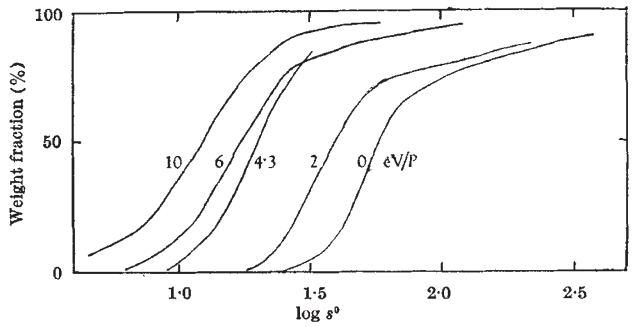

Fig. 2. Integral sedimentation coefficient distribution curves for irradiated thymus deoxyribonucleohistone. Solvent: 0·7 mM phosphate. Ordinate: weight fraction (per cent) of total nucleohistone with s^0 less than a given value. s^0 = sedimentation coefficient extrapolated to zero concentration. (Values for water at 20° C as solvent.) Dosages in eV/P indicated on each curve

A weight-average sedimentation coefficient ($\overline{s^0}$) was calculated (Table 1) from the distribution curves and was found to be related to M_L, also a weight average, by $\overline{s^0} = 9{\cdot}3 \times 10^{-6} M_L^{0{\cdot}66}$. The parallel displacement of the curves in Fig. 2 therefore implies that all the DNH molecules in the original heterogeneous preparation undergo the same fractional decrease in molecular weight, as well as in s^0. Thus all the DNH molecules are degraded to the same extent, and have the same proportionate loss of histone, down to $M_L = 5 \times 10^6$. This excludes the possibility that different DNH molecules in the original distribution have different sensitivities to the action of γ-rays. A similar conclusion was reached[2b] concerning the dissociability of histone from DNH by increasing the salt concentration. One notable feature of the s-distribution is the presence in the original DNA of about 20 per cent of DNH of very high s^0 ($> 150S$), which is manifest as a diffuse leading edge to the boundary, and is degraded to the same extent as the smaller molecules.

Density-gradient sedimentation. Centrifugation (at 44,770 r.p.m.) to equilibrium (22 h) in a gradient of 7·7 molal cæsium chloride discriminates between DNA molecules of different densities, by the position of the final DNA band, and of different molecular weights and composition, by the spreading of the band. In the present case, this technique has the added advantage of allowing effects on DNA in the DNH to be discerned since the latter will be fully dissociated at such a high ionic strength. The bands for un-irradiated DNA and DNH were shown to be identical in position (density) and shape. After irradiation there was a slight increase in density (Table 1) in the direction expected for denaturation (ρ for denatured DNA = 1·712), but the most noticeable change was the marked increase in width of the band. This change could be attributed to the general decrease in molecular weight or to an alteration in the density distribution of the DNA, for example, by traces of the less-dense histone remaining attached to DNA molecules after irradiation. Further experiments on other denatured and degraded DNA and DNH are needed to determine this point.

These observations are consistent with the original proposal[2a] that the initial effect of γ-irradiation on DNH in solution is to dissociate the histone, with at first little or no denaturation of the DNA. This suggestion is in agreement with a variety of radiobiological evidence[5] that ionizing irradiation 'labilizes' the protein–nucleic acid linkages. The proposal that this initial degradation of DNH is a one-hit process is now further supported, and the sedimentation investigations indicate that all the DNH molecules, whatever their initial size, are dissociated by the irradiation to the same extent. These observations will be reported more fully elsewhere.

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A COMPLETE INTRACELLULAR UNIT FOR INCORPORATION OF AMINO-ACID INTO STORAGE PROTEIN UTILIZING ADENOSINE TRIPHOSPHATE GENERATED FROM PHYTATE

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THE storage proteins of developing wheat endosperm accumulate in distinctive electron-dense granules, 0·5–20μ in diameter, as observed in thin sections of suitably prepared tissue[1–3]. By starch-gel electrophoresis, the protein components of these protein bodies may be distinguished from the protein components of the soluble supernatant ('cytoplasmic') fraction of endosperm cells, since the latter are relatively fast-moving as compared with the components of the protein bodies[3]. The sites of synthesis and characteristics of the systems catalysing formation of the storage and cytoplasmic proteins of the endosperm have now been studied and are described here.

