UC Berkeley
UC Berkeley Previously Published Works

Title
OSMOTIC REGULATION OF α-AMYLASE SYNTHESIS AND POLYRIBOSOME FORMATION IN ALEURONE CELLS OF BARLEY

Permalink
https://escholarship.org/uc/item/38d4p16p

Journal
Journal of Cell Biology, 59(2)

ISSN
0021-9525

Authors
Armstrong, Judith E
Jones, Russell L

Publication Date
1973-11-01

DOI
10.1083/jcb.59.2.444

Peer reviewed

OSMOTIC REGULATION OF α-AMYLASE
SYNTHESIS AND POLYRIBOSOME FORMATION
IN ALEURONE CELLS OF BARLEY

JUDITH E. ARMSTRONG and RUSSELL L. JONES

From the Department of Botany, University of California, Berkeley, California 94720

ABSTRACT

Water stress inhibits the gibberellic acid (GA₃)-induced synthesis of α-amylase in aleurone layers of barley (*Hordeum vulgare* L.). Electron microscope evidence indicates that the effect of water stress induced by 0.6 M solutions of polyethylene glycol (PEG) is to reduce the binding of ribosomes to the endoplasmic reticulum. This was confirmed by sucrose density gradient centrifugation of polyribosome preparations from stressed cells. The reduction in polyribosome formation does not result from reduced ribosome activity as measured by [³H]peptidylpuromycin formation. Thus, calculation of percent active ribosomes shows that osmoticum has little effect on the ability of ribosomes to incorporate puromycin into nascent protein. Water stress does not cause a marked decrease in the total RNA level of aleurone cells. Estimates of total RNA in postmitochondrial supernatant fractions from stressed cells show only a reduction of 8–9% relative to the control. Membrane synthesis measured by [¹⁴C]choline incorporation is depressed by 15% in cells stressed with 0.6 M PEG for 2.5 hours.

INTRODUCTION

Seeds of the grasses are characterized by the presence of a secretory tissue, the aleurone layer which surrounds the starchy endosperm. During germination the aleurone layer secretes several hydrolytic enzymes which serve to mobilize the stored substrates within the endosperm. This process of substrate mobilization has been shown to be triggered by gibberellins produced in the embryonic axis of the seed (26, 31). It is now established that several gibberellins including gibberellic acid (GA₃) induce the *de novo* synthesis of α-amylase (10), protease (15), and B-1,3-glucanase and ribonuclease (4) in isolated aleurone layers of barley. These hydrolytic enzymes are formed at different times after GA₃ treatment but they are all secreted from the aleurone cell and are active in the breakdown of endosperm reserves. During germination, the solutes produced in the endosperm are absorbed by the scutellum and are transported to the growing regions of the embryo.

Although gibberellins act as the initiators of hydrolase synthesis, enzyme production is also regulated by the osmotic pressure exerted by the end products of enzyme action. Thus glucose concentrations of 0.3 M are found in the endosperm of germinating seeds while the total solute concentration reaches levels of 0.6 M (20). These osmotically active solutes inhibit hydrolytic enzyme production by exerting water stress on the aleurone cells. The ability of various other osmotica to inhibit hydrolytic enzyme synthesis in isolated aleurone layers has also been demonstrated. Polyethylene glycol

(PEG) and mannitol at concentrations of 0.6 M cause an 80% inhibition of GA₃-induced α-amylase formation (19).

Evidence is accumulating that water stress affects enzyme levels in many plants. Bardzik and Marsh (3) showed that the levels of two out of three enzymes studied in maize seedlings under water stress decreased markedly. Several workers have related the stress-induced changes in enzyme levels to the conversion of polyribosomes to monoribosomes (13, 14, 35).

This study reports on an investigation into the effects of water stress on polyribosome formation in aleurone cells of barley. Isolated aleurone layers were stressed by incubation in 0.6 M PEG at various times of GA₃ treatment. The effects of the osmoticum were assessed by electron microscopy and by determining the effects of stress on polyribosome formation, ribosome activity, and membrane synthesis.

MATERIALS AND METHODS

Tissue Preparation

Barley (*Hordeum vulgare* L. c.v. Himalaya) seeds were deembryonated, surface sterilized for 20 min in a 10% solution of commercial bleach, and imbibed on sterile sand for 3 days (22). Aleurone layers were removed from imbibed seeds and incubated in a medium containing 1 mM sodium acetate buffer (pH 4.8), 20 mM $CaCl_2$, and 5 μM GA₃ (7). PEG (average molecular weight 400) was added to a final concentration of 0.6 M either with, or at specified times after, GA addition.

Electron Microscopy

Tissue pieces were fixed in a mixture of 3% glutaraldehyde and 2% formaldehyde, buffered with 100 mM sodium cacodylate (pH 7.0) for 24 h, and postfixed in 2% osmium tetroxide buffered with 50 mm sodium cacodylate (pH 7.0) for 6 h. After washing three times with 100 mM sodium cacodylate buffer (pH 7.0) the fixed tissue was stained in 0.5% aqueous uranyl acetate for 5 h at 4°C then dehydrated in ethanol and propylene oxide before embedding in an Epon resin mixture (21). Sections were stained with saturated aqueous uranyl acetate followed by Reynolds' lead citrate and viewed in a Zeiss EM 9 A or Siemens Elmiskop 1 A microscope.

Aleurone tissue was also stratified by ultracentrifugation before fixation. This technique serves to concentrate organelles in specified regions of the cell on the basis of relative density differences (5, 18). Aleurone layers were cut into pieces approximately 5 mm² and allowed to sediment to the bottom of a water-filled cellulose nitrate centrifuge tube. This procedure ensures that centrifugal force is always from the endosperm side to the seed coat side of the tissue. Tissue pieces were centrifuged in a SW 50.1 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 34,000 rpm for 90 min. Fixation and processing of the tissue for electron microscopy was carried out as described above.

Chemical Assays

α-Amylase activity in incubation media and tissue extracts was determined colorimetrically using the starch-iodine procedure (22). Aliquots of the incubation media were incubated with the substrate without prior purification. Tissue extracts were obtained by grinding aleurone layers with 100 mM sodium acetate buffer (pH 4.8) containing 20 mM $CaCl_2$ in a glass TenBroeck homogenizer. The homogenate was centrifuged at 2,000 g to remove cell debris and the supernate was used directly for enzyme assay. Total protein was determined using the method of Lowry et al. (25) using crystalline bovine serum albumin as standard.

