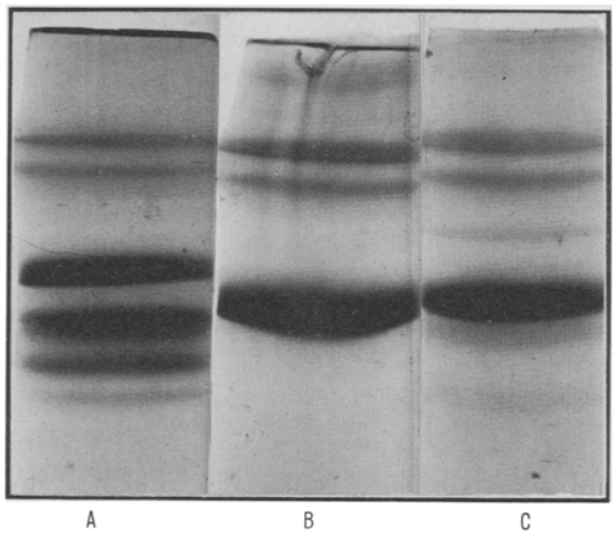

Fig. 2. Acrylamide gel electropherogram of A) calf thymus histones, B) nuclear histone, C) intact cell histones.

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.

Table II. Metabolic activity of isolated nuclei

Enzyme RNA polymerase	Activity 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.

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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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.

The elimination of walls is achieved most often by the action of various enzymes[5] (method E). However, a technique based on old observations[6,7] has recently been worked out[8], which makes it possible to obtain protoplasts mechanically by simply breaking the cell walls (method M). Method E allows an abundant production of large protoplasts, while method M does not. This last method, however, has the great advantage of avoiding any obviously disturbing[9] enzymatic action. Until now a comparative analysis of both these techniques has not led to any systematic comparisons. On the other hand, though cytological properties of protoplats are becoming well known[10], particularly at the level of their ultrastructure[11–13], their biochemical characteristics are far less known. A synthesis of their proteins[14] and their behaviour as regards a few growth hormones[15,16] have produced few publications.

The importance of transaminases in plant cells has been recognized for a long time[17]. They have been chosen as a measurement for the enzymatic activity of protoplasts, an activity which may be considered as one of the best means of evaluating their physiological reactivity[9].

Protoplasts are obtained from root cells of *Allium cepa* (10 cm). The method E is inspired by that of TAKEBE et al.[5]; it is based on the use of maceroenzyme and cellulase. The method M is, roughly speaking, that which PRAT and ROLAND[8] have worked out. Two transaminase systems will be examined here, the glutamic-oxalacetic-transaminase (GOT) and the glutamic-pyruvic-transaminase (GPT) transaminases. These enzymes are extracted and dosed according to the RELTMANN and FRANKEL[18] technique, adapted to root extracts[19]. The principle of determination is based on a spectrophotometric analysis of coloured hydrazones. The unity of transaminase activity is defined[20] as being the extracting activity able to produce at 37 °C in 60 min (GOT) or in 30 min (GPT) a quantity of ketonic acids corresponding to 1 µg of pyruvate (standard curve).

Table I. Comparative transaminase activities[a] (GOT and GPT) of protoplasts mechanically (M) and enzymatically (E) prepared from 10 cm roots of *Allium cepa*

Levels (cm)	GOT		GPT	
	M	E	M	E
0.5– 1.5	50.9	41.0	20.7	17.4
±	4.1	4.2	2.5	3.1
4.5– 5.5	27.6	24.1	12.4	16.1
±	3.5	3.2	2.0	2.5
9.0–10.0	10.2	7.9	6.0	5.8
±	2.1	2.7	1.9	2.1

Each result is the average of 14 values. [a] In µg equivalents ($\times 10^{-5}$) of pyruvate formed by 10^7 protoplasts.

Table II. Relative transaminase activities (GOT and GPT) of protoplasts mechanically (M) and enzymatically (E) prepared from the 10 cm roots of *Allium cepa* (level: 4.5–5.5)

	GOT		GPT	
	M	E	M	E
Activity[a] (A)	27.6	24.1	12.4	16.1
Mean volume (V) in µm³ per protoplast	2004	9670	2210	9305
Protoplasts counted	197	132	180	121
$10^4 \cdot$ A/V	137.7	24.9	56.1	17.3
M/E	5.5 ± 0.4		32 ± 0.3	

[a] see Table I.

On the other hand, since transaminases vary in activity according to the age of roots and the cell differentiation[21], it was interesting to compare transaminase activity in 3 different regions (0.5 to 1.5, 4.5 to 5.5 and 9.0 to 10 cm, starting from the root apex) of a root having served for preparing protoplasts.

These results (Table I) authorize the following few comments. Transaminase activity – which is always lower in the GPT system – is proportionately weaker as protoplasts originate from parts more distant from the apex. These values confirm those obtained with LENS[21] root cells. It shows without ambiguity, that enzymatic activity of protoplasts really does reflect that of those cells from which the protoplasts were prepared. Even though results obtained for both types of protoplasts appear to indicate generally that transaminase activity is stronger in protoplasts mechanically prepared (method M), these datas, however, are not significant. But, though the relative number of protoplasts obtained by both methods cannot be compared, the size of these protoplasts is also very different[9]. For this reason, values of transaminase activity were also compared with an unit of protoplasts volume. The results related to protoplasts at the level of 4.5 to 5.5 (Table II) show that the transaminase activity is stronger (5.5 times for GOT and 3.2 times for GPT) for protoplatsts prepared mechanically. Thus, these data indicate that the enzymatic preparation is not beyound criticism; by using enzymes which dissociate cell walls, several biochemical and physiological properties of the protoplasts were altered.

Résumé. Deux systèmes transaminasiques (GOT et GPT) de protoplastes, obtenus mécaniquement et enzymatiquement à partir de cellules de racines d'*Allium cepa*, sont analysés. Les résultats indiquent que l'activité transaminasique (GOT étant supérieur à GPT) des protoplastes est le reflet de celles des cellules à partir desquelles ils ont été préparés (et ceci notamment en rapport avec leur état de différentiation). Par ailleurs, les protoplastes formés mécaniquement sont plus actifs; ceci indique notamment que les enzymes employées pour dégrader les parois perturbent la biochimie et la physiologie des protoplastes obtenus.

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