Incorporation of amino-acids into proteins of intact tissue, homogenates and isolated cellular components. With intact wheat heads freshly collected at about day 20 after flowering, amino-acids labelled with carbon-14 were taken up from solutions in water and incorporated into the proteins of both the protein body fraction and the supernatant fraction prepared by differential centrifuging of homogenates of the endosperm cells. Incorporation into the protein body fraction was unaffected whereas incorporation into the supernatant protein was considerably inhibited when the cut heads were pretreated with 0·05 M sodium fluoroacetate[4].

When endosperm was homogenized in 0·2 M potassium phosphate buffer, *p*H 7·4, containing 6 mM magnesium chloride and 1 per cent (v/v) of non-ionic detergent ('Nonidet *P*40'), the fraction which in 30 min sedimented between about 5,000 and 20,000*g* ('protein body preparation') consisted principally of protein bodies and plastids. Some of the plastids retained fine structure characteristic of plastids observed by electron microscopy of intact tissue

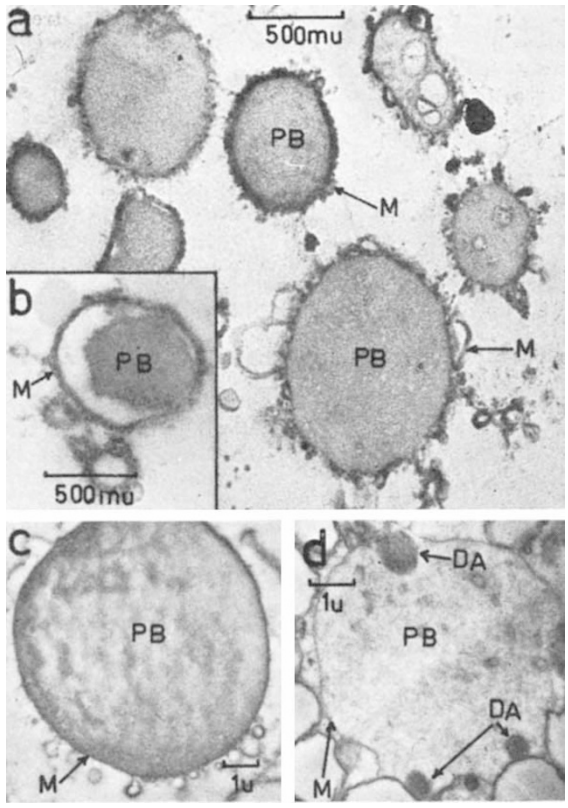

Fig. 1. Protein-forming plastids showing protein body (*PB*), 'dense areas' (*DA*) and membranes (*M*). Figs. 1*a* and 1*b* are of isolated plastids obtained from developing wheat endosperm. Fig. 1*c* is of intact wheat endosperm, and 1*d* is of intact peanut cotyledon. The electron micrographs are of thin sections of material fixed with formaldehyde and later treated with potassium permanganate (ref. 2)

(Fig. 1). The supernatant preparation was substantially devoid of protein bodies and plastids, but contained free ribosomes and lipoprotein material which sedimented at 105,000*g* in 90 min. When incubated with ^{14}C-amino-acids, tissue homogenates and the isolated protein body and supernatant preparations each incorporated amino-acids into protein components; both the protein body proteins and the supernatant proteins of the homogenate contained ^{14}C-amino-acids. Results obtained with homogenates and with the protein body and supernatant preparations showed that the incorporated ^{14}C-amino-acids were not confined to the N- or C-terminal positions; they were not displaced by incubation with an excess of non-isotopic amino-acid, or by prolonged dialysis against 2 M urea or water[5].