RNA content of aleurone cells was determined on nucleic acids prepared from postmitochondrial supernatant fractions using the methods of Schneider (33). The purified RNA was determined colorimetrically with the method of Mejbaum (27). The method of Warburg and Christian (36) was used for the determination of RNA in microsomal pellets.

Uptake and Incorporation of [¹⁴C]Choline

70 aleurone layers were incubated with GA₃ for 9 h, then transferred to a medium containing 10 mg casein hydrolyzate and 5 μCi of [¹⁴C]choline chloride (specific activity 8.25 Ci/mol), and kept on ice for 10 min to facilitate equilibration of label. Incubation was initiated by transferring them to a water bath at 25°C and oscillated at 100 cycles/min for 20 min. The tissue was washed with 100 ml of water for 5 min then transferred in groups of 35 into 10 ml of a medium containing 1 mM sodium acetate buffer (pH 4.8), 20 mM $CaCl_2$, 5 μM GA₃, and, where appropriate, 0.6 M PEG. After a further incubation period of 2.5 h at 25°C with shaking, the tissue was rinsed with water and a microsomal preparation prepared. Tissue was ground in 3 ml of a buffer containing 250 mM sucrose, 100 mM HEPES (pH 7.55), 50 mM KCl, 2 mM magnesium acetate, and 7 mM 2-mercaptoethanol and the homogenate was centrifuged three times at 0°C. The first two centrifugations were carried out in a Beckman J-21 centrifuge using a JA-21 rotor. The supernate of the first centrifugation at 4,000 rpm for 10 min was centrifuged for a further 10 min at 12,000 rpm. The

second supernate was decanted and spun for 18 h at 40,000 rpm in a Beckman SW 50.1 rotor at 0°C. The resulting microsomal pellet was suspended in 10% TCA, filtered on a Whatman glass fiber filter, and washed with 50 ml of cold 5% TCA containing 1 μM carrier choline chloride. Uptake of label into both acid-soluble and -insoluble fractions was determined on 0.1-ml aliquots of the initial homogenate.

Radioactivity in all fractions was determined by counting in a Beckman model LS-150 scintillation spectrometer. The glass fiber filters containing acid-insoluble counts were dried at 70°C and placed directly in fluor, while acid-soluble radioactivity was determined on aliquots mixed with Aquasol (Nuclear-Chicago, Des Plaines, Ill.) before counting.

Polyribosome Isolation

The procedures described by Evins (8) were followed for polyribosome isolation from aleurone layers. 25 layers were homogenized in 3 ml of the buffer described above for the isolation of microsomes. The homogenate was centrifuged in a JA-21 rotor for 10 min at 4,000 rpm to pellet cell debris and the resulting supernate centrifuged at 12,000 rpm for 10 min at 0°C. The second supernate was decanted and 0.3-0.4-ml aliquots were layered over 0.3-1.0 M isokinetic sucrose gradients as described by Noll (30). After centrifugation the gradients were displaced in an upward direction by pumping 50% sucrose through the bottom of the tube using a syringe pump (Harvard apparatus) at a speed of 0.82 ml/min. Samples were monitored continuously at 254 nm using a Chromatronix 230 UV minitor (Chromatronix Inc., Berkeley, Calif.) connected to a Varian model 20 recorder with a chart speed of 0.67 inches/min (Varian Associates, Palo Alto, Calif.).

Formation of [³H]Peptidylpuromycin

The reaction between [³H]puromycin and polyribosome-bound peptidyl-tRNA was used as a measure of the number of active ribosomes. A polyribosome preparation was prepared as described above with the exception that the second supernate was layered on a discontinuous sucrose gradient of 0.6-1.6 M and centrifuged in a SW 50.1 rotor at 40,000 rpm for 4 h at 0°C. The pellet was incubated at 37°C in 0.5 ml of a mixture containing 37.7 μM HEPES (pH 7.55), 18.8 μM KCl, 50 μM magnesium acetate, 7.3 mM 2-mercaptoethanol, 5 mM ATP, 50 μM GTP, 1 mM phosphoenolpyruvate, 10 μg pyruvate kinase, and 5 μCi of [³H]puromycin (specific activity 1.11 Ci/mmol). The reaction was terminated by addition of 5 ml of 10% TCA, filtered on a Whatman glass fiber filter and washed with 50 ml of 5% TCA containing 50 μM carrier puromycin. The filter was dried at 70°C and placed directly in fluor for count-

ing. The percent of active ribosomes was calculated from the incorporation of [³H]peptidylpuromycin into the acid-insoluble fraction (37). The amount of RNA in each of the microsomal pellets used for the determination of active ribosomes was measured spectrophotometrically by $A_{260}:A_{280}$ ratios (36).

RESULTS

The effect of the addition of osmoticum on the production of α-amylase from five aleurone layers incubated in 5 μM GA$_3$ for 14 h is shown in Table I. The addition of PEG for the last 2.5 h of GA$_3$ treatment produces a 42% inhibition of α-amylase production, and, when added for the last 5 h of GA$_3$ treatment, amylase production is inhibited 57%. If PEG is added at the same time as GA$_3$ (14 h total) an 81% inhibition of enzyme production results. It is presumed there is similar inhibition of the production of other hydrolytic enzymes with water stress as demonstrated by Jones and Armstrong (20).

Preliminary observations of aleurone cells with the electron microscope indicated that water stress caused a marked change in the number of ribosomes associated with the surface of the endoplasmic reticulum (ER). In order to obtain a more definitive estimate of the amount and appearance of the ER, cells were stratified by ultracentrifugation. Centrifugation of whole aleurone cells is an ideal tool for cytological analysis of these cells since they possess organelles of markedly different densities. Thus, as much as 30% of the volume of the cell is occupied by the dense protein bodies or aleurone grains, and a similar volume of the cell

TABLE I

Effect of Osmoticum on the Production of α-Amylase in Barley Aleurone Layers

Treatment	Amylase/five layers	% control
GA$_3$ 5 μM 14 h	55	100
GA$_3$ 11.5 h + GA and 0.6 M PEG 2.5 h	32	58.0
GA$_3$ 9.5 h + GA and 0.6 M PEG 5 h	24	43.5
GA$_3$ + 0.6 M PEG 14 h	10	18.2
H$_2$O	11	19.0

Five aleurone layers were incubated in acetate buffer with 5 μM GA$_3$ for 14 h. The osmoticum was added at various time intervals as indicated and the total amylase units were measured. Average of nine replicates.

is occupied by the less dense lipid bodies or sphero-somes (Fig. 1). The result of centrifugation is to produce cells where the ER, mitochondria, glyoxysomes, and nucleus are stratified in a discrete zone in the center of the cell while the protein bodies migrate centrifugally and the lipid bodies centripetally (Figs. 1 and 2).

