The Development of Polysomes in the Seed of *Pisum arvense*

By G. R. BARKER AND M. RIEBER

Department of Biological Chemistry, University of Manchester

(Received 19 April 1967)

1. Ribosomal preparations from dormant seed, cotyledon and growing tissue from *Pisum arvense* were examined. 2. Polysomes were obtained from growing tissues under all conditions used. 3. Such particles were obtained from seed immediately after imbibition only when 5 mM-zinc sulphate was included in the medium used for extraction. 4. No polysomes were obtained from dry seed. 5. Extracts of dry seed showed limited incorporation of phenylalanine into protein. 6. Extracts of seed after imbibition showed enhanced activity in incorporation of phenylalanine amounting to 72% of the activity found in extracts of growing tissue. 7. A nuclear fraction from dry seed was able to incorporate ATP into acid-precipitable material. 8. It is concluded that the protein-synthesizing system of the dry seed is limited by the low concentration of functional polysomes.

It has been reported (Young & Varner, 1959; Young, Huang, Vanecko, Marks & Varner, 1960) that phosphatase and α -amylase increase in activity in the cotyledons of pea seed during germination. In the early stages of germination, labelled glycine is incorporated into protein of the cotyledons *in vivo* (Young *et al.* 1960) and it is therefore possible that the development of the enzymic activities represents protein newly synthesized in the cotyledons. It was therefore decided to study the protein-synthesizing system of the seed during the early stages of germination.

Previous studies (Barker & Hollinshead, 1965) have shown that 70s ribosomes can be prepared from cotyledon tissue of *Pisum arvense* provided that the concentration of Mg^{2+} ions is sufficiently high. No detectable synthesis of RNA takes place in the cotyledon during germination (Barker & Hollinshead, 1964) and the question therefore arises whether polysomes are present in this tissue. In the experiments now reported, conditions are established for the preparation from germinating seed of particles believed to consist of polysomes, but no such material was detected in dry seed. It is demonstrated that dry seed possess a limited capacity for incorporating phenylalanine and ATP into acid-precipitable material. The results suggest that the small capacity for protein synthesis in dormant seed is due to the low concentration of ribosomes bound to messenger RNA.

MATERIALS AND METHODS

Seed of *P. arvense* (var. Minerva Maple) were a gift from Messrs Gartons (Warrington, Lancs.). Imbibition and germination were carried out as described by Barker &

Hollinshead (1964). L-[^{14}C]Phenylalanine and [8- ^{14}C]ATP were purchased from The Radiochemical Centre, Amersham, Bucks. The sodium salts of nucleotides and the potassium salt of polyuridylic acid were obtained from the Sigma (London) Chemical Co. Ltd., London, S.W. 6. Pyruvate kinase and sodium phosphoenolpyruvate were purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Amino acids and CsCl (AnalaR grade) were obtained from British Drug Houses Ltd., Poole, Dorset. Actinomycin D was a gift from Merck & Co. Inc., Rahway, N.J., U.S.A.

Preparation of tissue extracts. All operations were carried out in a cold-room at 2–4°. Tissue extracts were prepared with 50 mM-tris-HCl buffer, pH 7.3 (measured at room temperature), containing $MgCl_2$ and $ZnSO_4$ at final concentrations indicated for each experiment. In each experiment approx. 15 g. of fresh tissue was used. Dry seed was ground in a stainless-steel vessel in an Ato-Mix homogenizer (Measuring and Scientific Instrument Co., London, S.W. 1) and the powder was sieved through a 20-gauge sieve (Baird and Tatlock Ltd., London, E.C. 1). The sieved material was homogenized for 90 sec. with 1.0 ml. of the appropriate medium/g. of material. Tissue taken after imbibition was homogenized directly without previous grinding. Root and shoot tissue were ground in a mortar with acid-washed sand and extracted with 0.5 ml. of medium/g. of fresh tissue. Homogenates or crude extracts were filtered through cheese-cloth and centrifuged at 15000 g for 10 min. in the no. 30 rotor of the Spinco model L centrifuge. The pellet was discarded. The supernatant was centrifuged at 105000 g for 2 hr. in the no. 40 rotor of the same centrifuge. The supernatant was dialysed for 2.5 hr. against the buffer used in incorporation experiments (see below). Protein was determined in the dialysed solution by the method of Gornall, Bardawill & David (1949), with bovine plasma albumin as standard. The ribosomal pellet was suspended in the appropriate medium. RNA present in the suspension was determined by the method of Loening (1961).

