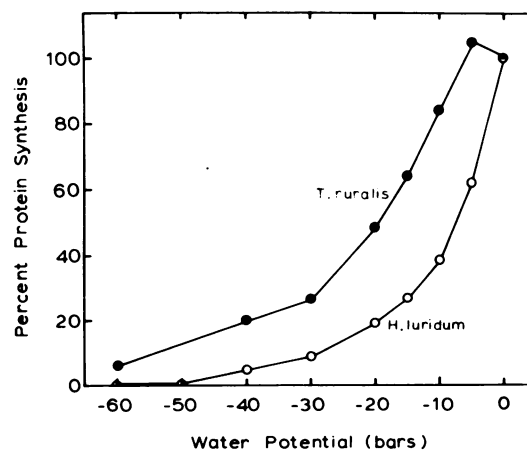


Fig. 1. Inhibition of protein synthesis by water stress due to decreasing water potential of bathing solution. Water potential was lowered by increasing concentrations of PEG. Samples of 250 mg moss were preincubated in 5 ml of respective solutions for 1.5 hr, then 10 μCi of ^3H -leucine were added. After 1 hr, each sample was washed in carrier leucine and incorporation of leucine into its proteins was determined. Values for 100% control were 680 cpm/ μg protein and 255 cpm/ μg protein for *T. ruralis* and *H. luridum*, respectively. Each value is a mean of two replicates.

synthesis of various subcellular fractions are shown in Table I. It can be seen that inhibition of incorporation in *T. ruralis* due to ψ_w of -10 bars is relatively less in the two DOC-soluble fractions (proteins dispersed by deoxycholate) than in the DOC-insoluble. The same stress (-10 bars) causes a strong inhibition of incorporation in all fractions of *H. luridum*, especially in the DOC-soluble cytoplasmic fraction where the inhibition is more than 80%. When ψ_w is lowered to -30 bars, inhibition of protein synthesis is nearly complete in all fractions of *H. luridum*. In *T. ruralis* also, increased stress is accompanied by an increased inhibition of incorporation into all subcellular fractions although inhibition is relatively less in the two DOC-soluble (wall and cytoplasmic) fractions than in others.

Separation of protein bands from undessiccated *T. ruralis* and *H. luridum* on polyacrylamide gels, and the distribution of radioactivity within them, are shown in Figure 2. It is clear that proteins of both mosses separate into about 30 bands during electrophoresis and that the incorporated radioactivity is well distributed among them.

The effects of two levels of water stress (-10 and -20 bars) on the pattern of synthesis of soluble proteins are shown in Figures 3 and 4 for *T. ruralis* and *H. luridum*, respectively. Figure 3 shows that water stress causes only minor changes in the double label ratios in *T. ruralis* treated with -10 bars of PEG (Fig. 3,A). The double label ratios are lower for proteins with greater electrophoretic mobility (slices 30-70). Increased stress (-20 bars) lowers the average double label ratio, but causes little variation in the ratios along the length of the gel (Fig. 3,B). The double label ratio plot from the control mixture (Fig. 3,C) is almost linear (as would be expected on theoretical grounds). In the case of *H. luridum* (Fig. 4), in contrast, both levels of water stress, -10 bars (A) and -20 bars (B) cause marked variations in the double label ratios along the length of the gel. The plot of double label ratios for the control mixture (C) is again nearly linear.

In order to determine whether the changes in pattern of protein synthesis during water stress were produced by protein breakdown rather than synthesis, a double label mixture was prepared which contained ^{14}C -labeled proteins from control moss and ^3H -labeled proteins from moss that had been labeled with ^3H -leucine and then chased with carrier leucine under a continuous stress of -20 bars. The double label ratio plot obtained (not shown) was similar to the corresponding curve C except that the average double label ratio was lower. Therefore, turnover was apparently taking place but it did not cause the observed variations in double label ratios. It appears that the differential inhibition of synthesis of proteins is the major cause of variations in double label ratios.

Since *H. luridum* fails to recover from desiccation (4), *i.e.* loss of 80% of its fresh weight, it was of interest to examine its ability to recover from a prolonged, steady state water stress (-60

Table I. Subcellular Distribution of Inhibition of Protein Synthesis by Various Levels of Steady State Water Stress in *T. ruralis* and *H. luridum*

Figures in parentheses represent per cent inhibition. Moss (0.5 g) was preincubated in respective PEG solutions for 1.5 hr and then incubated with $25 \mu\text{Ci}$ of $[4,5\text{-}^3\text{H}]\text{leucine}$ (42 Ci/m mole) in 5 ml solution for 1 hr. Each value is a mean of two replicates.