The ER of cells treated with GA is characterized by the presence of numerous ribosomes along its surface (Fig. 3). The ER of cells treated with PEG during the last 2.5 h of GA_3 treatment however is characterized by a marked reduction in ribosome number (Fig. 4). Exposure of cells to osmotic stress for longer time periods results in a further reduction in ribosomes on the ER (Fig. 5). In addition to reducing ribosome number stress periods of 5 h also cause the ER to fragment, producing smaller vesicular components (Fig. 5). These vesicular components are devoid of ribosomes and consequently possess a reduced density causing them to stratify in a more centripetal area of the cell (Fig. 5).

Attempts were made to quantitate the ribosomes on the surface of the ER by counting the number of ribosomes appearing in random 0.2 μm lengths of membrane cross section. This procedure is a

valid form of quantitation in centrifuged cells since stratification avoids the selection of particular ER stacks by placing all of the ER in the same region of the cell (Figs. 1 and 2). Estimates of ribosomes from control and stressed tissue are shown in Table II. It is clear that duration of water stress affects the association of ribosomes with the ER.

The effects of stress on both α-amylase formation and ribosome attachment are also reversible (Table II and Fig. 7). It is clear that the effects of stress are not completely reversible over a 2.5-h time period, however longer recovery periods do allow for complete restoration of enzyme production (19).

Polyribosome Isolation

Polyribosomes were isolated from aleurone layers incubated for 13 h in either 5 μM GA_3 or 5 μM GA_3 with 0.6 M PEG added during the last 4 h of GA_3 treatment. Polyribosome profiles from GA_3-treated and -stressed aleurone tissue are shown in Fig. 6. It is clear that water stress converts the polyribosomes to the monomeric form (Fig. 6). Incubation of polyribosomes from GA_3-
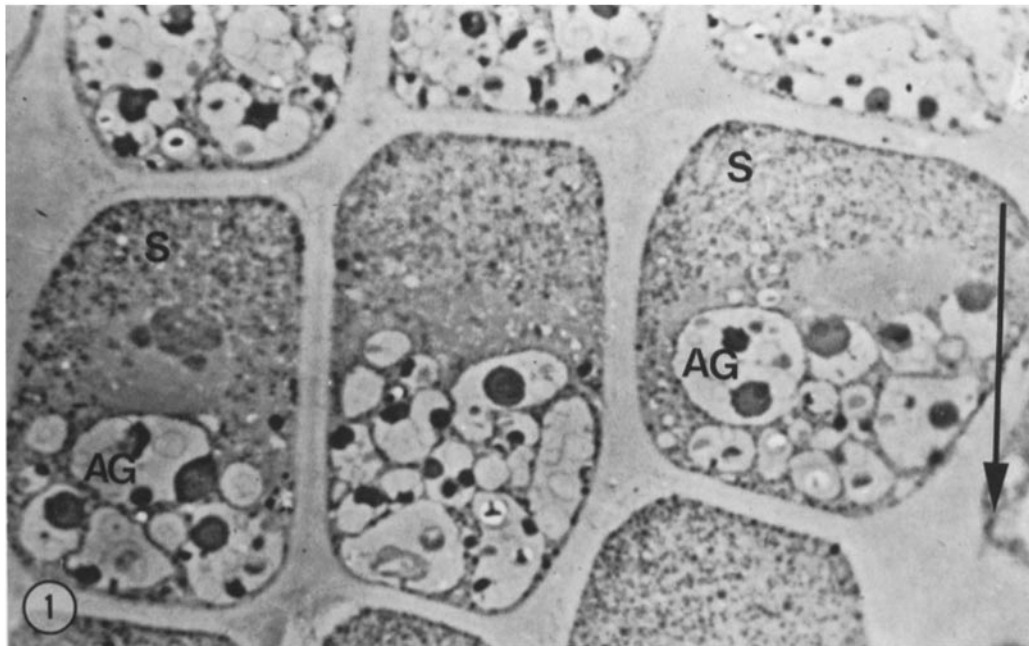

Figure 1 Light micrograph of stratified aleurone layer showing the distribution of spherosomes (S) at the centripetal zone of the cell and the aleurone grains or protein bodies (AG) at the centrifugal zone. The arrow indicates the direction of centrifugal force (g).

