THE DISTRIBUTION OF THE ENZYMES IN RESTING CEREALS

IV. A COMPARATIVE INVESTIGATION OF THE DISTRIBUTION OF ENZYMES AND MITOCHONDRIA IN WHEAT GRAINS*

by

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In the preceding communications (ENGEL[1], ENGEL AND HEINS[2], ENGEL[3]) we have given the results of the enzyme determinations on the different parts of the cereal grains. It was found possible to calculate the approximate enzyme content of each kind of tissue.

It now became pertinent to enquire whether it would be possible to connect some cytological structures with the distribution of the enzymes.

LINDERSTRØM-LANG[4], PHILIPSON[5], HOLTER[6] and DOYLE[7] have been able to demonstrate that the peptidase and catalase activity in the ova of marine invertebrates is not

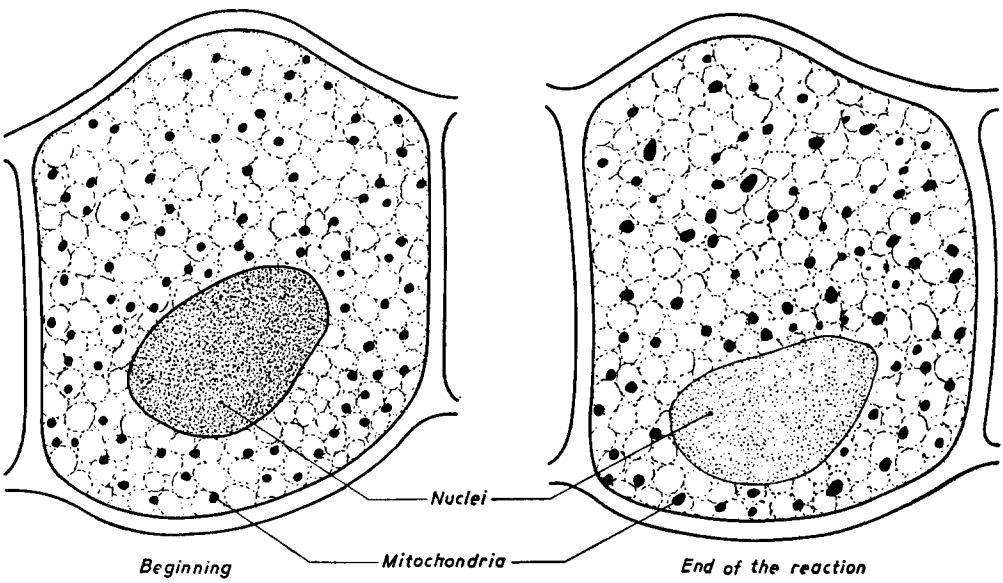

Fig. 1. The staining procedure of mitochondria with Janus green

bound to any microscopical granular constituent of the cell but that these enzymes were localized in the hyaline ground substance of the cytoplasm.

* For III, see CHR. ENGEL, *Biochim. Biophys. Acta* 1 (1947) 278.

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HOLTER AND DOYLE[8] found however that the amylase in amoeba is distributed differently in the protoplasm. The localization of the mitochondria seemed to be the same as that of the amylase.

For cereal grains the same problems have been discussed by HORNING AND PETRIE[9] and HORNING[10]. These authors found that the mitochondria were associated in some way with the enzymes.

In this paper we have investigated the distribution of mitochondria in the different tissues of wheat grains and have compared the results with those obtained on enzymes which have been described in the earlier communications of the first author[1,2,3].

EXPERIMENTAL

1. *Histological technique*

Grains of Manitoba wheat were enclosed in melted paraffin without preceding fixation and were cut with a microtome longitudinally and transversely in 10 μ thick slices. The latter were brought on object glasses which were moistened with a weakly acidified solution of Janus green B.

Not acidified solutions caused a fine granular precipitation in the slices owing to which it was impossible to identify the mitochondria. Staining with iron haematoxyline after fixation did not give good results because then much thinner (3–5 μ) sections were needed.