Preparation of DNA-containing fraction. An adaptation

of the method of Huang & Bonner (1962) was used. A 160 g. sample of dry seed was ground and sieved as described above. The sieved material was homogenized for 1.5 min. with 150 ml. of 50 mM-tris-HCl buffer, pH 8.0, containing 0.25 M-sucrose, 10 mM-2-mercaptoethanol and 1.0 mM-MgCl₂. The homogenate was filtered twice through cheese-cloth and centrifuged for 30 min. at 4000 g. The supernatant was discarded, and the sedimented gel layer was scraped from the underlying starch layer as described by Huang & Bonner (1962) and suspended in 50 mM-tris-HCl buffer, pH 8.0, containing 10 mM-2-mercaptoethanol. The suspension was centrifuged for 15 min. at 100 000 g and the pellet resuspended in the same buffer. This process was repeated once more. Then 5 ml. of the resulting suspension was layered on 25 ml. of 1.7 M-sucrose containing 10 mM-2-mercaptoethanol and centrifuged for 105 min. at 22 000 rev./min. in the SW25 rotor of the Spinco model L centrifuge. The pellet was homogenized with 4.5 ml. of 4 M-CsCl containing 10 mM-2-mercaptoethanol and the suspension was centrifuged for 20 hr. at 35 000 rev./min. in the SW39 rotor of the Spinco model L centrifuge. For enzymic experiments, the pellet was suspended in 50 mM-tris-HCl buffer, pH 8.0, containing 10 mM-2-mercaptoethanol, and dialysed for 1 hr. against the same buffer. DNA was determined in the resulting solution by the method of Dische (1955), with calf thymus DNA as standard. For purposes of characterizing the DNA, the pellet obtained in the SW39 rotor was resuspended in 4.5 ml. of 4 M-CsCl containing 10 mM-2-mercaptoethanol and centrifuged as before. The pellet was suspended in a solution containing 15 mM-NaCl and 1.5 mM-sodium citrate and dialysed for 1 hr. against the same solution.

Sedimentation experiments. Ribosomal preparations were examined in the Spinco model E centrifuge with schlieren optics. Measurements were made at 4° in 1.2 cm. cells with concentrations of RNA not greater than 4 mg./ml. Values for sedimentation coefficients were corrected to infinite dilution and to 20° as described by Schaechter (1963). DNA was examined in a similar way with ultraviolet optics.

Incorporation of phenylalanine into protein. The incubation mixture contained, in a final volume of 1 ml., tris-HCl buffer, pH 7.3 (50 μ moles), ATP (1 μ mole), GTP (0.3 μ mole), 2-mercaptoethanol (5 μ moles), KCl (30 μ moles), sodium phosphoenolpyruvate (10 μ moles), pyruvate kinase (40 μ g.), [¹⁴C]phenylalanine (0.1 μ mole; 0.5 μ Ci), dialysed ultracentrifugal supernatant (containing approx. 2.5 mg. of protein), ribosomal suspension (containing approx. 0.5 mg. of RNA) and 0.1 μ mole of each of the following amino acids: L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. In addition, the mixture contained MgCl₂ at a final concentration equal to that used in preparing the ribosomal and supernatant fractions. Solutions were incubated at 37° for 1 hr. The reaction was stopped by cooling the tubes rapidly to 0° and adding 0.5 ml. of 0.1 M-phenylalanine and 5 ml. of 10% (w/v) trichloroacetic acid. Control experiments were performed in which the reaction was stopped at zero time. After standing at 0° for 15 min. the precipitated protein was centrifuged and washed twice by resuspension and centrifugation with 5 ml. of 5% (w/v) trichloroacetic acid. The residue was heated at 90° for 30 min. with 5% (w/v) trichloroacetic acid, centrifuged, washed once with 5 ml. of ethanol-ether (1:1, v/v) and then

4 ml. of ether and dried in air. The dry residues were dissolved in 1 ml. of Hyamine, transferred to vials and radioactivity was measured in a Nuclear Enterprises Scintillator Spectrometer with NE213 scintillator [Nuclear Enterprises (G.B.) Ltd., Edinburgh]. Counting efficiency was 40%. Counting was continued until the statistical error was less than 5%.

Assay of RNA nucleotidyltransferase activity. The incubation mixture contained, in a final volume of 1 ml., tris-HCl buffer, pH 8.0 (50 μ moles), sodium phosphoenolpyruvate (10 μ moles), pyruvate kinase (40 μ g.), MgCl₂ (5 μ moles), MnCl₂ (1 μ mole), 2-mercaptoethanol (10 μ moles), DNA-containing fraction (containing approx. 220 μ g. of DNA), [8-¹⁴C]ATP (0.4 μ mole; 0.62 μ Ci) and 0.4 μ mole each of GTP, CTP and UTP. Solutions were incubated at 37° for 1 hr. and the reaction was stopped by adding 0.2 ml. of 5 M-ATP and 5 ml. of 10% (w/v) trichloroacetic acid. After standing at 0° for 15 min. the precipitate was centrifuged and washed twice with 5 ml. of 5% (w/v) trichloroacetic acid and once with 4 ml. of ethanol-ether (1:1, v/v). The precipitate was dissolved in 0.3 ml. of 0.5 N-NaOH, and 5 ml. of 5% (w/v) trichloroacetic acid was immediately added. The precipitate was collected on a Millipore filter (Millipore Filter Corp., Bedford, Mass., U.S.A.), washed twice on the filter with 5 ml. of 5% (w/v) trichloroacetic acid and transferred to a vial for measurement of radioactivity as described above.