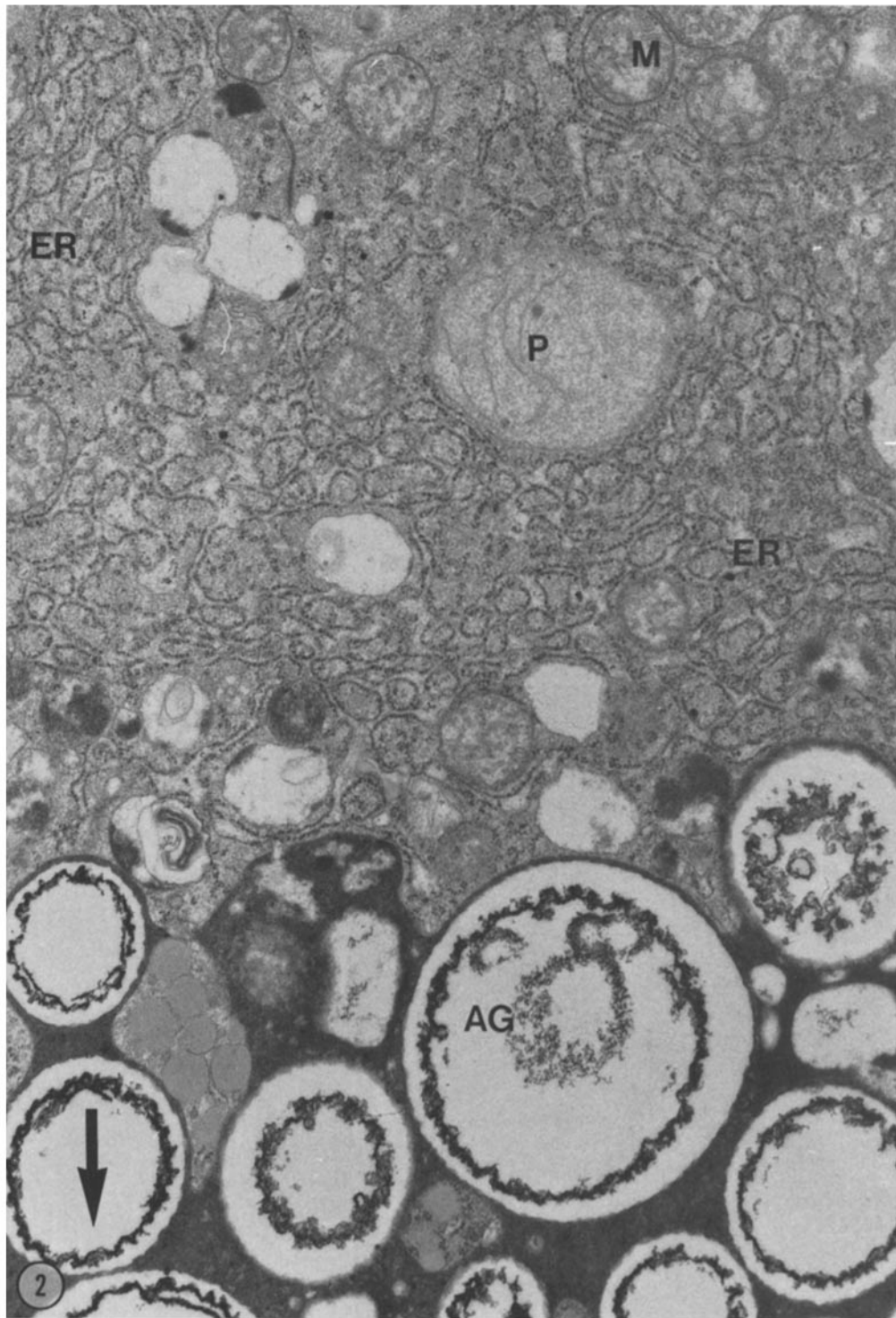FIGURE 2 Electron micrograph of stratified aleurone cell treated with GA₃ (5 μM) for 14 h showing the general distribution of ER, aleurone grains (AG), mitochondria (M), and plastids (P). Arrow indicates direction of g. × 22,750.
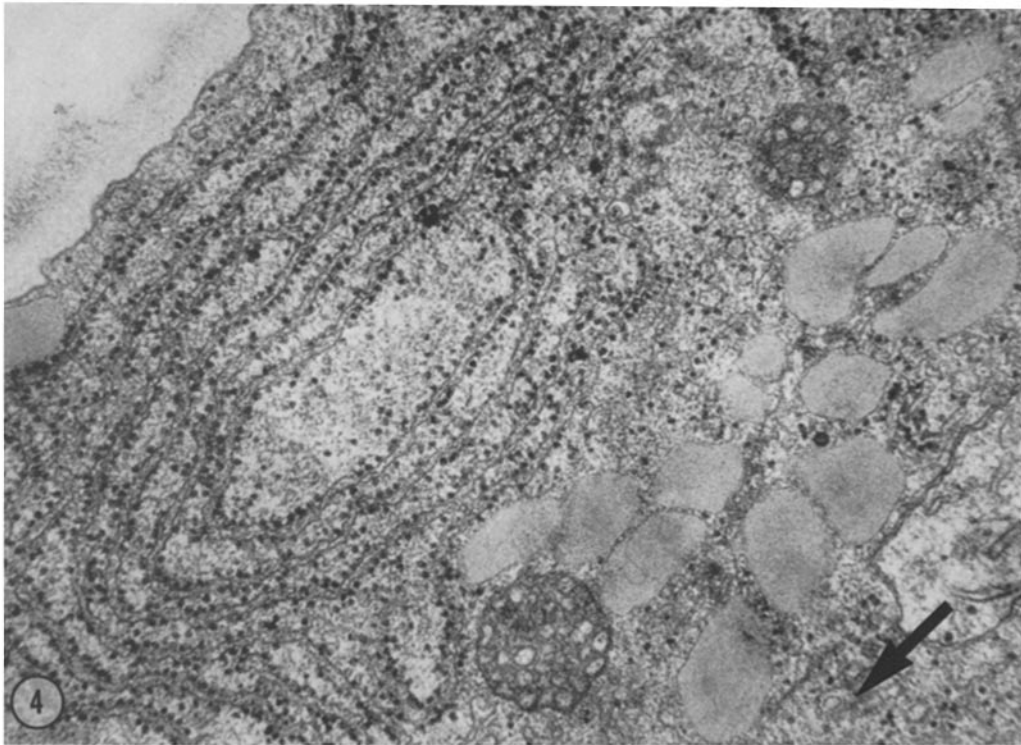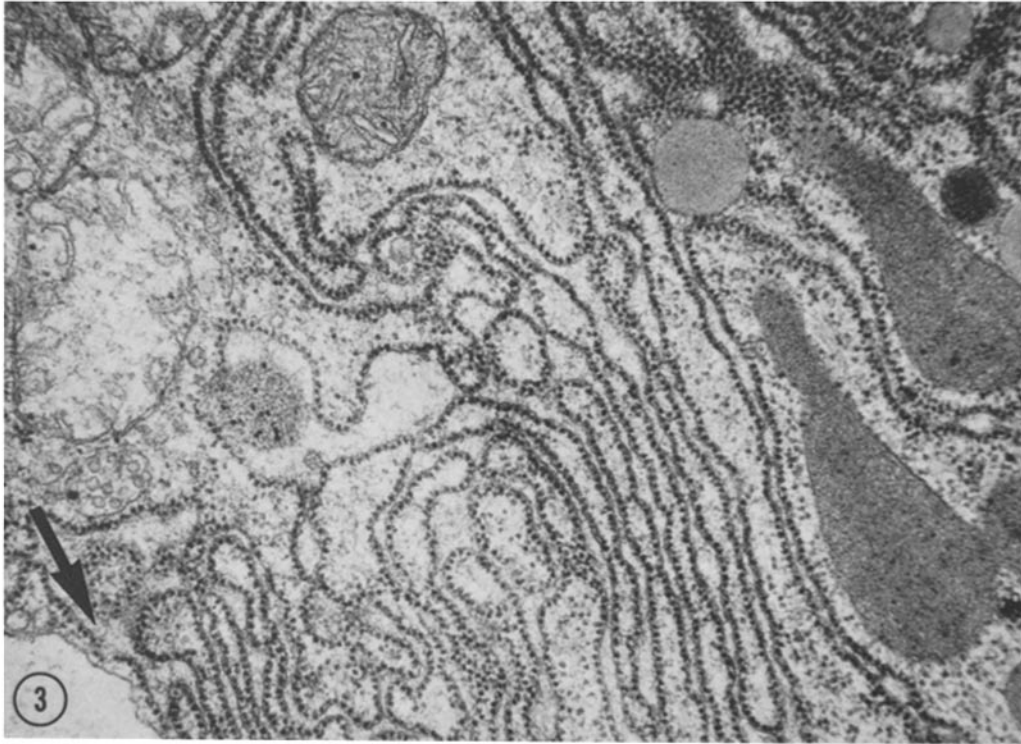
FIGURE 3 Detail of ER after stratification of aleurone cell treated with GA₃ (5 µM) for 14 h. Note the dense association of ribosomes on the ER surface. Arrow indicates the direction of *g*. × 53,200.