The dehydrated cells of the slices quickly take up the Janus green solution and successively the different structures were stained, in the beginning only the cellwalls and later also the mitochondria and the nuclei. During the staining procedure the volume of the mitochondria increases and at the end of the staining period they may be observed as big granula or globules, cf. Fig. 1.

After half an hour the mitochondria are deeply coloured; after 1 to $1^1/_2$ hour the intensity of the colour diminishes and changes via red to colourless. In the nucleus the same phenomena may be observed in an earlier stage. The course of the colouration was in accordance with our experience from animal material. In experiments with other dyes than Janus green, as neutral red, nillblue sulfate and gentian violet only the latter stained the mitochondria, this forms a support for the opinion that they are really the mitochondria.

2. *Counting of the mitochondria*

By staining intra vitam with Janus green in the described manner the mitochondria are well identified and when they are deeply coloured it is possible to count them in white or pale blue light.

For this purpose a chequered eyepiece micrometer was used. The size of one square was determined with the help of an objective micrometer and was 290 μ^2. As the slices were 10 μ thick the content of each volume unit was 10 $\mu \cdot 290\ \mu^2 = 2900\ \mu^3$. The number of mitochondria was counted in 50 volume units and was calculated per μl.

The principle of the counting is demonstrated in Fig. 2.

In Fig. 3 some detailed pictures are given of the staining of mitochondria in the different cells.

In the aleurone layer the highest number of mitochondria was counted, cf. Table I. The starch containing cells of the subaleurone and inner endosperm did only contain small islands of protoplasm and a strongly deformed nucleus. The amount of mitochondria is rather high but less than in the aleurone cells.

TABLE I

NUMBER OF MITOCHONDRIA PER μl OF TISSUE

Kind of tissue	Mitochondria per μl
Aleurone layer cells	$9.4 \cdot 10^6$
Subaleurone cells	$2.5 \cdot 10^6$
Starch cells	$1.7 \cdot 10^6$
Epithelial layer of scutellum	$3.4 \cdot 10^6$
Scutellum	$5.7 \cdot 10^6$

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COMPARISON OF THE DISTRIBUTION OF MITOCHONDRIA AND ENZYMES

In the first place it must be remarked that the determinations of enzymes and mitochondria have been carried out on different grains. The enzyme content of each grain is not the same, but qualitatively however the distribution over the various tissues

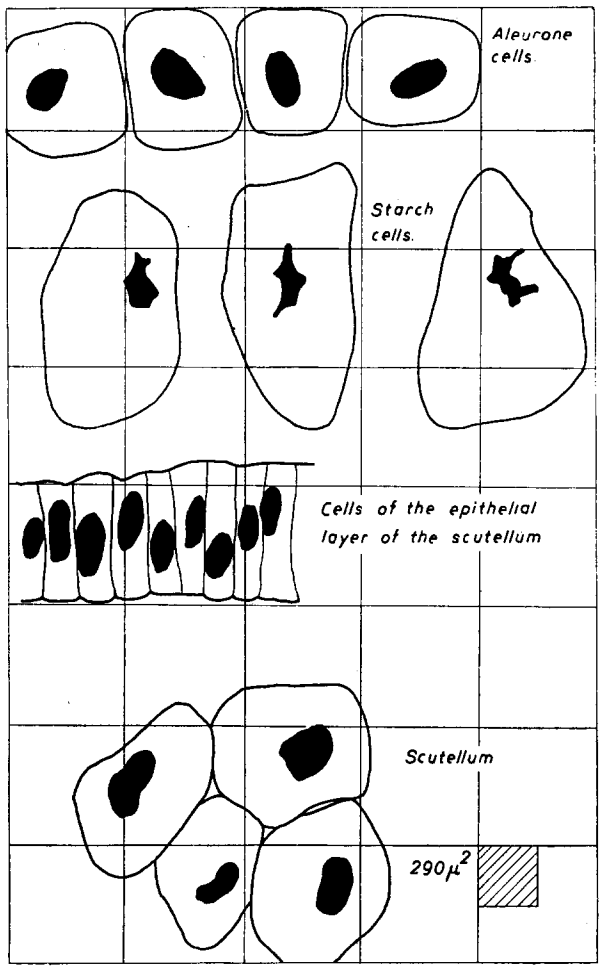

Fig. 2. Method of counting

is for all grains comparable. Therefore, the distribution of mitochondria may be qualitatively compared with that of enzymes.