Treatment	Wall Fractions		Cytoplasmic Fractions		
	DOC - soluble	DOC - insoluble	Buffer soluble	DOC - soluble	DOC - insoluble
	cpm/ μg protein				
<i>Tortula ruralis</i>					
control	633(0)	232(0)	486(0)	310(0)	196(0)
-10 bars	615(2)	130(44)	425(13)	265(15)	105(46)
-30 bars	335(47)	69(70)	50(90)	125(60)	36(82)
<i>Hygrohypnum luridum</i>					
control	332(0)	105(0)	380(0)	292(0)	217(0)
-10 bars	165(50)	25(76)	168(56)	52(82)	94(57)
-30 bars	14(96)	2(98)	5(00)	6(98)	10(95)

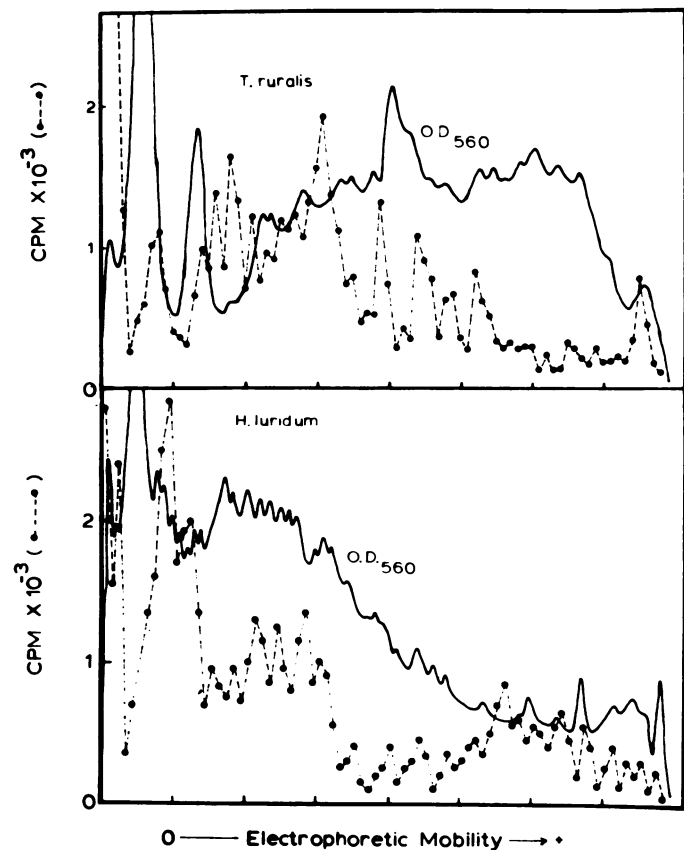


FIG. 2. Separation of soluble proteins by disc electrophoresis on polyacrylamide gels and distribution of radioactivity. Five hundred mg moss were incubated with $50 \mu\text{Ci}$ of ^3H -leucine (42 Ci/m mole) contained in 5 ml solution for 1 hr. An extract of soluble proteins was prepared as described in the text and a $100\text{-}\mu\text{l}$ aliquot was separated on 7.5% polyacrylamide gel.