FIGURE 4 Detail of ER after stratification of aleurone cell treated with 5 µM GA₃ for 14 h with PEG (0.6 M) added for the last 2.5 h of GA₃ treatment. Ribosome density of the ER is considerably reduced relative to the control (cf. Fig. 3). Arrow indicates the direction of *g*. × 58,500.
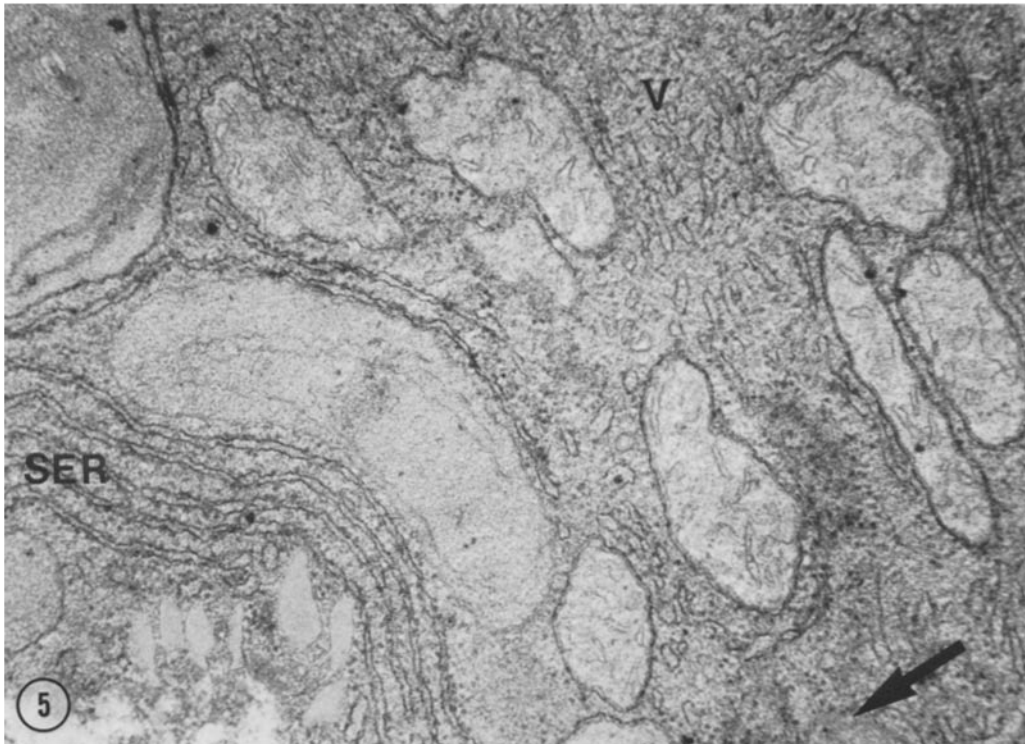
FIGURE 5 Stratified aleurone cell treated with 5 μM GA₃ for 14 h with 0.6 M PEG added for the last 5 h of GA₃ treatment. Ribosome aggregation is further reduced relative to control. Also note the smooth surfaced ER (*SER*) and associated vesicles (*V*) which are clearly separated from the stacked *ER*. Arrow indicates direction of *g*. × 56,000.

TABLE II

Number of Ribosomes in Plane Section of 1-μm Length of ER

Treatment	No. of ribosomes attached/μmER
GA₃ 5 μM 14 h	52
GA₃ 5 μM 14 h + 0.6 M PEG last 2.5 h	38
GA₃ 5 μM 14 h + 0.6 M PEG last 5 h	10
GA₃ 5 μM 14 h + 0.6 M PEG 9.0–11.5 h post GA	43

treated tissue with 30 μg of RNase at 30°C for 5 min results in the degradation of the polyribosomes to the monomer form (Fig. 6).

As a control for the effect of PEG on polyribosome formation, polyribosomes were isolated from aleurone tissue stressed with 0.6 M glycerol which has been previously shown to inhibit hydrolytic

enzyme production (19). The data (not shown) parallels that observed with polyribosomes isolated from tissue stressed with PEG (Fig. 6).

Determination of Active Ribosomes

Using labeled puromycin it is possible to determine if the microsomal fraction retains the ability to synthesize nascent protein, including nascent peptidyl tRNA as a structural part of the ribosome under osmotic stress. Puromycin added to microsomal preparations inhibits protein synthesis but allows an active ribosome to complete one peptide bond releasing the nascent puromycinpeptide.