RESULTS

Effect of composition of medium on ribosomal preparations. Sedimentation coefficients of ribosomal particles are shown in Table 1. The results recorded under each set of conditions were obtained in a number of experiments on different occasions. It is seen that, with a medium containing 10 mM-magnesium chloride, 3-day-old axis tissue from which cotyledons had been removed (Expt. 1) and root and shoot tissues from 6-day-old plants (Expts. 2 and 3) yielded particles having sedimentation coefficients in excess of 110s. In contrast, preparations from dry seed (Expt. 4) from cotyledons of 6-day-old plants (Expt. 5) and from seed after imbibition for 17 hr. (Expt. 6) gave no particles with sedimentation coefficients higher than 110s. By using seed after imbibition, it was confirmed that omission of magnesium chloride from the extraction medium results in some dissociation of ribosomal particles (Expt. 7) (cf. Barker & Hollinshead, 1965), and it was found that increasing the concentration of magnesium chloride to 20 mM (Expts. 8 and 9) did not result in the appearance of larger particles. Addition of magnesium chloride at a final concentration of 10 mM and zinc sulphate at a final concentration of 1.0 mM to the extraction medium had no effect on the sedimentation coefficients of the particles obtained from seed after imbibition (Expt. 10), but increasing the concentration of zinc sulphate to 5 mM resulted in the appearance of particles having sedimentation coefficients 140s and 170s (Expt. 11). Under the

Table 1. *Sedimentation coefficients of ribosomal particles prepared from Pisum arvense with different media*

Tissue from *P. arvense* taken at different stages of germination was homogenized as described in the text with 50mM-tris-HCl buffer, pH 7.3, containing MgCl₂ and ZnSO₄ at the concentrations indicated. Ribosomal particles were isolated as described in the Materials and Methods section, resuspended in the same medium and examined in the Spinco model E centrifuge.

Expt. no.	Tissue examined	Concn. (mM)		Sedimentation coefficients (s)
		MgCl ₂	ZnSO ₄	
1	3-day-old axis	10	0	170, 140, 110, 80, 60
2	6-day-old root	10	0	140, 110, 80, 60
3	6-day-old shoot	10	0	170, 140, 110, 80, 60
4	Dry seed	10	0	110, 80, 60
5	Cotyledons from 6-day-old plants	10	0	110, 80, 60
6	Seed after imbibition for 17hr.	10	0	110, 80, 60
7	Seed after imbibition for 17hr.	0	0	80, 60, 40
8	Seed after imbibition for 17hr.	15	0	110, 80, 60
9	Seed after imbibition for 17hr.	20	0	110, 80, 60
10	Seed after imbibition for 17hr.	10	1	110, 80, 60
11	Seed after imbibition for 17hr.	10	5	170, 140, 110, 80, 60
12	3-day-old axis	10	5	170, 140, 110, 80, 60
13	Dry seed	10	5	110, 80, 60
14	Seed after imbibition for 17hr.	5	5	110, 80, 60
15	Seed after imbibition for 17hr.	10	10	No sedimenting particles
16	Seed after treatment with actinomycin D	10	5	170, 140, 110, 80, 60
17	3-day-old axis after treatment with actinomycin D	10	5	170, 140, 110, 80, 60

same conditions of extraction, 3-day-old axis tissue gave identical results (Expt. 12), but no faster-sedimenting material was obtained from dry seed (Expt. 13). Decreasing the concentration of magnesium chloride even in the presence of 5mM-zinc sulphate resulted in loss of faster-sedimenting material from extracts of seed after imbibition (Expt. 14), and increasing the concentration of zinc sulphate to 10mM resulted in flocculation and the absence of any observable particulate material from the ultracentrifugal pattern (Expt. 15).

In all experiments, the component having a sedimentation coefficient of 80s exhibited the strongest peak. The 140s and 170s components, when observed, were always present in very small amounts, the latter sometimes being only just perceptible. It is possible that some heavy particles may have escaped detection in some experiments. Figs. 1(a), 1(b) and 1(c) illustrate the patterns obtained in Expts. 6, 7 and 11 respectively.

