As to the question of the origin and development of the plastid structure (lamellae and grana), the following contradictory observations have been previously reported: (1) on the one hand, the grana are differentiated after formation of the lamellae (WETTSTEIN[2], HODGE[4]), and (2) on the other, precisely the opposite opinion (MÜHLETHALER et al.[5]). In the case of the chloroplasts found in the roots of this fern, the grana-*like* structures seem to be differentiated after formation of the lamellar structures.

Figure 1 shows the central portion of one root near the tip where the cells are in the more undifferentiated state. Plastids in various developmental stages can be observed in each cell. The young undifferentiated plastids of simpler structure consist of only 2 or 3 lamellae (Figure 1). They would be regarded as the typical proplastids by MÜHLETHALER et al.[5] and BOPP-HASSENKAMP[6], although there are some opposing arguments by several investigators as to the proper definition of proplastid.

Some, but not all, of the plastids contain starch grains (Figures 1 and 3). The one shown in Figure 3 is filled with large grains. Such plastids may be called amyloplasts? They also clearly possess the lamellar and grana-*like* structure. Therefore, they have the appearance of both amyloplasts and chloroplasts. Herein they are called chloroplasts tentatively: only the plastids, in which starch grains are included and other structural elements cannot be positively recognized, are called amyloplasts.

It should be noted further that the plastids of the roots of this fern are all of a chloroplast nature and some of them seem to have the functions both of photosynthesis (proper only to chloroplasts) and of starch reservoirs (proper only to amyloplasts). The roots of higher plants normally lack the function of photosynthesis; i.e., they have not chloroplasts: the plastids of their roots include proplastids, leucoplasts and amyloplasts.

However, since the fern studied here always floats on the water, it seems very likely that the roots suspended as they are in the water are accessible to light and would possess the function of photosynthesis. The light microscope reveals furthermore that almost all plastids are yellowish-green, like the ordinary chloroplasts, and that they react positively to I_2-KI as well as to Lillie's polysaccharide staining. It may be supposed, therefore, that there might be some relationship between the grana-*like* structure and the starch-containing power of the chloroplasts and the above-mentioned habitat.

Zusammenfassung. Alle Plastiden in den Wurzelzellen von *Azolla imbricata* erweisen sich elektronenmikroskopisch als von Chloroplastiden-Natur. Sie zeigen granenartige Strukturen und enthalten zum Teil Stärkekörner. In einigen Zellen der Wurzelmitte werden auch Proplastiden festgestellt.

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The Action of a Phenothiazine Derivative on Enzymes of the Pentose Phosphate Pathway[1]

Phenothiazine derivatives have been implicated as inhibitors of many enzyme systems[2-4]. It has also been observed that they are inhibitors of human erythrocyte metabolism in the presence of methylene blue[5], presumably by inhibiting an enzyme of the pentose phosphate pathway. The present report extends the observations on the pentose pathway by studying the effect of phenothiazine derivatives on glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD) in human erythrocytes.

Blood from healthy donors was collected in tubes containing heparin. The cells were washed three times with 0.14 *M* NaCl and packed by centrifugation after each washing. They were subsequently taken up in distilled water and hemolyzed by alternate freezing and thawing. This preparation was used as a source of enzymes and was prepared fresh daily. All operations were carried out at 4°. Oxidation of glucose-6-phosphate (G-6-P) and 6-phosphogluconate (6-PG) was followed by conventional Warburg techniques[6].

Observations showed that the phenothiazine derivative (thioperazine) exerted an inhibitory effect when either G-6-P or 6-PG was used as substrate (Table I). Preincubation of the phenothiazine with the enzyme at 27° for 30 min prior to the addition of G-6-P or triphosphopyridine nucleotide (TPN) increased the inhibition. TPN, on the other hand, when preincubated with the phenothiazine partially protected the enzyme system against inhibition. In those cases where 6-PG was used as substrate, the results obtained on preincubation were equivocal and are still under investigation.

The TPN effect was further studied by varying the TPN concentration in the system. It can be noted from Table II that an increase in the TPN concentration had a significant effect on the inhibition produced by the phenothiazine. For example, a system containing 0.46 μmoles of TPN in the presence of a constant amount of phenothiazine resulted in an inhibition of 49% of the G-6-PD activity, whereas 1.84 μmoles of TPN decreased the inhibition to 19%.

Tab. I. Effect of thioperazine[a] on G-6-PD activity

Conditions	% Inhibition (range)
No preincubation	31–33
Preincubation 30 min with inhibitor	49–59
Preincubation 30 min with inhibitor plus TPN	28–38

Assay System: 0.10 ml hemolyzate; 0.50 ml glycylglycine buffer, pH 7.4; 0.31 μmoles methylene blue; 40 μmoles $MgCl_2$; 0.92 μmoles TPN; 15 μmoles G-6-P; 1.5 μmoles phenothiazine. Total vol. 3.0 ml.