Microsomal preparations were isolated from aleurone cells incubated in either 5 μM GA₃ or 5 μM GA₃ with 0.6 M PEG added during the final 2.5 h of the 12-h incubation period. The effect of osmoticum on the formation of [³H]peptidyl-puromycin is shown in Table III. PEG causes a reduction in the amount of RNA in the micro-
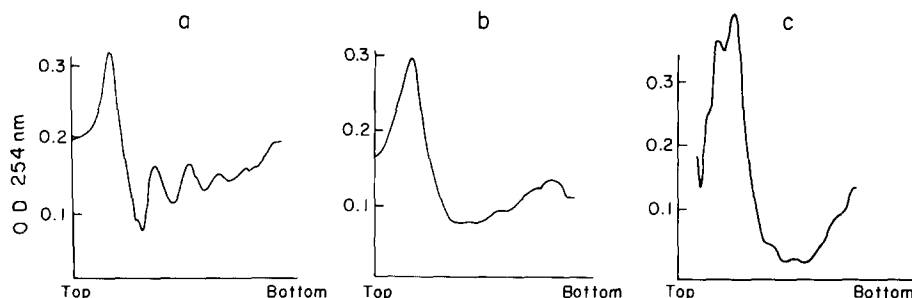
FIGURE 6 (a) Isokinetic density gradient profile of a polyribosome preparation from aleurone layers treated with 5 μM GA₃ for 12 h. (b) Polyribosome profile from aleurone layers treated with 5 μM GA₃ for 12 h with 0.6 M PEG added for the last 3 h of GA₃ treatment. (c) Isokinetic density gradient profile of polyribosomes isolated from aleurone cells treated with 5 μM GA₃ for 12 h after 5-min treatment with 30 μg/ml of rNase at 30°C.

somal fraction and a similar reduction in [³H]puro-mycin incorporation. Calculation of the percent of active ribosomes, however, shows that addition of osmoticum has little effect on the ability of ribosomes to incorporate puromycin into nascent protein.

Total RNA

An estimate of the total RNA pool of GA₃-treated and -stressed cells was obtained by colorimetric determination of the RNA level of post-mitochondrial supernatant fractions. 10 aleurone layers were incubated in 5 μM GA₃ or 5 μM GA₃ and 0.6 M PEG for 16 h. RNA levels were determined after 6, 10, 12, and 16 h (Fig. 8). Total RNA reaches a peak at 12 h of GA₃ treatment coinciding with the period of maximum α-amylase synthesis (8). Total RNA levels of stressed tissue show a similar time course although the level of RNA is 8–9% less than that seen in the tissue treated with GA₃ alone (Fig. 8).

Choline Chloride Incorporation

The incorporation [¹⁴C]choline chloride into TCA-insoluble fractions of a microsomal preparation has been shown to provide an estimate of ER synthesis (9). Data for the uptake and incorporation of [¹⁴C]choline chloride into acid-soluble and -insoluble fractions of aleurone homogenates and microsomal fractions are shown in Table IV. Although control tissue shows greater incorporation of label into the acid-insoluble component of the microsomal pellet, correction of the data for uptake differences show that water stress depresses ER synthesis by a factor of only 15%. Similarly, [¹⁴C]choline chloride incorporation into acid-

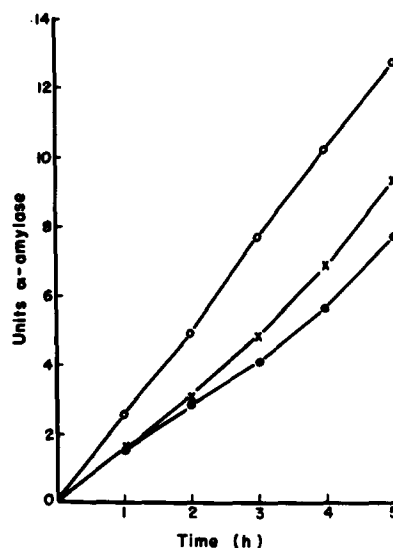

FIGURE 7 Recovery of α-amylase synthesis in aleurone layers treated with PEG (0.6 M) for 1 and 2 h. Aleurone layers were removed from seeds incubated in GA₃ (5 μM) for 9 h and incubated in osmoticum for the specified time. After stressing, the layers were washed with 1 mM acetate buffer (pH 4.8) containing 20 mM CaCl₂ and returned to a medium containing GA₃ alone. O——O, GA₃; X——X, PEG for 1 h; ●——●, PEG for 2 h.

soluble and -insoluble fractions of the initial homogenate is little affected by osmotic stress (Table IV).

DISCUSSION

Earlier studies on the response of barley aleurone layers to GA₃ have established that membrane synthesis and polyribosome formation are essential

TABLE III

Effect of Osmoticum on the Activity of Ribosomes in the Barley Aleurone Layer

	GA₃ 14 h	GA₃ 14 h + PEG 3 h
μg RNA	13.5	5.5
Molecules RNA	3.6×10^{12}	4.5×10^{11}
CPM [³H]puromycin	3600	1835
Molecules puromycin	8.8×10^{11}	1.48×10^{12}
% active ribosomes	24.5	30.2

A microsomal fraction from 35 aleurone layers was incubated in the presence of puromycin. One fraction was from aleurone cells treated with GA₃ 5μM for 14 h, and the second fraction from cells treated with GA and 0.6 M PEG for the final 3 h. The percent of active ribosomes is calculated from the incorporation of [³H]puromycin into acid-insoluble fraction.
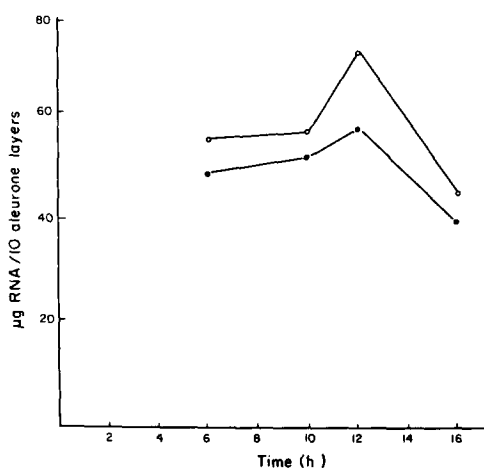

FIGURE 8 RNA level of postmitochondrial supernatant fractions from aleurone layers treated with 5 μM GA₃ (O-O) and 5 μM GA₃ with 0.6 M PEG (●-●). 10 aleurone layers were sampled at each time point and the RNA was determined spectrophotometrically.