To determine the nature of the effect of Zn²⁺ ions, the following experiments were carried out. Two ribosomal preparations were made from the same batch of seed after imbibition for 17hr. In preparation A, seed was homogenized with 50mM-tris-hydrochloric acid buffer, pH 7.3, containing 10mM-magnesium chloride and the ribosomal pellet was resuspended in 50mM-tris-hydrochloric

acid buffer, pH 7.3, containing 10mM-magnesium chloride and 5mM-zinc sulphate. In preparation B, the above medium containing both Mg²⁺ and Zn²⁺ ions was used for homogenization and the Zn²⁺-free medium was used for resuspension of the particles. Ultracentrifugation showed the presence of particles having sedimentation coefficients 110s and 80s in preparation A and 140s, 110s and 80s in preparation B.

The possibility that the formation of faster-sedimenting particles during the process of imbibition might be due to the synthesis of messenger RNA was tested as follows. Seed was soaked for 17hr. in water containing 20μg. of actinomycin D/ml., and ribosomal particles were prepared from half of the seeds with 50mM-tris-hydrochloric acid buffer, pH 7.3, containing 10mM-magnesium chloride and 5mM-zinc sulphate for homogenization and resuspension. The other half of the seed, after treatment with actinomycin D, was planted in vermiculite and allowed to germinate for 3 days. Ribosomal particles were prepared from the axis tissue as described above. Ultracentrifugal patterns observed are shown in Table 1 (Expts. 16 and 17). The results of experiments carried out at the same time with seed that had not been treated with actinomycin D were the same as in Table 1 (Expts. 11 and 12).

To confirm that the faster-sedimenting particles are polysomes, the sedimentation pattern obtained with axis tissue prepared in Expt. 1 was compared

Fig. 1. Ultracentrifugation of ribosomal preparations from *P. arvense*. (a) After imbibition for 17 hr. seed was homogenized in a medium containing 10 mM-MgCl₂ and no ZnSO₄. Ultracentrifugation was carried out at 35600 rev./min.; time, 20 min. after attaining speed. (b) Seed was examined under the same conditions except that neither MgCl₂ nor ZnSO₄ was added to the medium. (c) Seed was examined under the same conditions except that the medium contained 10 mM-MgCl₂ and 5 mM-ZnSO₄. (d) 3-day-old axis tissue was homogenized in a medium containing 10 mM-MgCl₂. Ultracentrifugation was carried out at 29500 rev./min.; time, 10 min. after attaining speed. (e) Ribosomal suspension was prepared as in (d) and incubated for 5 min. at 37° with pancreatic ribonuclease before centrifugation. Ultracentrifugation was carried out as in (d).

with that obtained with similar material that had been incubated for 5 min. at 37° with crystalline pancreatic ribonuclease at a final concentration of 5 µg./ml. The results are shown in Table 1 (Expts. 18 and 19) and in Figs. 1(d) and (e).

Incorporation of phenylalanine into protein. Ribosomal and supernatant fractions were prepared as described in the Materials and Methods section from seed after imbibition for 17 hr., with 50 mM-tris-hydrochloric acid buffer, pH 7.3, containing 10 mM-magnesium chloride, 5 mM-zinc sulphate and 5 mM-2-mercaptoethanol for homogenization. The ribosomal pellet was resuspended in 50 mM-tris-hydrochloric acid buffer, pH 7.3, containing various concentrations of magnesium chloride. Corresponding supernatant fractions were dialysed against the same buffer as that used for homogenization except that zinc sulphate was omitted. Incorporation of [¹⁴C]phenylalanine into protein was measured as described in the Materials and Methods section and the results are shown in Table 2. It is seen from Expts. 1, 2 and 3 that incorporation is optimum in the presence of 10 mM-magnesium chloride, in agreement with the results of Earl & Korner (1965).

Under the same conditions, incorporation of [¹⁴C]phenylalanine was investigated with preparations from dry seed and 4-day-old axis tissue. The results are shown in Table 2 (Expts. 4 and 5).

The effect on incorporation of [¹⁴C]phenylalanine by extracts of dry seed of the addition of polyuridylic acid at a final concentration of 100 µg./ml. is shown in Table 2 (Expts. 6, 7 and 8).

Demonstration of RNA nucleotidyltransferase activity in dormant seed. The DNA-containing fraction was prepared from dry seed as described in the Materials and Methods section. A solution of the material in saline-citrate medium had a melting temperature of 71° and a sedimentation coefficient of 22s. Huang & Bonner (1962) reported corresponding values of 70–71° and 18s for material from pea embryos prepared and examined in the same

Table 2. Incorporation of [¹⁴C]phenylalanine into protein by extracts of *Pisum arvense*

Ribosomal and supernatant fractions were prepared from tissue of *P. arvense* and incorporation of [¹⁴C]phenylalanine was measured as described in the text. Incubations were carried out at 37° for 1 hr. except in control experiments in which the reactions were stopped after zero time.