Characteristics of incorporation by the supernatant preparation. The supernatant preparation contained ribosomes (85 per cent of which had a sedimentation coefficient of 80*s*; cf. Table 2), some fragments of lipoprotein membranes and soluble protein. Incorporation of amino-acids was strongly inhibited by chloramphenicol, hydroxylamine and fluoroacetate (Table 1). By starch-gel electrophoresis[6], it was shown that radioactivity was associated with each of the characteristic protein components. Supernatant preparations were inactive after storage of grain for only 3 months, whereas active protein body preparations (see below) were obtained from grain stored at $-15°$ C for up to 7 months[5].

Characteristics of incorporation by the protein body preparation. Incorporation by this system was strongly inhibited by chloramphenicol and by puromycin, but only slightly by hydroxylamine and fluoroacetate (Table 1). There was no inhibition by 0·5 mM actinomycin *D*. Radioactivity was associated with each of the several protein components separated by starch-gel electrophoresis[5].

Incorporation of amino-acid was maintained for up to 3 h in some preparations and was not dependent on the addition either of an adenosine triphosphate (ATP)-generating system or of amino-acids. After incubation for 1 h at 30° C, the addition of an ATP-generating system slightly stimulated incorporation. There was no stimulation of incorporation by addition of the supernatant fraction and supernatant ribosomes. As shown here, the lack of dependence of incorporation on addition of any substrate, of ATP, or an ATP-generating system, or on aerobiosis, is due to the presence of protein-bound phytate and of an ATP : phosphoinositol phosphotransferase.

From protein bodies disrupted by ultrasonic vibration (20 kc/s for 3 min in an ice bath), ribosomes, amino-acid-activating enzymes, and transfer RNA were isolated and identified[7,8]. About 83 per cent of the ribosomes had a sedimentation coefficient of 76*s* under similar conditions to those used for the ribosomes from the supernatant preparation (Table 2).

The supernatant obtained from a protein body preparation after ultrasonic disruption and centrifuging at 20,000*g* for 30 min at 2° C contained about 20 per cent of the total nitrogen of the preparation. This supernatant catalysed incorporation of ^{14}C-amino-acids into non-dialysable components which were separated by chromatography on diethylaminoethyl-cellulose into protein and ribonucleic acid. Both the protein and the ribonucleic acid contained radioactivity; that associated with the ribonucleic acid was alkali-labile, indicating an aminoacyl linkage with the ribonucleic acid[8].

Electron microscopy and identification of protein-forming plastids of endosperm. By fixation of endosperm with 3 per cent neutral formaldehyde, and subsequent treatment with potassium permanganate[2], there was satisfactory retention of fine structure in thin sections of endosperm cells. By electron microscopy it was observed

Table 1. Effects of Various Agents on the Incorporation of Amino-acids into the Proteins of the 'Protein Body Preparation' and the 'Supernatant Preparation' of Wheat Endosperm

Reagent	Conc. (mM)	Inhibition (per cent)	
		Protein body preparation	Supernatant preparation
Hydroxylamine	10	14	72
Fluoroacetate	10	0	84
Chloramphenicol	0·3	57	78
	0·7	99	—
Puromycin	1	47	—
	5	68	—
Actinomycin *D*	0·3	0	—

The protein body preparation (ref. 5) contained predominantly plastids and free protein bodies; the supernatant preparation contained protein, ribosomes and some membrane material. The preparations were separately incubated with ^{14}C-proline or with a mixture of ^{14}C-proline, ^{14}C-glutamate, ^{14}C-glycine and ^{14}C-leucine at 30° C for 1 h. The reactions were stopped by addition of urea (final conc., 2 M). Measurement was made of the radioactivity incorporated into the non-dialysable components of the protein body preparation, and into the non-dialysable components (soluble after centrifuging at 105,000*g* for 90 min) of the supernatant preparation.