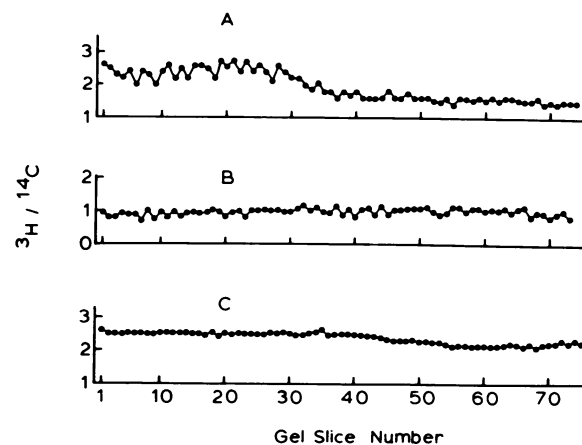


FIG. 3. Effects of water stress on the pattern of protein synthesis in *T. ruralis*. Five hundred mg control moss were labeled with either $50 \mu\text{Ci}$ ^{14}C -leucine or $100 \mu\text{Ci}$ ^3H -leucine. Samples of moss stressed with either -10 or -20 bars of water potential were labeled with ^3H -leucine. After 75 min of incorporation, soluble protein extracts were prepared. Double labeled mixtures were prepared by mixing equal volumes of ^{14}C -labeled extract from control moss and ^3H -labeled extract from either control moss or moss stressed with -10 or -20 bars of water potential. A $100\text{-}\mu\text{l}$ aliquot of each double labeled mixture was separated on polyacrylamide gel. The gel was cut into 1-mm slices and $^3\text{H}/^{14}\text{C}$ ratio in each was determined. A: ^{14}C -labeled extract of control + ^3H -labeled extract of control moss; B: ^{14}C -labeled extract of control + ^3H -labeled extract of stressed (-10 bars) moss; C: ^{14}C -labeled extract of control + ^3H -labeled extract from stressed (-20 bars) moss.

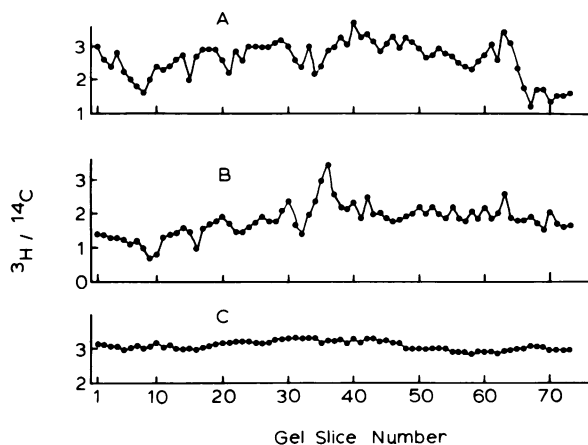


FIG. 4. Demonstration that water stress causes major qualitative changes in the pattern of protein synthesis in *H. luridum*. Protocol of experiment and explanation of symbols are the same as in Figure 3.

bars). Moss samples (1 g of each) were incubated in PEG solution (-60 bars) for 6 hr and the loss in fresh weight determined as described elsewhere (9). In order to monitor their ability to recover, as indicated by the rate of amino acid incorporation into proteins, 250-mg samples of fresh moss were incubated in PEG solution (-60 bars) for 6 hr, thoroughly washed, and then placed in distilled H₂O. At different times, duplicate samples were taken, washed, and incubated for 20 min in 10 μ Ci [4,5-³H]leucine (38 Ci/mmol) contained in 5 ml of solution. Unstressed moss was used as the control. After incorporation, specific radioactivity of the extracted proteins was determined. *T. ruralis* and *H. luridum* lost, respectively, 50 and 55% of their fresh weight under the administered steady state water stress. Figure 5 shows that *H. luridum* was able to recover completely from this degree of water stress, although its recovery was slower than that of *T. ruralis*. This ability of *H. luridum* to recover from steady state water stress (i.e. loss of about 55% of its fresh weight) is in sharp contrast to its inability to recover from complete desiccation (i.e. air drying) during which it loses about 80% of its fresh weight.

Stability of Proteins during Desiccation. Total protein content does not seem to change much during rapid or slow desiccation of both *T. ruralis* and *H. luridum* (Table II). As the labeled fresh moss is allowed to desiccate slowly, the specific activity of proteins increases, indicating that protein synthesis continues for some time during drying of both mosses. It should be noted that during desiccation of *T. ruralis*, the decrease in soluble radioactivity is greater than the increase in protein radioactivity, indicating a net decrease in the total radioactivity in the tissue. This decrease in the total radioactivity in the tissue is more pronounced in slowly desiccated than in rapidly desiccated *T. ruralis*. A similar but less pronounced decrease in total radioactivity occurs during slow, but not during rapid, desiccation of *H. luridum*. This decrease is apparently due to a slow decarboxylation of leucine (Malek and Bewley, unpublished) and the consequent removal of leucine from the amino acid pool and the entry of its products into other metabolic pathways.