Expt. no.	Tissue examined	Concn. of MgCl ₂ in incubation mixture (mM)	Concn. of polyuridylic acid in incubation mixture (µg./ml.)	Incorporation of phenylalanine (µmoles/mg. of RNA)
1	Seed after imbibition for 17 hr.	5	0	107
2	Seed after imbibition for 17 hr.	10	0	138
3	Seed after imbibition for 17 hr.	15	0	117
4	Dry seed	10	0	21
5	4-day-old axis tissue	10	0	208
6	Dry seed	5	100	285
7	Dry seed	10	100	428
8	Dry seed	15	100	518

way. Determination of the incorporation of ATP into acid-precipitable material as described in the Materials and Methods section resulted in the incorporation of 736 μmoles of ATP when the complete system was used. Under identical experimental conditions, but with CTP, GTP and UTP omitted from the medium, 93 μmoles of ATP were incorporated.

DISCUSSION

The results shown in Table 1 indicate that growing tissue from germinated seed yields, under all the experimental conditions used, particles having sedimentation coefficients in excess of 110s, which we regard as polysomal. No such particle was obtained from dormant seed, from seed after imbibition for 17 hr. or from cotyledons of growing plants with media containing 10mM-magnesium chloride for homogenization and resuspension of particles. Further, variation of the concentration of added magnesium chloride between zero and 20mM did not result in the appearance of faster-sedimenting material. The apparent absence of polysomes from cotyledon tissue was tentatively ascribed to the high activity of ribonuclease (Holden & Pirie, 1955; Ingle & Hageman, 1965; Barker & Hollinshead, 1967). Since addition of sucrose to the medium used for homogenization had no influence on the sedimentation patterns obtained, the effect of Zn^{2+} ions, which are known to inhibit pea ribonuclease (Holden & Pirie, 1955), was examined. It is seen from Table 1 (Expt. 11) that, with seed after imbibition for 17 hr., extraction with a medium containing 10mM-magnesium chloride and 5mM-zinc sulphate yielded particles that were indistinguishable in the ultracentrifuge from those obtained from growing tissue either in the presence or absence of Zn^{2+} ions. The effect of the Zn^{2+} ions is clearly not due to their replacing Mg^{2+} ions in the ribosomal complex, since in the first place the same effect was not obtained by increasing the concentration of magnesium chloride to 20mM. Further, faster-sedimenting particles were not obtained with 5mM-magnesium chloride and 5mM-zinc sulphate. Comparison of the results obtained in Expts. 8 and 11 rule out the possibility that the effect is due to change in the concentration of bivalent cations relative to the constant concentration of univalent cations. Addition of 1.0mM-zinc sulphate to a medium containing 10mM-magnesium chloride had no effect on the nature of the particles obtained; increasing the concentration of zinc sulphate to 10mM resulted in flocculation, as reported by Petermann, Mizen & Hamilton (1953) for mammalian ribosomes.

The presence of particles having sedimentation coefficients higher than 110s could be due either to

polysomes, or to non-specific aggregation of ribosomes under the influence of Zn^{2+} ions. It is considered that the second possibility is excluded by the fact that addition of Zn^{2+} ions to a ribosomal suspension prepared in the absence of Zn^{2+} ions gave no material sedimenting faster than the 110s particles. The faster-sedimenting particles are therefore tentatively described as polysomes, and it is considered probable that the preservative effect of Zn^{2+} ions is due to inhibition of RNA-degrading enzymes in the tissue extracts. In contrast with the results obtained with seed after imbibition, addition of Zn^{2+} ions to the extraction medium did not result in the appearance of faster-sedimenting particles in extracts of dry seed, and it is concluded that polysomes are absent from, or present only in small concentrations in, extracts of dry seed.

To confirm that the faster-sedimenting particles obtained from seed after imbibition are functional polysomes, incorporation of phenylalanine into protein was measured under identical conditions with cell-free extracts of dry seed, seed after imbibition and growing tissue. The results show that incorporation by extracts of dry seed amounted only to 13% of that obtained with extracts of growing tissue. The corresponding value for extracts of seed after imbibition was 72%. Thus the presence or absence of polysomes in extracts of seed is paralleled by the activity in incorporating phenylalanine. We believe that the low concentration of functional polysomes is at least the major reason for the low incorporation of phenylalanine with extracts of dry seed, and this is supported by the fact that addition of polyuridylic acid to extracts of dry seed markedly increased the degree of incorporation (cf. Table 2, Expts. 4 and 8). In agreement with the results of Arnstein, Cox & Hunt (1962), the stimulation by polyuridylic acid was dependent on the concentration of Mg^{2+} ions (see Table 2, Expts. 7, 8 and 9). It thus appears that the cell-free system from dry seeds responds normally to the addition of polyuridylic acid and is therefore believed to possess the expected components of a protein-synthesizing system but to be deficient in messenger-bound ribosomes. This conclusion is in essential agreement with those of Marcus & Feeley (1964) on ungerminated peanuts and wheat seed.

