technique used. In the present paper it has been shown that water extracts of separated aleurone layers contain glutamate decarboxylase activity in easily detectable amounts. Thus barley aleurone appears to have the capacity to produce GABA as does the embryo[1]. It does not follow, however, that the functions of GABA are the same in both tissues: in embryos the enzyme glutamate decarboxylase increases in activity as germination proceeds and considerable quantities of GABA are synthesised[1], whereas in aleurone cells the glutamate decarboxylase activity declines steeply after a few hours of germination, and GABA cannot at present be detected. It has been suggested that in the developing embryo glutamate decarboxylase and GABA are important in the build-up and functioning of the Krebs cycle[1,3] whereas in the aleurone cells it seems possible that GABA may serve a regulatory function.

The discovery of a possible control function for GABA in plants suggests that GABA may have the same two broad functions in plants as in animals[12–14]. Namely as an intermediary metabolite concerned with the functioning of the Krebs cycle and as part of a system controlling the development and functioning of the overall organism[15].

Résumé. L'enzyme glutamate décarboxylase se trouve dans les extraits d'eau de l'aleurone de l'orge. Au début de la germination, l'activité de cette enzyme augmente, mais après, quand apparait l'enzyme α-amylase, elle diminue. Quoiqu'au dessous d'une concentration de $2\times10^{-6}M$ l'acide γ-amino butyrique (GABA) n'affecte pas l'activité de l'α-amylase in vitro, la GABA à $5\times10^{-6}M$ peut provoquer une inhibition de 25% qui n'est pas modifiée par des concentrations de GABA comprises entre $5\times10^{-6}M$ et $10^{-3}M$.

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Characterization of Nuclei from Immature Barley Endosperm

The ultrastructure of the developing barley endosperm has been described by BUTTROSE[1]. Little is known, however, of the biochemical events following anthesis and, in particular, of the role of the nucleus in these events.

Few convenient methods for the isolation of nuclei from higher plants have been reported, mainly because of the presence in the selected tissues of chloroplasts, amyloplasts and mechanically resistant cell walls. In the cereal grain, however, immediately after anthesis, rapid division of the triploid endosperm nucleus takes place without accompanying cell wall formation[2]. The nuclei are close packed with only a few undifferentiated spherical bodies present. Thus a method could be easily devised for the isolation of relatively uncontaminated and intact nuclei. This paper then describes some of their properties in terms of chemical composition, metabolic activity and histone content.

Materials and methods. The 2 row barley, *Hordeum distichum* (L.) Lam. CV. MARIS BALDRIC, was used and was grown either in quantity on the University farms or in the greenhouse under lights.

100 grains, never older than 3 days after anthesis were individually squashed in an ice-cold agate mortar in 2 ml of M sorbitol containing 30% glycerol and $0.001\,M$ Ca^{2+}. The mixture was filtered through 2 layers of muslin on to 2.0 ml of $1.8\,M$ sorbitol, containing 30% glycerol and $0.001\,M$ Ca^{2+} in a 5 ml polypropylene centrifuge tube. Thus 2 layers were formed. A glass rod was passed once through the interface to give a slight mixing of the layers. This was centrifuged for 20 minutes at $200\times g$ and the nuclei accumulated in the lower layer. The top layer and interface were discarded. The lower layer was then transferred to another 5 ml centrifuge tube and centrifuged at $200\times g$ for 2 h. The pellet was further compacted by accelerating to $1{,}000\times g$ for 1 min at the end of the 2 h. This pellet was used without further treatment in the experiments to be described.

The purification was monitored routinely with a Vickers Patholux microscope using phase contrast lenses and a magnification of 1,000 diameters. A Leitz microscope was used to take phase contrast photomicrographs at a magnification of 700 diameters.

DNA, RNA and protein were determined in the nuclear pellet as described by ROZIJN and TONINO[3]. RNA polymerase was assayed as described by WEISS[4] using boiled nuclei as a control. The pH of assay was 7.8. NAD pyrophosphorylase was assayed by the method of GINSBURG-TIETZ et al.[5] and the NAD synthesized measured by the method of KLINGENBERG[6]. Fumarase was assayed by the method of PIERPOINT[7] and succinoxidase spectrophotometrically as described by VEEGER et al.[8].

Histones were extracted from the nuclear pellet by a modification of the method of MURRAY et al.[9]. The nuclei were extracted with 3.0 ml of a KCl-HCl buffer ionic strength, 0.1, pH 2.8, and the suspension left at 4 °C for 20 min before centrifuging at $5{,}000\times g$ for 10 min. The supernatant was discarded and the pellet washed once with 3.0 ml of the same buffer. The washed pellet was then

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extracted with 3.0 ml 0.25 *M* HCl and the suspension centrifuged at 5,000 × *g* for 10 min. The supernatant was removed with a Pasteur pipette and 9 volumes of acetone at −18 °C added to it. The mixture was left overnight at −18 °C and the resulting precipitate pelleted and washed 3 times with acetone before drying under vacuum.