Table 2. Comparative Sedimentation Coefficients of Major Components of a Disrupted 'Protein Body Preparation' and of a 'Supernatant Preparation'

Sedimentation coefficient and percentage of each component			
From protein body preparation		From supernatant preparation	
S_w	Per cent	S_w	Per cent
35	5	40	10
58	2	60	1
76	83	80	85
107	9	120	3
		132	1

In each case, the pellet sedimented at 105,000*g* for 90 min was resuspended in 0·1 M *tris*–hydrochloric acid buffer, *p*H 7·4, containing 1 per cent of detergent ('Nonidet *P*40'), and 6 mM magnesium chloride, and again sedimented at 105,000*g* for 90 min. The pellet so obtained was suspended in 0·1 M *tris*–hydrochloric acid buffer, *p*H 7·4, containing 6 mM magnesium chloride. Sedimentation coefficients were estimated at 4° C with a Spinco model *E* analytical ultracentrifuge.

that the protein bodies were localized within characteristic morphological structures (plastids) which have a distinctive outer membrane, a reticular network and ribosomes within the membrane[7]. The protein body preparation consisted predominantly of protein bodies (many of which retained fragments of associated membranes), disrupted plastids, and some intact plastids characteristic of those observed in whole tissue[7]. This cell-free preparation, even after repeated washing and sedimentation, or prolonged dialysis against water, retained some amino-acids, amino-acid activating enzymes, ribosomes, organic phosphates, and ATP : phosphoinositol phosphotransferase. This retention of components is attributable to the presence of structures having associated membranes which thereby prevent loss of enzymes and substrates. Since these morphological units catalyse incorporation of amino-acid into proteins of the protein bodies, it is proposed that they be named 'protein-forming plastids' or 'proteoplasts'. Fig. 1 shows typical proteoplasts as isolated from wheat endosperm.

The proteoplast is evidently a structural unit within which storage protein accumulates—a process previously described by us as 'internal secretion'[1]. Several other types of plastids have been described in plants[9,10]; the chloroplast, for example, is a complete unit which has the highly complex structure and organization necessary for the total process of photosynthesis[11]. The proteoplast of plant seeds herein described appears to be an analogous structure having the complex organization and the enzymes and other components necessary for the complete incorporation of amino-acids into storage protein, and it is very probable that it catalyses net synthesis of the storage proteins.

The interpretation of the structure as a plastid is largely based on the ability to isolate the distinctive morphological units having specific functional activity for incorporation of amino-acids into characteristic storage proteins, and to obtain from these, by ultrasonic disruption, enzymes, transfer-ribonucleic acid (*t*-RNA) and ribosomes in a fraction not sedimented at 20,000*g* for 30 min. This supernatant material retains ability to incorporate amino-acid into protein. It is possible that the proteoplasts are formed by development from other cellular structures such as the so-called Golgi apparatus, the endoplasmic reticulum, or the mitochondria. The origin of the analogous amyloplasts and chloroplasts is not yet clearly established.

ATP : phosphoinositol phosphotransferase of wheat endosperm. The protein body preparation from wheat endosperm contained about 0·5 per cent of an acid-stable, organic phosphate which was tentatively identified as phytate. The protein bodies are particularly electron-dense after fixing tissue in potassium permanganate[2]—an unusual finding for protein (Fig. 2). Furthermore, many bodies have areas of high electron density after permanganate treatment; these we have previously described as 'dense areas'[1,2]. It seemed likely that the appearance of protein bodies in electron micrographs could be attributed to protein-bound phytate. Calcium and sodium phytate was therefore separately added to different preparations of 2 per cent gelatin. After the gel had set, pellets were fixed with potassium permanganate and thin sections were cut and examined by electron microscopy. Whereas the electron density of the untreated gelatin was little above background, the material containing phytate was similar in density to that of protein bodies fixed with potassium permanganate (Fig. 2). It is concluded that the protein-bound phytate accounts for the general appearance of permanganate-fixed protein bodies, and that the 'dense areas' are probably areas of accumulation of phytate.