Stability of Proteins during Rehydration. It was considered useful to examine the total protein content and specific activity of proteins during rehydration of a prelabeled desiccated moss. For this purpose, replicate samples similar to those from which data in Table II were obtained were rehydrated in the presence of carrier leucine. Carrier leucine was used because the experiment was primarily designed to study protein turnover during desiccation and rehydration. After regular time intervals, samples were removed and their total protein content, protein radioactivity, soluble radioactivity, and the radioactivity in the bath-

ing solution (leaked out from the tissue) were determined. Total protein content did not show any consistent change during rehydration (Table III). Specific activity increased in *T. ruralis* but not in *H. luridum*. It should be remembered that at zero time these samples contained similar amounts of protein and soluble radioactivities to those given for corresponding treatments in Table II. The increase in specific activity of proteins during rehydration of prelabeled desiccated *T. ruralis* apparently means that the leucine pool in the dried moss must be saturated and that the carrier leucine does not get in to replace it all. For this reason, a turnover study was not possible. However, it is noteworthy that the total protein content did not decrease during rehydration.

During rehydration, radioactivity leaked out of the cells into the bathing solution; the amount of leakage was different in the two mosses and depended on whether the moss was rapidly or

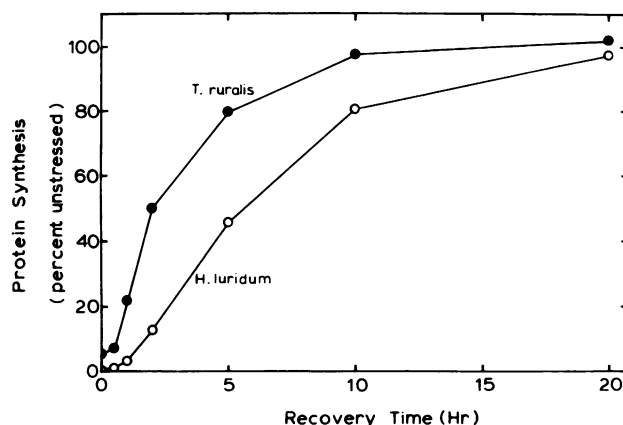


FIG. 5. Recovery of protein synthesis following a 6-hr treatment with -60 bars of water potential. At zero time, moss was removed from PEG solution and allowed to recover in distilled H₂O. At different times during recovery, 20-min incorporation of amino acid was carried out. Similar incorporation in unstressed moss was used as 100% control which was 180 cpm/ μ g protein and 65 cpm/ μ g protein, respectively, in *T. ruralis* and *H. luridum*. Each value is a mean of two replicates.

Table II. Distribution of ³H-Leucine Radioactivity during Desiccation of Prelabeled *T. ruralis* and *H. luridum*

For experimental protocol see under "Materials and Methods." Each value is a mean of two replicates.

Desiccation Treatment	Total Protein	Protein Specific Activity	Protein Radio-activity	Soluble Radio-activity	Total Radio-activity
	mg	cpm/ μ g	cpm $\times 10^{-6}$	cpm $\times 10^{-6}$	cpm $\times 10^{-6}$
<i>T. ruralis</i>					
Fresh (control)	5.25	148	0.78	2.8	3.5
Rapidly dried	4.75	143	0.68	2.1	2.8
Slowly dried	4.88	196	0.96	1.5	2.5
<i>H. luridum</i>					
Fresh (control)	5.18	100	0.52	1.8	2.3
Rapidly dried	4.84	110	0.54	1.8	2.3
Slowly dried	5.14	125	0.64	1.5	2.1

Table III. Changes in Protein Content and Specific Activity during Rehydration of Prelabeled, Desiccated Moss

Each sample was equivalent to 250 mg fresh weight. Figures in parentheses represent cpm/ μ g protein. Each value is a mean of two replicates. SD: slowly dried; RD: rapidly dried.

Rehydration Time (min)	Protein Content (mg)			
	<i>Tortula ruralis</i>		<i>Hygrohypnum luridum</i>	
	SD	RD	SD	RD
0	4.88 (196)	4.75 (143)	5.14 (125)	4.84 (110)
60	4.75 (206)	4.68 (158)	5.00 (110)	4.20 (112)
120	4.75 (240)	4.70 (185)	4.85 (115)	4.50 (105)

slowly dried. The changes in total radioactivity contained in the tissue (protein plus soluble) and in the bathing solution during rehydration are shown in Figures 6 and 7 for *T. ruralis* and *H. luridum*, respectively. Figure 6 shows that total radioactivity in the tissue decreases on rehydration for 30 min in slowly dried, and for 60 min in rapidly dried *T. ruralis*. After these times, radioactivity in the tissue starts increasing again. Radioactivity in the bathing solution increased for 30 min and 60 min, respectively, for slowly and rapidly dried *T. ruralis*. After these times