Histones were extracted from whole cells by squashing 60 whole grains in 2 ml of HCl-KCl buffer pH 2.8, ionic strength 0.1, in an agate mortar. After 20 min at 4 °C the mixture was spun at 5,000 × *g* for 20 min without filtering. The supernatant was discarded and the pellet washed with a further 3 ml of the same buffer. The pellet was then homogenized in a glass homogenizer with 3 ml 0.25 *M* HCl, left for 20 min at 4 °C, and centrifuged at 5,000 × *g* for 20 min. The histones were precipitated from the supernatant as described above.

Polyacrylamide gel electrophoresis was carried out by the method of JOHNS[10] using the Shandon disc electrophoresis apparatus and Vokam power unit.

Results and discussion. The isolated nuclei are extremely fragile and rupture under even the slight mechanical pressure of a cover slip. Thus, since they are obtained as a concentrated suspension rather than as a pellet and applied to the slide in a larger than ideal volume, it is difficult to obtain a uniform focal plane for more than a few nuclei (Figure 1). The nuclei are large, 3–5 μm and very similar to those seen in fixed and stained sections of the original tissue. Examination of the nuclear preparations by phase contrast microscopy revealed little cytoplasmic contamination. The mitochondrial enzymes succinoxidase and fumarase, often used as an indication of cytoplasmic contamination, were not detected in the nuclear pellet. Thus the preparation satisfies the criteria for morphological appearance under phase contrast, nuclear enzyme activity and low cytoplasmic activity suggested by ROODYN[11].

The nuclei are characterized by a relatively low DNA: RNA ratio compared to pea seedling nuclei[12] (Table I). In whole grain sections fixed and stained with Feulgen, endosperm nuclei have a much lower colour intensity than those in the surrounding tissue. This is consistent with the low nuclear DNA concentration. Furthermore the endosperm nuclei are associated with a rapidly developing tissue and soon after the period of free nuclear division, cell walls are formed, amyloplasts quickly fill the cells and the nuclei disappear. Endosperm protein synthesis still continues in their absence[13]. Thus the nuclei, at the time of isolation must be actively synthesizing both ribosomal and messenger RNA and this might account for a high nuclear RNA content.

The isolated nuclei are metabolically active and have appreciable RNA polymerase and NAD pyrophosphorylase activity (Table II). That the RNA polymerase was DNA dependent was shown by a 5-fold decrease in activity following a 20 min preincubation of the nuclei with deoxyribonuclease. A significant decrease in activity was also observed by a 5 min post-incubation treatment with ribonuclease showing that the radioactive product was indeed RNA. The results for RNA polymerase and NAD pyrophosphorylase compare favourably with those for pea[12] and yeast[14] nuclei, respectively. Results with animal cell nuclei[15] have shown that RNA polymerase is firmly bound to DNA and that NAD pyrophosphorylase is associated with nuclear ribosomes isolated after disruption of the nuclear membrane[16]. An RNA polymerase in plant cell nuclei has been demonstrated by RHO and CHIPCHASE[17].

The disc electrophoretic patterns of histones from endosperm nuclei (B) and intact endosperm cells (C) run simultaneously with a control of calf thymus histone are shown in Figure 2. The first 2 bands are impurities found at the origin and may be ignored. Only one histone component can be seen in the nuclear extract. This has a mobility between that of the first and second calf thymus histones. Whole cells have 3 additional components of which 2 are

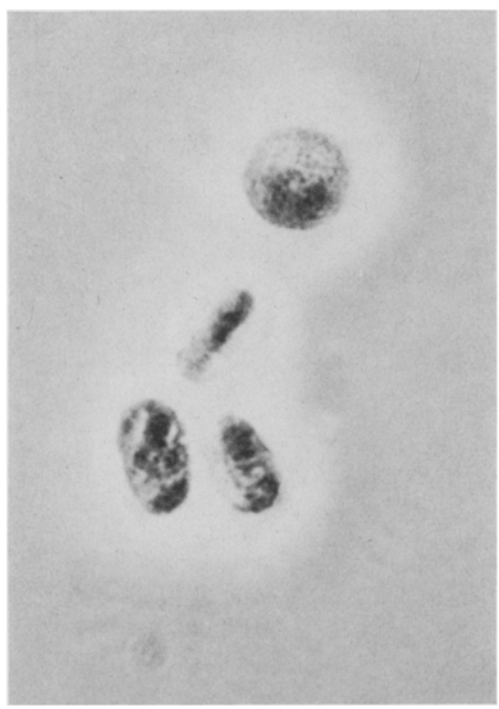

Fig. 1. Phase contrast micrograph of barley endosperm nuclei isolated by the method described in the text.