The presence of protein-bound phytate would account for the incorporation of amino-acids into storage protein without requirement for an ATP-generating system, if ATP were formed *in situ* according to the reaction:

$$\text{inositol hexaphosphate} + \text{adenosine diphosphate} \overset{\text{phospho transferase}}{\rightleftharpoons} \text{phospho-inositol} + \text{adenosine triphosphate} \quad (1)$$

and evidence for this reaction was therefore sought.

A protein body preparation containing 4 mg/ml. of nitrogen was disrupted in 0·1 M potassium phosphate buffer, *p*H 7·4, at 20 kc/s for 2 min at about 2° C. The supernatant obtained by centrifuging at 20,000*g* for 30 min at 2° C catalysed incorporation of ^{14}C-proline into protein and there was no need for an ATP-generating system.

As shown in Table 3, this supernatant (preparation *A*, Table 3) also catalysed phosphorylation of ADP, in the presence and absence of added phytate. Similar activity was present in a fraction (preparation *B*, Table 3) precipitated from an homogenate of whole endosperm between 30 and 50 per cent of saturation with ammonium sulphate; preparation *B* contained 5·4 mg of protein per ml. When glucose and hexokinase were included in the reaction solutions, so that ADP was regenerated (Table 3, series I), only catalytic amounts of ADP were needed for formation of glucose-6-phosphate. Moreover, the amount of adenosine monophosphate (AMP) formed was always less than one-fifth of the amount of ATP formed. Thus the low ATP: adenylate phosphotransferase activity did not account for the formation of ATP. Although not dependent on the addition of phytate, formation of ATP was markedly stimulated by phytate, especially with preparation *C* (Table 3), which was obtained by incubating a portion of preparation *B* with an excess of ADP for 90 min at 30° C before dialysis. These results suggest that a bound phosphoryl donor was present in the dialysed enzyme preparations. These preparations were found to contain phytate. The formation of ATP was stimulated by the addition of a portion of the enzyme preparation which had been heated at 100° C for 30 min in either 0·1 N hydrochloric acid or in 0·1 N sodium hydroxide; this stimulation

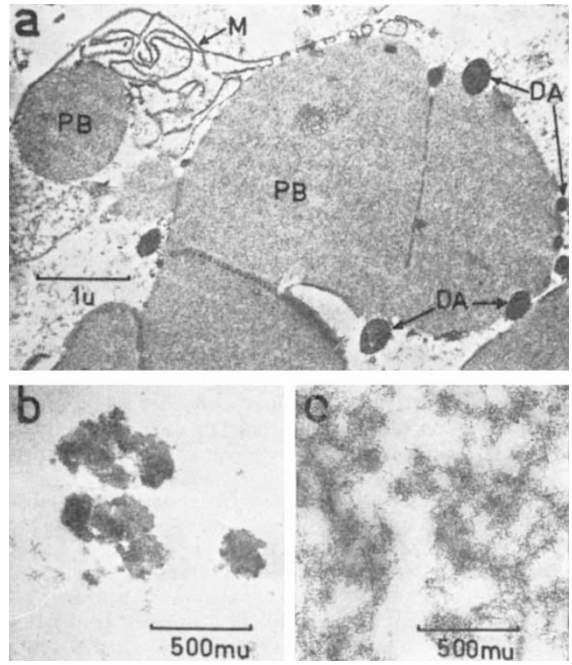

Fig. 2. Comparative electron density of protein bodies of intact wheat endosperm, and of gelatin containing added phytate; all material was treated with 2 per cent of potassium permanganate. Fig. 2*a* is of intact endosperm, and shows protein bodies (*PB*) and 'dense areas' (*DA*); some disruption of the lipoprotein membranes (*M*) of the plastid is apparent. Fig. 2*b* and 2*c* are of gelatin containing 0·5 per cent of calcium phytate (*b*) and of sodium phytate (*c*), respectively. Gelatin without added phytate showed no electron dense material