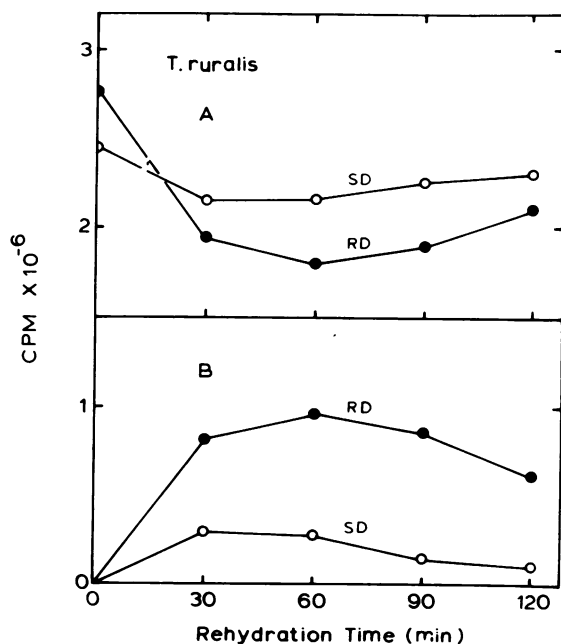


FIG. 6. Changes in total radioactivity in the moss (A) and in radioactivity leaked into the bathing solution (B) during rehydration of pre-labeled, desiccated *T. ruralis*. Each value is a mean of two replicates. SD: slowly dried; RD: rapidly dried.

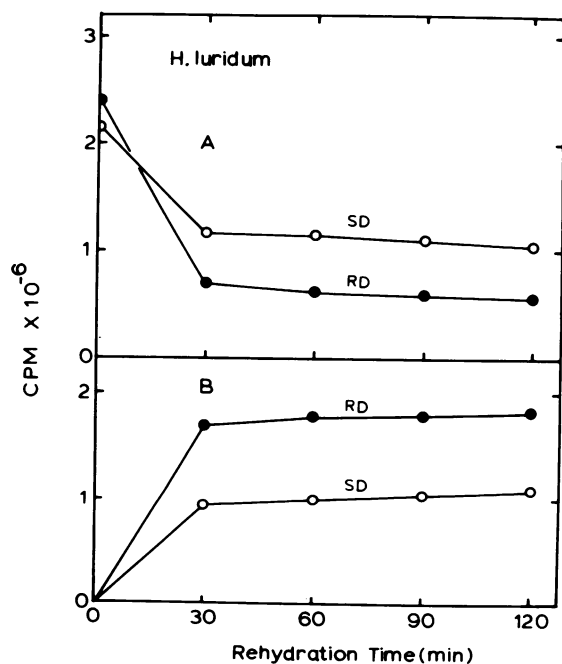


FIG. 7. Changes in total radioactivity in the moss (A) and in radioactivity leaked into the bathing solution (B) during rehydration of pre-labeled, desiccated *H. luridum*. Each value is a mean of two replicates. SD: slowly dried; RD: rapidly dried.

of rehydration, the radioactivity in the bathing solution starts decreasing. We have determined that the radioactivity that leaks into the bathing solution is trichloroacetic-acid-soluble. It is clear from Figure 6 that the initial increase and subsequent decrease in the radioactivity in the bathing solution are reciprocally related to the initial decrease and subsequent increase in the total radioactivity of the tissue. The difference in the amount of leakage of radioactivity from slowly and rapidly dried *T. ruralis* is worthy of note. After 1 hr of rehydration, radioactivity leaked from slowly dried and rapidly dried *T. ruralis* was, respectively, 18 and 45% of the soluble radioactivity present in the tissue at zero time. When labeled fresh moss was placed in a solution of carrier leucine, 15% of its soluble radioactivity leaked into the solution. Thus, there is little difference in membrane permeability between fresh and slowly dried *T. ruralis*. We also found that the observed decrease in total radioactivity of the tissue was entirely due to a decrease in the soluble radioactivity. The subsequent increase in total tissue radioactivity was largely due to an increase in protein radioactivity. These results further support the observations presented in Table III and show that protein synthesis and leakage of soluble radioactivity occur simultaneously.