To compare the results of mitochondria countings and the determinations of enzymes from the previous communications, the enzyme content must be calculated per μl of tissue of every kind.

This calculation was carried out in the following way.

Amylase, cf. ENGEL[1] Fig. 2, 7 A en 7B.

The first slice (25 μ) was pericarp and testa and did not contain any amylase.

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The second and third slice (50 μ) contained aleurone cells and were also without amylase activity.

The amylase content of the subaleurone layer ($\pm$ 100 μ thick) was calculated as the average of the amylase content of the subaleurone cells of the next four 25 μ slices. Slice nr 4 contained 23% subaleurone tissue (curve III) and had an amylase content

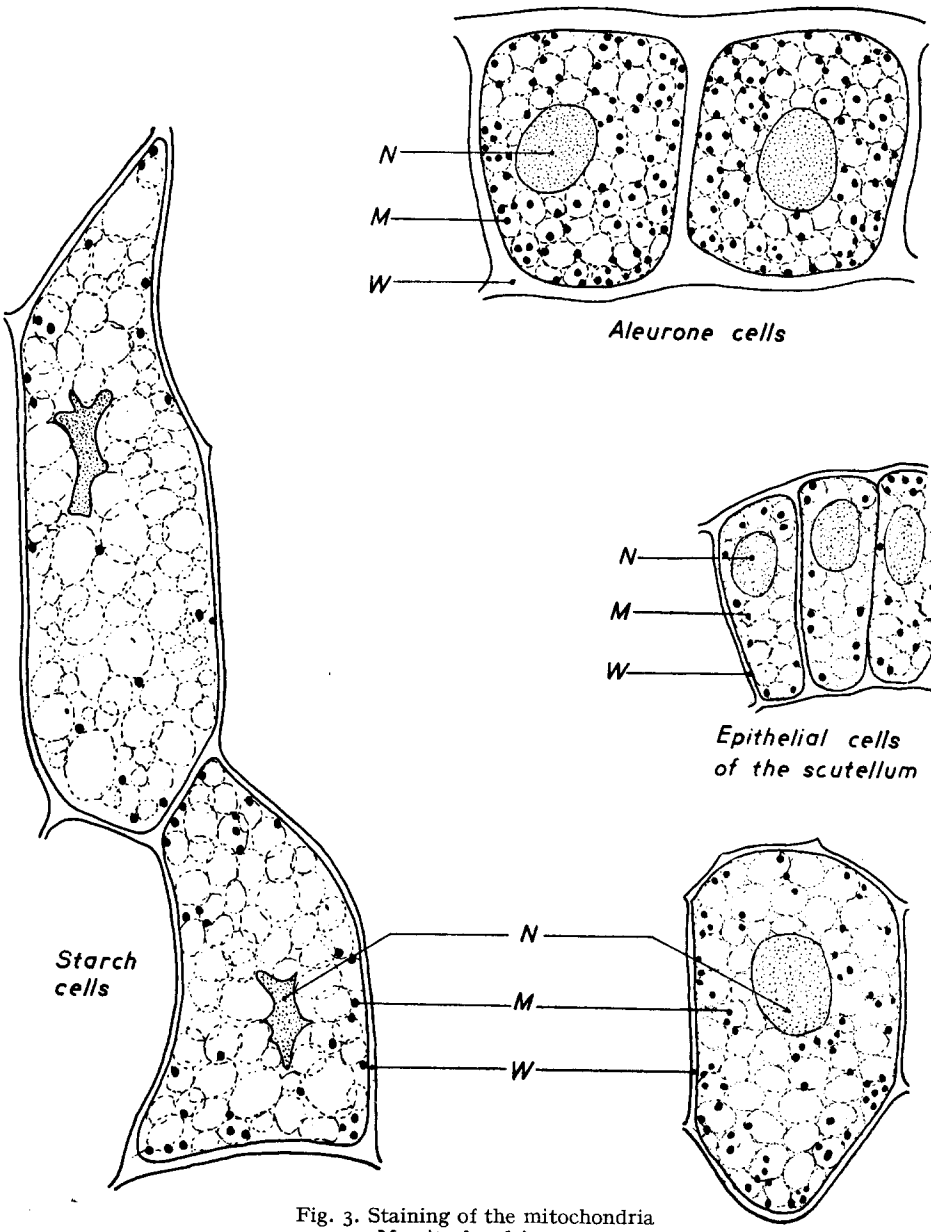

Fig. 3. Staining of the mitochondria
M mitochondria
N cell nucleus
W cell wall

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of 27.1 total amylase and 19.4 free active amylase (curve II and I). The subaleurone part of this slice contained therefore per μl: $\frac{100}{23} \cdot 27.1 = 116.5$ total amylase and $\frac{100}{23} \cdot 19.4 = 83.4$ free active amylase. For the slices nr 5, 6 and 7 the same calculation was carried out. The subaleurone part of these slices contained respectively: 49.6, 63.0 and 71.3 total amylase per μl and 35.0, 31.9 and 31.8 free active amylase per μl. The average value for the subaleurone layer is therefore 75.1 total amylase and 45.6 free