for hydrolytic enzyme production (8, 9, 16, 17, 23). Further, it has been shown that water stress can function to regulate GA₃-induced hydrolytic enzyme production (19, 20). The evidence presented above suggests that water stress regulates hydrolytic enzyme production by affecting the process of translation. Our preliminary evidence showing a reduction in the number of membrane-associated ribosomes in water-stressed tissue has been confirmed at the electron microscope level in stratified cells (Figs. 3 and 4). Since stratification separates organelles on the basis of relative density differences, this technique allows the partial separation of vesicular and smooth ER from semi-

TABLE IV

[¹⁴C]*Choline Chloride Incorporation into TCA-Soluble and Insoluble Fractions of Aleurone Cell Homogenates*

Fraction	cpm total uptake	cpm/mg protein	cpm/mg protein corrected for uptake
TCA-soluble GA₃ homogenate	156,000	3,987	3,987
TCA-soluble GA₃ + PEG homogenate	106,000	2,373	3,488
TCA-insoluble GA₃ homogenate	84,060	2,123	2,123
TCA-insoluble GA₃ + PEG homogenate	73,980	1,700	2,422
TCA-insoluble GA₃ microsomal	460	740	740
TCA-insoluble GA₃ + PEG microsomal	358	480	624

See Materials and Methods section for experimental details.

rough and rough ER. Because the various components of the ER occupy different positions within the stratified cell it can be argued that the observed changes in the rough ER are not artefacts of fixation and tissue preparation since cells are fixed after centrifugation. If fixation did result in selective removal of ribosomes then both smooth, semirough, and rough ER would occupy the same relative position in the stratified cell.

Although other workers (28, 29) have presented

evidence suggesting that osmotic stress reduces the number of ribosomes on the ER, these studies have not been correlated to the appearance of specific enzymes. In barley aleurone cells, however, the synthesis and secretion of α-amylase after treatment with GA$_3$ has been shown to be directly related to the number of membrane-bound polysomes (8). Thus the concomitant inhibition of α-amylase synthesis and decreased ribosome association with the ER after water stress points to a direct effect of this stress on protein synthesis.

The proportion of ribosomes which can be isolated as polyribosomes from aleurone cells is also affected by stress. The conversion of polyribosomes to the monomer form could account for the reduction in ribosome density of ER. This conclusion is based on the fact that few free (nonmembrane-bound) polyribosomes are seen in aleurone cells whereas ribosomes associated with the ER of this cell appear in configurations considered characteristic of polyribosomes (17). Evidence from the study of the synthesis of other secretory proteins also suggests that they are synthesized on membrane-bound polyribosomes (1, 11, 32, 34).

Water stress has been shown to stimulate the conversion of polyribosomes to monoribosomes in other plant cells (13, 14) and this conversion has also been related to the reduction in the synthesis of certain enzymes (3). It thus seems likely that the conversion of polyribosomes to monoribosomes in the aleurone cell under osmotic stress could account for the reduction in ribosome density on the ER and for the concomitant reduction in the synthesis of the secreted hydrolases.

Several hypotheses are available to explain the decrease in the number of membrane-bound polysomes after stress. Among them are suggestions that water stress affects peptide chain initiation (14, 24), RNA pool size (6, 12), ribosome activity, and membrane synthesis. Although it was beyond the scope of this initial investigation to examine all the possible controls of polyribosome formation an investigation was made of ribosome activity, total RNA level, and membrane synthesis in control and stressed cells.

It is apparent that if ribosomes are impaired in their ability to synthesize nascent protein, then polyribosome formation and membrane binding would be affected (2). The observed effects of water stress could thus be interpreted via an effect on ribosome activity. Ribosomes from stressed tissue can however form [^{3}H]peptidylpuromycin as actively as ribosomes isolated from control tissue,

indicating that they have the same potential for protein synthesis.

Since rRNA accounts for 85% of aleurone RNA, the RNA content of postmitochondrial supernatant fractions provide a good estimate of rRNA. The total RNA level of stressed cells differs from the control by only 8–9%, a difference which would be insufficient to account for the reduction in enzyme synthesis or polyribosome formation after stress. Further, ribosome profiles after density gradient centrifugation of postmitochondrial supernates from stressed cells show only a change in the aggregation of ribosomes with no decrease in the amount of RNA (Fig. 6). Differences observed in the RNA levels of microsomal pellets from control and stressed tissue used to determine [^{3}H]peptidylpuromycin formation (Table III) do not reflect changes in rRNA between the two treatments since nonmembrane-bound ribosomes and ribosomal subunits would be excluded from such preparations.

The rate of synthesis of aleurone cell ER as measured by [^{14}C]choline incorporation has been shown to be regulated by GA$_3$ (9). Changes in the rate of membrane synthesis could thus affect ribosome attachment and the formation of membrane-bound polyribosomes. PEG, however, does not have a significant effect of [^{14}C]choline incorporation into the microsomal fraction of aleurone cells, suggesting that membrane is not limiting the binding of ribosomes. This data is in agreement with the electron microscope observations indicating that in cells stressed for 2.5 h the smooth ER does not disappear and is probably available for ribosome binding. Longer stress periods of up to 5 h may, however, result in membrane modification as indicated by a reduction in the amount of stacked ER and the formation of vesicular components which are probably derived from the smooth ER (Fig. 5).

These results establish a close relationship between the binding of polyribosomes to the ER and the control of the synthesis of a secretory enzyme in barley aleurone cells during water stress. Evidence suggests that the effects of water stress on membrane-bound polyribosome formation is not mediated through effects on the activity of bound ribosomes, RNA pool size, or membrane synthesis.

BIBLIOGRAPHY

1. ANDREWS, T. M., and J. R. TATA. 1971. Protein synthesis by membrane bound and free ribosomes of secretory and non-secretory tissue. *Biochem. J.* 121:683.

2. BAGLIONI, C., I. BLEIBERG, and M. ZAUDERER. 1971. Assembly of membrane-bound polyribosomes. *Nature (Lond.).* 232:8.

3. BARDZIK, J. M., and H. V. MARSH. 1970. Effects of water stress on the activities of three enzymes in maize seedlings. *Plant Physiol.* 82:821.