Total radioactivity of the tissue also decreased when slowly or rapidly dried *H. luridum* was rehydrated and a corresponding amount of radioactivity leaked into the bathing solution (Fig. 7). The leaked radioactivity represents a soluble component of the tissue radioactivity and is not due to degradation of proteins (Table III). The decrease in total radioactivity of the tissue and increase in the radioactivity in the bathing solution were not reversed with time. At the end of 1 hr of rehydration, radioactivity in the bathing solution accounted for about 70 and 95% of the zero time soluble radioactivity present in slowly dried and rapidly dried *H. luridum*, respectively. Leakage from labeled fresh *H. luridum* was found to be 16% of its soluble radioactivity. Thus, there is little difference in membrane permeability between fresh *T. ruralis* and fresh *H. luridum*.

DISCUSSION

It has been reported that on rehydration following complete desiccation, *T. ruralis* is able to reform polysomes and resume protein synthesis (3), whereas *H. luridum* is unable to do so (4). The biochemical and/or biophysical bases underlying this difference between these two mosses are not clearly understood. Results presented here show that both quantitatively (Fig. 1) and qualitatively (Figs. 3 and 4), protein synthesis is modulated more by water stress in the drought-sensitive *H. luridum* than in the drought-tolerant *T. ruralis*. Even moderate water stress of -10 bars causes a strong inhibition of protein synthesis in *H. luridum*. In several subcellular fractions, this inhibition is equal to, or greater than, the inhibition caused by -30 bars in *T. ruralis* (Table I). Since *H. luridum* fails to recover from complete desiccation, *i.e.* following an 80% loss of fresh weight (4), but does recover completely from a steady state stress of -60 bars, *i.e.* following a 55% loss of fresh weight (Fig. 5), it is apparent that *H. luridum* has to lose more than 55% of its fresh weight in order to sustain permanent, irreversible damage. This was also suggested in a previous study (4).

It is apparent from our studies that the differential responses of *T. ruralis* and *H. luridum* to desiccation are not due directly to differential stability of their proteins during desiccation and subsequent rehydration.

Leakage of solutes from dry seeds, pollen, and other plant tissues during rehydration is a well documented phenomenon and is considered to be a marker or measure of membrane damage (14, 17, 21). It is accepted that such leakage during rehydration of the dried tissues reflects membrane damage, in particular to the plasmalemma (14), although damage to other membrane systems *e.g.* endoplasmic reticulum, mitochondrial,

chloroplastic and vacuolar membranes, cannot be ruled out. Changes in organelle morphology (20, 22) and function (5, 6), and disruption of organelle membranes due to water stress (23) have been reported; it is doubtful whether such damage would be reflected by leakage into the medium surrounding the cells unless the integrity of the plasmalemma itself was also in some way altered. Leakage of radioactivity from *T. ruralis* is more pronounced following rapid desiccation than following slow desiccation (Fig. 6). It is possible that the moss membranes may undergo certain reversible conformational changes during desiccation in order to retain their integrity. Such protective conformational changes are perhaps only partially accomplished during rapid desiccation due to speed of dehydration and cessation of cellular events. Slow desiccation alleviates this restriction. In the resistant conformation, membrane may also be less susceptible to attack by hydrolytic enzymes on rehydration. A correlation between membrane organization and susceptibility to phospholipase attack has been reported for rat erythrocytes (12).

It has been previously suggested (11) that the primary action of water stress might be to disrupt membrane-bound polysomes by causing a shrinkage of membranes, thereby reducing the number of attachment sites for polysomes. Certainly, there is a reduction in membrane-bound polysomes in response to water stress (1), and there is a loss of polysomes which is not mediated through increased ribonuclease activity during water stress or desiccation (8, 9, 15, 16). On rehydration of slowly dried moss, where there is apparently only limited membrane damage, there are probably enough polysome attachment sites to allow sufficient protein synthesis for the resumption of normal cellular activities. Extensive membrane damage would restrict resumption of protein synthesis. As might be expected, the rate of protein synthesis during rehydration of *T. ruralis* is greater following slow desiccation than following rapid desiccation (13). The damage to *H. luridum* membranes during desiccation and subsequent rehydration is apparently too extensive to allow protein synthesis to effect its repair.

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