Incorporation of phenylalanine into protein by extracts of seed after imbibition is considerably in excess of that obtained with extracts of dry seed and is of the same order as that observed with extracts of axis tissue. Further, seed after imbibition resembles axis tissue in containing polysomes. The question therefore arises as to the processes taking place during imbibition whereby recognizable polysomes are formed and an active protein-synthesizing system is developed. The possibility exists that polysomes are present in dry seed and are either

broken during the necessary vigorous mechanical treatment or are firmly attached to membranes and not discernible. The use of detergents did not result in the appearance of polysomal material. Deoxycholate gave rise to precipitates of the type described by Kirsch (1962) and by Pogo *et al.* (1962), and addition of Triton X had no effect on the ultracentrifugal patterns obtained (cf. Marcus & Feeley, 1965). It therefore seems probable that polysomes are either absent from dry seed or are present in amounts below the level of detection. Evidence presented in the Appendix (Chapman & Rieber, 1967) supports this conclusion. This possibility is not susceptible to experimental test by biochemical methods, but relevant information is discussed in the Appendix (Chapman & Rieber, 1967). Alternative explanations are either that messenger RNA in the seed is not associated with ribosomes until after imbibition, or that messenger RNA is synthesized during this process. The experiments reported here indicate the presence of a nuclear enzyme system in dry seed that is capable of RNA synthesis. On the other hand, no RNA synthesis in the cotyledons *in vivo* could be detected by using labelled orthophosphate (Barker & Hollinshead, 1964). It therefore appears that the RNA-synthesizing system is not fully operative. This is similar to the situation obtaining in bacterial spores (Kerjan & Szulmajster, 1966). An alternative explanation is that *in vivo* the DNA of the cotyledon is not able to act as template in RNA synthesis, but that during the process of isolating the nuclear fraction, involving the removal of histone (Huang & Bonner, 1962), the DNA is converted into an active form. In view of the fact that no RNA synthesis has been detected in the cotyledons, the possibility must be considered that during imbibition monomeric ribosomes become associated with pre-existing messenger RNA.

The existence of long-lived messenger RNA in plants has been previously suggested on the basis of experiments with actinomycin D by Morton & Raison (1964) and by Dure & Waters (1965). In the latter experiments treatment of cotton seed with the drug produced no alteration of the proportion of polysomes to ribosomes, although incorporation of radioactive phosphate into RNA was inhibited. In the present experiments, treatment of the seed with actinomycin D during imbibition did not affect the ultracentrifugal pattern of ribosomal preparations. However, since no synthesis of RNA can be detected in pea cotyledons during germination, it cannot be assumed with certainty that the drug enters the cells. Although protoplasts prepared from *Escherichia coli* are sensitive to actinomycin D (Mach & Tatum, 1963), whole cells are not affected by the drug without prior treatment with EDTA (Haywood & Sinsheimer, 1963; Hurwitz,

Furth, Malamy & Alexander, 1962; Leive, 1965). It has been shown (Chipchase & Birnstiel, 1963) that actinomycin D inhibits the synthesis of RNA in a nuclear fraction from pea seedlings, but, since in the present work the drug had no adverse effect on subsequent growth, too much significance must not be attached to experiments involving its use. However, it is possible that the situation is analogous to that found in cells of *E. coli* and spores of *Bacillus cereus* (Aronson, 1965).

From the results presented, it is concluded that in the dormant seed a potentially fully operative system for protein synthesis is present, but that its operation is limited by the absence or low concentration of functional polysomes. Although enzymes necessary for RNA synthesis are present in the dormant seed, they appear not to be operative and it is therefore tentatively concluded that the dormant seed either contains a long-lived messenger RNA unassociated with ribosomes, or possesses polysomes that are not detected by normal methods, owing possibly to being bound to membranes.

M. R. is on leave of absence from Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas, Venezuela, and is indebted to the British Council for the award of a Scholarship. The authors are indebted to Miss D. E. Pinion for carrying out the ultracentrifugal analyses.