Table I. Chemical composition of isolated nuclei

Tissue	DNA (%)	RNA (%)	Protein (%)
Nuclei	7 ±2.9 (5)	16±5 (5)	77±4 (5)
Whole endosperm cells	1.0±0.5 (3)	24±8.5 (3)	75±7.6 (3)
Pea seedling nuclei (BIRNSTIEL et al.[12])	13	10	77

For each experiment the sum of DNA, RNA and protein is set at 100. The results are expressed as percentages of this sum, with standard deviations. The figures for whole endosperm cells and pea seedling nuclei are given for comparison. The number of experiments are given in parenthesis.

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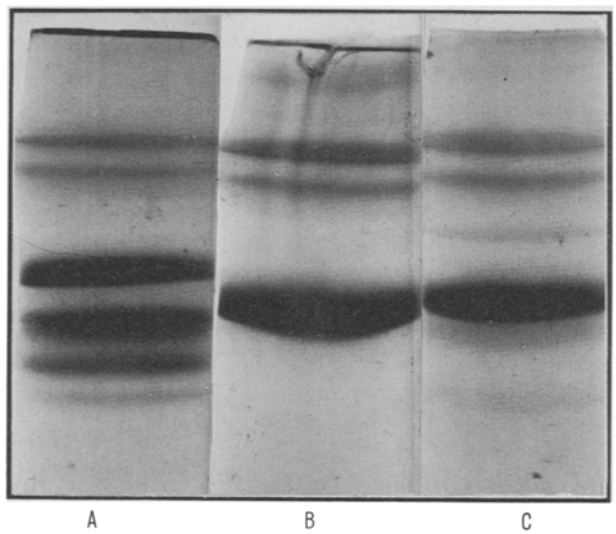

Fig. 2. Acrylamide gel electropherogram of A) calf thymus histones, B) nuclear histone, C) intact cell histones.

of greater and 1 of less mobility than the nuclear component.

The chemistry and biology of pea histones has been reviewed by SMITH et al.[18]. It was considered that while there may be no histone fraction specific to any pea tissue, reproducible quantitative differences may be observed in the histone fractions from different tissues[19].

From the present work one must conclude that there appears to be only one histone present in endosperm nuclei at this stage in their development. This histone corresponds in mobility to histone II extracted from purified pea chromatin as described by SMITH et al.[18].

The additional 3 components in the extract of whole cells may be either basic proteins, possibly ribosomal or, since the preparation almost certainly contains non-endosperm tissue, histones derived from embryo or seed coat nuclei. The mobility pattern of the whole cell histones is certainly similar to that associated with histones from pea tissue chromatin.

It may well be that the pattern for pea tissue chromatin is also a composite one derived from nuclei at different developmental stages. In the present case, however, the histone fraction was prepared form a fairly homogenous preparation of nuclei at the same developmental stage. Since endosperm nuclei disappear and presumably disintegrate after cell wall formation and amyloplast accumulation, the presence of a single histone may be related to their metabolic decline[20, 21].

Résumé. Les noyaux d'endosperme ont été isolés de grains très jeunes d'orge. Le DNA, RNA et la protéine offraient entre eux les rapports 7:16:77. Les enzymes RNA polymérase (Nucléoside triphosphate: RNA nucléotidyle transférase, E.C. 2.7.7.6) et NAD pyrophosphorylase (ATP: NMN adényl transférase, E.C. 2.7.7.1) ont été observés dans les noyaux isolés. Un seul histone peut être trouvé dans la préparation des noyaux.

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Table II. Metabolic activity of isolated nuclei

Enzyme	Activity
RNA polymerase	nmoles of (^{14}C) ATP incorporated into RNA/mg protein/40 min
Normal system	2.7
0.05 ml ribonuclease (1 mg/ml) for 5 min after incubation	2.0
Nuclei + 0.05 ml deoxyribonuclease (1 mg/ml) for 20 min before incubation	0.5
NAD pyrophosphorylase	nmoles NAD/mg protein/30 min
	0.16

Each figure is the mean of 2 experiments.

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Transaminase Activities of Root Protoplasts

By eliminating walls of bacterial[1] cells, it has been possible to obtain physiologically intact forms named respectively for the Gram bacteria – and[2] spheroplasts and protoplasts. Such structures are of the greatest interest for both biochemistry and genetics. And it is understandable that one may be tempted to prepare similar forms in other organisms, and particularly in higher plants. Roots of Tomato were the first to be used[3] and the word 'protoplast'[4] was attributed to cells deprived of their skeletal envelope.