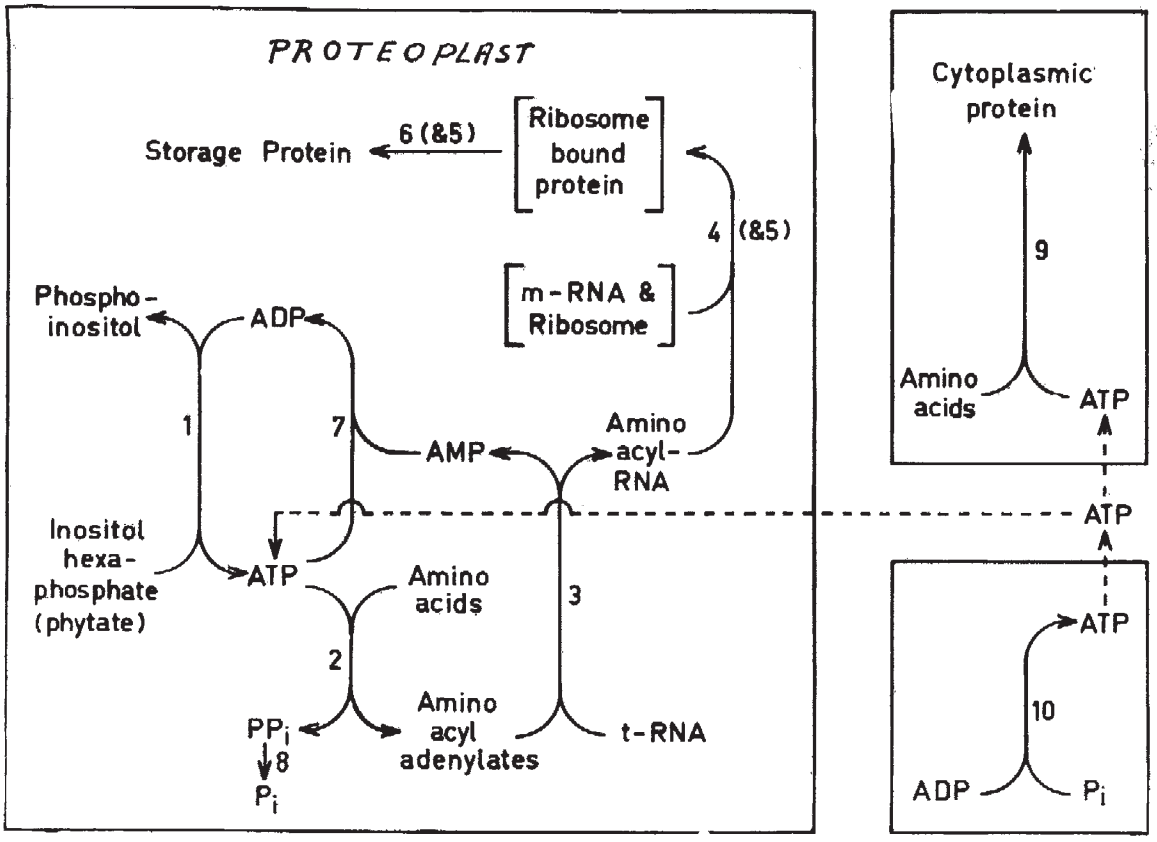

Fig. 3. Diagrammatic representation of compartments in endosperm cells. Each of reactions 1–8 have been identified in isolated proteoplasts (in the protein body preparation), and are catalysed by the following enzymes or enzyme systems: (1) ATP : phosphoinositol phosphotransferase; (2) amino-acid activating enzymes; (3) amino-acid activating enzymes and *t*-RNA; (4) proteoplast ribosomes (presumably functional as ergosomes, and containing *m*-RNA); (5) ATP : nucleosidediphosphate phosphotransferase, catalysing formation of guanosine triphosphate, probably required for reactions 4 and 6; (6) system for release of completed protein, which accumulates within the proteoplast as a protein body; (7) ATP : adenylate phosphotransferase; (8) inorganic pyrophosphatase