4. BENNETT, P. A., and M. J. CHRISPEELS. 1972. *De novo* synthesis of ribonuclease and β-1,3 glucanase by the aleurone cells of barley. *Plant Physiol.* 49:445.

5. BOUCK, G. B. 1963. Stratification and subsequent behavior of plant organelles. *J. Cell Biol.* 18:441.

6. CHEN, D., S. SARID, and E. KATCHALSKI. 1968. The role of water stress in the inactivation of messenger RNA from wheat embryos. *Proc. Natl. Acad. Sci. U.S.A.* 61:1378.

7. CHRISPEELS, M. J., and J. E. VARNER. 1967. Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. *Plant Physiol.* 42:398.

8. EVINS, W. 1971. Enhancement of polyribosome formation and the induction of tryptophan-rich proteins by gibberellic acid. *Biochemistry* 10:4295.

9. EVINS, W., and J. E. VARNER, 1971. Hormone controlled synthesis of endoplasmic reticulum in barley aleurone cells. *Proc. Natl. Acad. Sci. U.S.A.* 68:1631.

10. FILNER, P., and J. E. VARNER. 1967. A test for *de novo* synthesis of enzymes: density labeling with H_2O^{18} of barley amylase induced by gibberellic acid. *Proc. Natl. Acad. Sci. U.S.A.* 58:1520.

11. GANOZA, M. C., and C. A. WILLIAMS, 1969. *In vitro* synthesis of different categories of specific protein by membrane bound and free ribosomes. *Proc. Natl. Acad. Sci. U.S.A.* 63:1370.

12. GATES, C. T., and J. BONNER. 1959. The response of the young tomato plant to a brief period of water shortage. IV. Effects of stress on the ribonucleic acid metabolism of tomato leaves. *Plant Physiol.* 34:49.

13. GENKEL, P. A., N. A. SATARIVA, and E. K. TVORUS. 1967. Effects of drought on protein synthesis and the state of ribosomes in plants. *Sov. Plant Physiol.* 14:754.

14. HSIAO, T. C. 1970. Rapid changes in levels of polysomes in *Zea mays* in response to water stress. *Plant Physiol.* 46:281.

15. JACOBSEN, J. V., and J. E. VARNER, 1967.

16. JOHNSON, K. D., and H. KENDE. 1971. Hormonal control of lecithin synthesis in barley aleurone cells: regulation of the CDP-choline pathway by gibberellin. *Proc. Natl. Acad. Sci. U.S.A.* 68:2674.

17. JONES, R. L. 1969. Gibberellic acid and the fine structure of barley aleurone cells. II. Changes during the synthesis and secretion of α-amylase. *Planta (Berl.).* 88:73.

18. JONES, R. L. 1969. The effect of ultracentrifugation on fine structure and α-amylase production in barley aleurone cells. *Plant Physiol.* 44:1428.

19. JONES, R. L. 1969. Inhibition of gibberellic acid induced α-amylase formation by polyethylene glycol and mannitol. *Plant Physiol.* 44:101.

20. JONES, R. L., and J. E. ARMSTRONG. 1971. Evidence for osmotic regulation of hydrolytic enzyme production in germinating barley seeds. *Plant Physiol.* 48:137.

21. JONES, R. L., and J. PRICE. 1970. Gibberellic acid and the fine structure of barley aleurone cells. III. Vacuolation of the aleurone cell during the phase of ribonuclease release. *Planta (Berl.).* 94:191.

22. JONES, R. L., and J. E. VARNER. 1967. The bioassay of gibberellins. *Planta (Berl.).* 72:155.

23. KOEHLER, D., K. D. JOHNSON, J. E. VARNER, and H. KENDE. 1972. Differential effects of mannitol and gibberellin regulated phospholipid synthesis and enzyme activities of the CDP-choline pathway in barley aleurone cells. *Planta (Berl.).* 104:267.

24. LIN, C. Y., and J. L. KEY. 1967. Dissociation and reassembly of polyribosomes in relation to protein synthesis in the soybean root. *J. Mol. Biol.* 26:237.

25. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265.

26. MACLEOD, A. M., and G. H. PALMER. 1967. Gibberellins from barley embryos. *Nature (Lond.).* 216:1342.

27. MEJBAUM, W. 1939. Uber die bestimmung kleiner pentosemengen, insbesondere in derivaten der adenylsaure. *Z. Physiol. Chem. (Hoppe-Seyler's).* 258:117.

28. NIR, I. S., S. KLEIN, and A. POLJAKOFF-MAYBER. 1969. Effect of moisture stress on submicroscopic structures of maize roots. *Aust. J. Biol. Sci.* 22:17.

29. NIR, I. S., S. KLEIN, and A. POLJAKOFF-MAYBER. 1970. Changes in the fine structure of root

Gibberellic acid induced synthesis of protease by isolated aleurone layers of barley. *Plant Physiol.* 42:1596.

cells from maize seedlings exposed to water stress. *Aust. J. Biol. Sci.* **23**:489.

30. NOLL, H. 1967. Characterization of macromolecules by constant velocity sedimentation. *Nature (Lond.).* **215**:360.

31. RADLEY, M. 1967. Site of reproduction of gibberellin-like substances in germinating barley embryos. *Planta (Berl.).* **75**:164.

32. ROSHBACH, M., and S. PENMAN. 1971. Membrane associated protein synthesis of mammalian cells. II. The two classes of membrane associated ribosomes. *J. Mol. Biol.* **59**:227.

33. SCHNEIDER, W. C. 1945. I. Extraction and estimation of deoxypentose nucleic acid and of pentose nucleic acid. *J. Biol. Chem.* **161**: 293.

34. SIEKEVITZ, P., and G. E. PALADE. 1966. Distribution of newly synthesized amylase in subfractions of guinea pig pancreas. *J. Cell Biol.* **30**:519.

35. STURANI, E., S. COCUCCI, and E. MARRE. 1968. Hydration dependent polysome-monosome interconversion in germinating castor bean endosperm. *Plant Cell Physiol.* **9**:783.

36. WARBURG, O., and W. CHRISTIAN. 1942. Isolierung und kristallisation des garungsferments enolase. *Biochem. Z.* **310**:383.

37. WOOL, I. G., and K. KURIHARA. 1967. Determination of the number of active muscle ribosomes: effects of diabetes and insulin. *Proc. Natl. Acad. Sci. U.S.A.* **58**:2401.