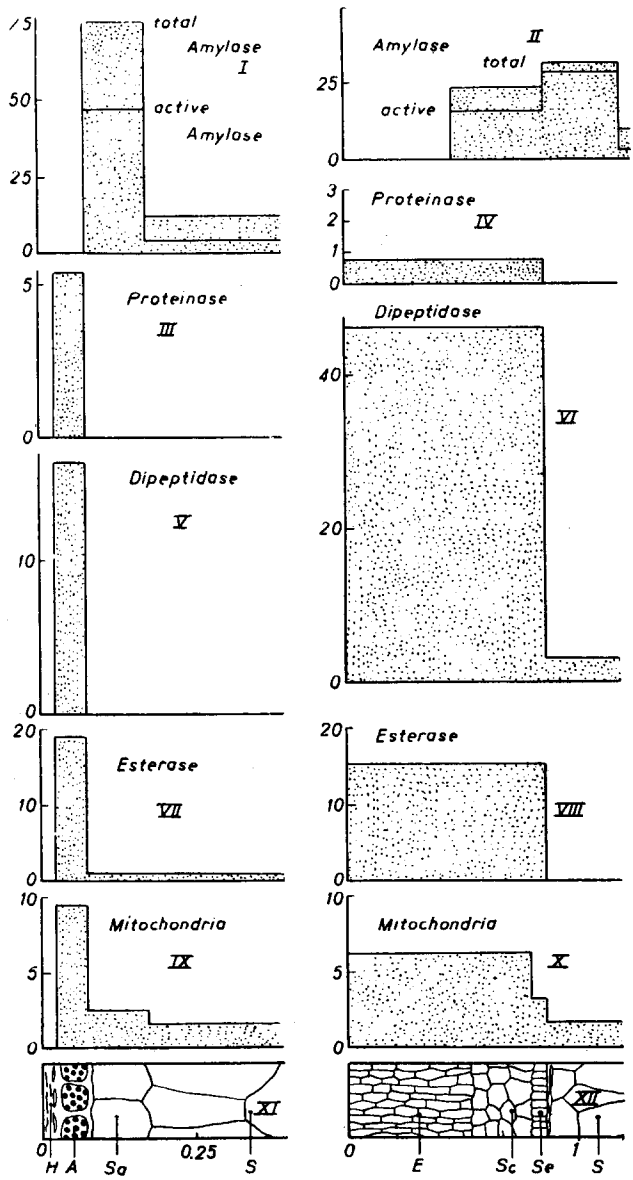

Fig. 4. The distribution of enzymes and mitochondria in the various tissues of the wheat grain.
The abscissae are in all figures the distance from the surface in mm.
The ordinates in I and II: mg maltose per μl of tissue per hour.
In III and IV: μl N/10 NaOH per μl of tissue per 24 hours.
In V and VI: μl N/10 NaOH per μl of tissue per hour.
In VII and VIII: μl N/20 HCl per μl of tissue per 24 hours.
In IX and X: number of mitochondria $\cdot 10^{-6}$ per μl of tissue.
In XI and XII: schemes of the various tissues have been given. H = pericarp and testa; A = aleurone cells; Sa = subaleurone cells; S = starch cells; E = germ tissue; Sc = scutellum; Se = epithelial cells of the scutellum.

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active amylase per μl. In the endosperm cells the amount of total amylase was found to be 11.1 and that of free active amylase 4.3 per μl.

In the scutellum, Fig. 7 B curve IV and V, in the slices nr 32 and 36 a free amylase amount was found of 16.5 and 15.4 and a total amylase amount of 26.0 and 22.2.

As these sections contain only scutellum tissue, the average values for the scutellum is total amylase 24.1 and active amylase 16.0 per μl.

The endosperm near the scutellum contains 28.7 active and 33.2 total amylase per μl average value of the sections 41, 46 and 49. The germ tissue did not contain amylase.

Proteinase, cf. ENGEL AND HEINS[2]. Fig. 2 and Table II.

The pericarp and testa and the endosperm do not contain proteinase. In the aleurone cells as average value from three determinations was found 5.4 per μl. In the total germ, the content was 0.82 per μl.

Dipeptidase, cf. ENGEL AND HEINS[2]. Fig. 3 and 4.

The pericarp and testa and the endosperm were without dipeptidase. In the aleurone layer was found as average value from three sections 16.4 per μl. In the germ tissue as mean value from all determinations 46.2 per μl was found.

Esterase, cf. ENGEL[3]. Fig. 1.

The pericarp and testa were without esterase. The aleurone layer contained 19.1 per μl, as an average of 2 slices. The endosperm tissue contained 1.0 per μl. The germ 15.4 per μl.