Reaction 9 summarizes the ATP-linked reactions (in the supernatant preparation) leading to formation of cytoplasmic proteins outside the proteoplast. Reaction 10 summarizes the formation of ATP linked to the oxidation of substrates in the endosperm; this is the probable site of action of fluoroacetate which gives selective inhibition of incorporation of amino-acids into cytoplasmic proteins. The possible movement of ATP is represented thus (- - - - →); phytate in the proteoplast acts as a buffer of the ATP–ADP system

was similar to that obtained with phytate (Table 3). It therefore appears that protein-bound phytate, or a derivative of phytate, acts as a substrate for a phosphotransferase present in the protein body preparations, and in the fractions isolated from whole endosperm.

Since there is some uncertainty as to the true structure of phytate and of its derivatives[12], we propose that the enzyme herein described should be known at present by the trivial name 'ATP : phosphoinositol phosphotransferase' (cf. Enzyme Commission Report[13]). The terminology 'phosphoinositol' indicates uncertainty as to the exact nature of the donor and of the product formed, although these are probably *myo*-inositol hexaphosphate and pentaphosphate respectively. Further properties of the enzyme, determination of the nature of the products, the position of the equilibrium in the reaction and the biosynthesis of phytate in wheat endosperm are being studied[14].

Role of phytate as a phosphagen. Phytate occurs in relatively high concentration in plant seeds[15,16], nucleated erythrocytes[17] and other tissues.

Atkinson and Morton[18] proposed that phytate possibly provided a reserve of reactive phosphoryl groups, since charge repulsion between adjacent groups could result in a relatively high phosphoryl transfer potential. 'Aleurone grains' and protein bodies of peanut (*Arachis hypogea*) cotyledons, like those of wheat endosperm, contain phytate[15]. These observations suggest that the phytate–phosphoinositol system acts as a buffer of the ATP–ADP system in the protein-forming plastids, maintaining the concentration of ATP at the optimum for synthetic functions. Thus phytate appears to be a localized phosphagen in plant storage organs, and may have a similar function in animal tissues such as the nucleated erythrocyte—a structure which, like the proteoplast, accumulates storage protein (hæmoglobin). Fig. 3 is a diagram representing the localization of the reactions which have been demonstrated to be associated with the proteoplast, and with other components of endosperm cells.

Occurrence and distribution of proteoplasts. Electron micrographs[19] of developing cotyledons from a wide range of leguminous seeds have shown the presence of protein bodies within structures comparable with the proteoplasts of wheat endosperm (Fig. 1). Although functional activity has thus far only been demonstrated in proteoplasts isolated from wheat endosperm, it is very likely that the structures of leguminous seeds have similar activity.

A striking feature of the isolated protein-forming plastids of wheat endosperm is the marked stability of the system for incorporation of amino-acids. Presumably, the plastid ribosomes carry a messenger-ribonucleic acid (*m*-RNA) having the code for formation of storage protein. This *m*-RNA, however, appears to be relatively stable. The failure of actinomycin *D* to inhibit the incorporation of amino-acids into the storage protein (Table 1) suggests that the incorporation does not depend on a deoxyribonucleic acid (DNA)-linked synthesis of *m*-RNA. Since formation of storage protein in the seed leads to an accumulation of reserves at the end of the life-cycle of the