REFERENCES

- Arnstein, H. R. V., Cox, R. A. & Hunt, J. A. (1962). *Nature, Lond.*, **194**, 1042.
- Aronson, A. (1965). *J. molec. Biol.* **13**, 92.
- Barker, G. R. & Hollinshead, J. A. (1964). *Biochem. J.* **3**, 78.
- Barker, G. R. & Hollinshead, J. A. (1965). *Biochim. biophys. Acta*, **108**, 323.
- Barker, G. R. & Hollinshead, J. A. (1967). *Biochem. J.* **103**, 230.
- Chapman, J. A. & Rieber, M. (1967). *Biochem. J.* **105**, 201.
- Chipchase, M. I. H. & Birnstiel, M. L. (1963). *Proc. nat. Acad. Sci., Wash.* **49**, 692.
- Dische, Z. (1955). In *The Nucleic Acids*, vol. 1, p. 294. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.
- Dure, L. & Waters, L. (1965). *Science*, **147**, 410.
- Earl, D. C. N. & Korner, A. (1965). *Biochem. J.* **94**, 721.
- Gornall, A. G., Bardawill, C. S. & David, M. (1949). *J. biol. Chem.* **177**, 751.
- Haywood, A. M. & Sinsheimer, R. L. (1963). *J. molec. Biol.* **6**, 247.
- Holden, M. & Pirie, N. W. (1955). *Biochem. J.* **60**, 53.
- Huang, R. C. & Bonner, J. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1216.
- Hurwitz, J., Furth, J. J., Malamy, M. & Alexander, M. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1222.
- Ingle, J. & Hageman, R. H. (1965). *Plant Physiol.* **40**, 48.
- Kerjan, P. & Szulmajster, J. (1966). *C.R. Acad. Sci., Paris*, **262**, 312.
- Kirsch, J. F. (1962). *Biochim. biophys. Acta*, **55**, 541.
- Leive, L. (1965). *Biochem. biophys. Res. Commun.* **18**, 13.
- Loening, U. E. (1961). *Biochem. J.* **81**, 254.

- Mach, B. & Tatum, E. L. (1963). *Science*, **139**, 1051.
 Marcus, A. & Feeley, J. (1964). *Proc. nat. Acad. Sci., Wash.*, **51**, 1075.
 Marcus, A. & Feeley, J. (1965). *J. biol. Chem.* **240**, 1675.
 Morton, R. K. & Raison, J. K. (1964). *Biochem. J.* **91**, 528.
 Petermann, M. L., Mizen, N. A. & Hamilton, M. G. (1953). *Fed. Proc.* **12**, 254.
 Pogo, A. O., Pogo, B. G. T., Littau, V. C., Allfrey, V. G., Mirsky, A. E. & Hamilton, M. G. (1962). *Biochim. biophys. Acta*, **55**, 849.
 Schaechter, M. (1963). *J. molec. Biol.* **7**, 561.
 Young, J. L., Huang, R. C., Vanecko, S., Marks, J. D. & Varner, J. E. (1960). *Plant Physiol.* **35**, 288.
 Young, J. L. & Varner, J. E. (1959). *Arch. Biochem. Biophys.* **84**, 71.

APPENDIX

Distribution of Ribosomes in Dormant and Imbibed Seeds of *Pisum arvense*: Electron-Microscopic Observations

BY J. A. CHAPMAN AND M. RIEBER

Rheumatism Research Centre and Department of Biological Chemistry, University of Manchester

(Received 19 April 1967)

This paper describes electron-microscopical studies of dormant and imbibed seed of *Pisum arvense* undertaken in conjunction with the biochemical investigation described in the main paper (Barker & Rieber, 1967).

METHODS

Specimens were selected from the embryos of dry (dormant) and imbibed seed. Imbibition of seed was carried out for 17 hr. as previously described (Barker & Hollinshead, 1964). Preparation of material for electron microscopy used standard procedures, with initial fixation for 2 hr. in cacodylate-buffered glutaraldehyde (Gordon, Miller & Bensch, 1963), washing overnight in sucrose-cacodylate buffer, post-fixation in osmium tetroxide and embedding in Araldite. Thin sections cut with a diamond knife on an LKB Ultratome III were stained with lead hydroxide and examined in a Siemens Elmiskop I instrument.

RESULTS

In dry (dormant) seeds the ribosomes are confined to limited cytoplasmic regions in association with mitochondria. In these regions the electron-opaque ribosomal particles are closely packed together, either in contact with one another or separated by a small gap (Plate 1a). It is difficult to obtain a quantitative assessment of the packing density from a 600 Å-thick section, but a rough estimate gives a value approaching 10^5 particles/ μ^3 . It is to be noted that hexagonal close-packing of uniform spheres with this packing density would require the sphere diameters to be 240 Å; this is not much greater than the reported dimensions of ribosomal

particles from pea seedlings (Ts'o, Bonner & Vinograd, 1956, 1958) and also from rabbit reticulocytes (Mathias, Williamson, Huxley & Page, 1964). So far as could be judged the distribution was uniform with no evidence of ribosomes grouped in clusters or rosettes, although the dense packing precludes any firm assertion that polysomes are absent in dry seeds.