In Fig. 4 all the results are demonstrated. From comparison with the number of mitochondria it is clear that the distribution of the amylase does not agree with that of the mitochondria at all.

The proteinase and dipeptidase distribution over the tissues slightly resembles that of the mitochondria. Quantitatively however there is too great a difference to suppose that this is caused by the individual variations of the grains, because the proteinase results were obtained from 8 different grains. The dipeptidase content in the germ is so high that for this reason alone the distribution differs from that of the mitochondria.

The esterase seems to be distributed qualitatively almost on the same way as the mitochondria. Quantitatively however, for the various kinds of tissue, the ratio $\frac{\text{esterase amount}}{\text{mitochondria number}}$ per μl is for aleurone $\frac{19.1}{9.4} = 2.0$, for the germ $\frac{15.4}{5.7} = 2.7$ and for endosperm $\frac{1.0}{1.7} = 0.6$.

The ratio is not constant, therefore quantitatively the distribution of mitochondria and the enzyme is not the same.

SUMMARY

No relation was revealed between the number of mitochondria and the amount of the enzymes amylase, proteinase and esterase calculated per μl of various tissues of the wheat grain. By the method used it is not possible to decide if there is any relation between the mitochondria and the enzymes.

RÉSUMÉ

Aucune relation entre le nombre de mitochondries et la quantité d'amylase, de protéinase, de dipeptidase et d'estérase calculée par μl de divers tissus du grain de blé n'a pu être établie. Avec la

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méthode employée, il n'est pas possible de déterminer s'il existe ou non une relation entre les mitochondries et les enzymes.

ZUSAMMENFASSUNG

Eine Beziehung zwischen der Mitochondrien-Zahl und der Menge von Amylase, Proteinase, Dipeptidase und Esterase pro μl von verschiedenen Weizenkorngeweben ist nicht festgestellt worden. Mit der angewandten Methode ist es unmöglich festzustellen ob eine Beziehung zwischen den Mitochondrien und den Enzymen besteht.

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Received April 30th, 1946