Table 3. ATP : PHOSPHOINOSITOL PHOSPHOTRANSFERASE ACTIVITY OF ENZYME PREPARATIONS FROM WHEAT ENDOSPERM

Enzyme preparation	Reactants (μmoles per 3 ml. of reaction solution)		Period of reaction at 30° C (min)	ATP determined as glucose-6-phosphate (μmoles per 3 ml. of reaction solution)		Remarks
	ADP	Phytate		I	II	
(*A*) 1 ml.	0	1·0	30	0·44	—	Supernatant fraction from protein body preparation
	0·1	0	30	0·47	—	
	0·1	0·25	30	0·52	—	
	0·1	0·50	30	0·65	—	
	0·1	1·0	30	0·69	—	
(*B*) 0·2 ml.	0	2	30	0·10	0·07	Dialysed protein fraction from whole endosperm; *a* and *b* were additions of 0·4 ml. of enzyme (preparation *B*) which had been pre-treated at 100° C for 30 min with 0·1 N HCl and 0·1 N NaOH, respectively; *c*, phytate added after 60 min incubation
	1·2	0	30	0·25	0·27	
	1·2	2	0	0	0	
	1·2	2	10	0·26	0·22	
	1·2	2	20	0·34	0·27	
	1·2	2	30	0·38	0·40	
	1·2	*a*	30	—	0·46	
	1·2	*b*	30	—	0·41	
	6·0	2	0	—	0	
	6·0	0	60	—	0·68	
	6·0	0	240	—	0·62	
	6·0	2	240	—	1·04	
	6·0	2*c*	120	—	1·14	
(*C*) 0·4 ml.	1·8	0	30	0·68	—	Protein fraction from whole endosperm pre-incubated with ADP and dialysed
	1·8	0·5	30	2·34	—	
	1·8	1·0	30	2·38	—	
	1·8	2·0	30	1·79	—	
	1·8	3·0	30	1·37	—	

The reaction solutions (final volume, 3 ml.) contained the dialysed enzyme preparation (0·2–1·0 ml.), ADP, sodium phytate, magnesium chloride (6 μmoles) and potassium phosphate buffer (100 μmoles) at *p*H 7·4. Formation of ATP was measured enzymatically (refs. 23, 24) after conversion to glucose-6-phosphate with added glucose (200 μmoles) and hexokinase (0·8 mg of Type II, Sigma Chemical Co., United States). Glucose and hexokinase were present during the reaction with the endosperm enzyme in series I, but were omitted in series II. Adenosine monophosphate (AMP) and ADP were also determined enzymatically (ref. 24), and changes in adenine nucleotides were also determined qualitatively after separation by paper electrophoresis (ref. 24).

plant, there is no obvious requirement for active control of the process, and this may be reflected in the apparent stability of the *m*-RNA.

As yet, our investigations have been confined to plant seeds, but it seems likely that structures with similar functions may occur in animal cells, particularly in oocytes of gastropods, amphibia and other animals. These oocytes contain yolk platelets, consisting of protein which is apparently formed in specialized structures[20–22]. These 'Golgi bodies'[21] or 'modified mitochondria'[20,22] may well have similar functions to those of the phytate-containing protoplasts described here.

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INTERFERON RESPONSES OF CHICK EMBRYO FIBROBLASTS TO NUCLEIC ACIDS AND RELATED COMPOUNDS

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IT has been demonstrated that the production of interferon by chick cells exposed to virus is a response to the nucleic acid component[1]. This led to the hypothesis that other nucleic acids might also provide such a stimulus, and we have found that several common nucleic acids will induce monolayer cultures of chick embryo tissue to produce interferon. These observations confirm and extend those recently reported by Rotem, Cox and Isaacs[2], who found that nucleic acids foreign to chick or mouse embryo fibroblasts inhibited virus multiplication when the added RNA was present in cell cultures during infection.

Assays of interferon activity were carried out in tube cultures of chick fibroblasts against vaccinia virus collected from chick embryo tissue cultures. The rubber-stoppered tubes contained 0·9 ml. of Eagle's basal medium with Earle's salts (EBME) maintaining monolayers of 24-h cultures incubated in a stationary position at 37° C. To a series of three tubes was added 0·2 ml. of test material in 2-fold dilution and, after incubation for 24 h, vaccinia virus (10 $TCID_{50}$ per 0·1 ml.) was added. Following three additional days of incubation, monolayers were examined for vaccinia cytopathic effect. The cultures showing no lesions were recorded, the titre of interferon calculated by the Reed and Muench method[3], and expressed as the reciprocal of dilution which would protect 50 per cent of the cultures. When protection was found only in those