Imbibed seed appears to contain roughly the same total number of ribosomes, but dispersed over much more extensive cytoplasmic regions with the particles separated from one another by correspondingly greater distances (Plate 1b). Assuming a section thickness of 600 Å, the mean packing density in this specimen is about 4×10^3 particles/ μ^3 ; a comparable ribosomal concentration has been reported in other metabolically active cells (Mathias *et al.* 1964; de Petris, Karlsbad, Pernis & Turk, 1966; Chapman, Elves & Gough, 1967). Some specimens showed a higher concentration of ribosomes, but in no case were the ribosomes in imbibed seed embryos packed as closely as in dry seeds.

Polysomes occur in imbibed seed, but, as might be expected from the biochemical evidence presented in the main paper (Barker & Rieber, 1967) the

EXPLANATION OF PLATE 1

(a) Closely packed ribosomes in a dormant seed of *Pisum arvense*. m, Mitochondrion, obliquely sectioned; c, cytoplasmic membrane. Magnification $\times 120000$. (b) Typical distribution of ribosomes in a 17 hr.-imbibed seed. Possible polysomes are indicated by arrows; er, endoplasmic reticulum. Magnification $\times 120000$.

proportion of ribosomes associated into polysomal clusters is small after only 17 hr. imbibition and most fields of view show only isolated ribosomal particles. Occasional elongated endoplasmic reticulum cisternae were noted in imbibed seed, particularly in those specimens in which the free ribosomal particles were widely separated. In dry seed endoplasmic-reticulum profiles were infrequent and invariably short.

CONCLUSIONS

The ultrastructural evidence shows that the different biochemical behaviour of ribosomes from dormant and imbibed seed reported in the main paper (Barker & Rieber, 1967) is paralleled by pronounced morphological differences. Cells of imbibed seed, demonstrated to possess particles active in protein synthesis, show a spatial distribution of ribosomes resembling that encountered in other metabolically active cells. In the dry seed, however, the ribosomes are condensed into regions consisting of closely packed particles. Similar concentrated masses of ribosomes have been noted in the nuclear caps of zoospores of aquatic fungi (Lovett, 1963) and in fungal gametangia and sporangia (Blondel & Turian, 1960; Chapman & Vijućić, 1965). Plant meristematic cells also contain a high concentration of ribosomal particles (Lund, Vatter & Hanson, 1958; Buvat, 1963), although the degree of close-packing appears to be less than that occurring in dormant tissue. Thus the biochemical and morphological evidence presented in this Appendix and the main paper (Barker &

Rieber, 1967) supports the view, expressed by Lovett (1963), that a dense packing of ribosomal particles can be linked with a latent potential for protein synthesis. This is shown to greatest effect in dormancy.

The authors are grateful to Mr S. Grundy and Miss R. Driver for technical assistance and to the Nuffield Foundation for financial support. M. R. is on leave of absence from Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas, Venezuela, and is indebted to the British Council for the award of a Scholarship.

REFERENCES

- Barker, G. R. & Hollinshead, J. A. (1964). *Biochem. J.* **93**, 78.
 Barker, G. R. & Rieber, M. (1967). *Biochem. J.* **105**, 1195.
 Blondel, B. & Turian, G. (1960). *J. biophys. biochem. Cytol.* **7**, 127.
 Buvat, R. (1963). *Int. Rev. Cytol.* **14**, 41.
 Chapman, J. A., Elves, M. W. & Gough, J. (1967). *J. Cell. Sci.* **2**, 359.
 Chapman, J. A. & Vijućić, R. (1965). *J. gen. Microbiol.* **41**, 275.
 de Petris, S., Karlsbad, G., Pernis, B. & Turk, J. L. (1966). *Int. Arch. Allergy, Basel*, **29**, 112.
 Gordon, G. B., Miller, L. R. & Bensch, K. G. (1963). *Exp. Cell Res.* **31**, 440.
 Lovett, J. S. (1963). *J. Bact.* **85**, 1235.
 Lund, H. A., Vatter, A. E. & Hanson, J. B. (1958). *J. biophys. biochem. Cytol.* **4**, 87.
 Mathias, A. P., Williamson, R., Huxley, H. E. & Page, S. (1964). *J. molec. Biol.* **9**, 154.
 Ts'o, P. O. P., Bonner, J. & Vinograd, J. (1956). *J. biophys. biochem. Cytol.* **2**, 451.
 Ts'o, P. O. P., Bonner, J. & Vinograd, J. (1958). *Biochim. biophys. Acta*, **30**, 570.