[a] This particular phenothiazine used because of its solubility at pH 7.4.

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The results reported confirm previous studies on the intact erythrocyte. In addition, it is now possible to be more specific regarding the enzyme systems involved. Inhibition was noted when either G-6-P or 6-PG was used as substrate. Since preincubation favors increased inhibition, it would appear, at least in the case of G-6-PD, that the phenothiazine blocks sites necessary for the action of the enzyme. By preincubating in the presence of TPN the inhibition is decreased, suggesting the preferential affinity of the apoenzyme for the TPN, thus preventing the enzyme-phenothiazine interaction. Alternatively, it is also possible that TPN forms a complex with the phenothiazine, thus preventing its binding to the apoenzyme.

It has been previously reported[7] that TPN stabilizes partially purified G-6-PD from erythrocytes. More recently, MARKS et al.[8] have presented evidence to indicate that TPN or G-6-P protects purified G-6-PD against inactivation by heat. The latter authors suggest that the substrate and coenzyme are important to the stability of the enzyme structure. In view of this, it is conceivable that phenothiazine inhibits the enzyme by influencing the TPN-enzyme interaction.

Similar findings have been reported in studies of other coenzymes. LASSLO and MEYER[9] have reported a phenothiazine-enzyme complex with the *D*-aminoacid oxidase system and point to the structural analogy between the *N*-alkyl substituted phenothiazine and the *N*-ribityl substituted isoalloxazine moiety of flavin adenine dinucleotide (FAD), the coenzyme for this system. In addition, YAGI et al.[10] have demonstrated the formation of complexes of chlorpromazine and flavins. They suggest that such an effect may have important *in vivo* significance, particularly in view of previous work which indicated that modifications in electroencephalogram patterns induced by chlorpromazine could be reversed by FAD[11].

Tab. II. Effect of TPN concentration on inhibition of Glucose-6-phosphate dehydrogenase

TPN (μmoles)	% Inhibition
0.46	49
0.92	42
1.38	30
1.84	19

Phenothiazine Concentration 1.5μmoles. Assay: same as under Table I.

Résumé. La thiopérazine, qui est un derivé de la famille de la phénothiazine, a le pouvour d'inhibition sur l'activité du glucose-6-phosphate déhydrogénase et du 6-phosphogluconate déhydrogénase dans les globules rouges. Ce pouvoir est modifié si on ajoute du TPN dans le système enzymatique. Le degré de cette modification correspond à la dose de TPN employée.

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Étude cytochimique des groupes sulfhydriles au cours des modifications de la détermination embryonnaire chez l'œuf de l'oursin *Paracentrotus lividus*

Le développement de l'œuf d'oursin est gouverné par les relations mutuelles et compétitives entre un gradient animal et un gradient végétal[1]. L'équilibre entre ces deux gradients peut être modifié expérimentalement par des méthodes opératoires ou par l'action d'agents chimiques appropriés, avec comme conséquence l'extension des structures entomésodermiques (végétalisation) ou ectodermiques (animalisation).

Nous nous proposons d'examiner et de comparer la distribution des groupes sulfhydriles dans les embryons végétalisés, animalisés et témoins. Les considérations suivantes font ressortir l'intérêt de l'étude des groupes sulfhydriles au cours des modifications expérimentales de la détermination embryonnaire. L'animalisation est en effet provoquée par certaines substances capables de réagir avec les groupes sulfhydriles telles que l'acide *o*-iodosobenzoïque[2], un dérivé organique du mercure, le Salyrgan[3], ainsi qu'un dérivé sulfhydrilé, l'acide thiomalique[4]. D'autre part, l'animalisation et la végétalisation interfèrent avec les gradients de réduction mis en évidence par CHILD[5] et HÖRSTADIUS[6] au cours du développement embryonnaire; ces gradients peuvent correspondre à une répartition inégale de substances réductrices parmi lesquelles on peut considérer les dérivés sulfhydrilés[7].

Méthodes. Les œufs de l'oursin *Paracentrotus lividus* sont utilisés dans ces expériences. Les œufs fécondés et lavés sont divisés en trois groupes. L'un de ces groupes constitue les embryons témoins que l'on fixe à la fin de la gastrulation, au moment de la formation du mésenchyme secondaire. L'animalisation est provoquée en traitant les œufs fécondés par une solution contenant 0,004% de chlorure de zinc dans l'eau de mer[8]. La végétalisation est obtenue en traitant les œufs fécondés par une solution de chlorure de lithium à la concentration 0,026 *M*. Après 24 h les embryons sont enlevés des solutions, lavés et reportés dans l'eau de mer normale où ils poursuivent leur développement pendant 20 h. Toutes les cultures sont faites à la température du laboratoire. Les embryons animalisés sont des blastulas hyperciliées présentant une extension considérable de l'épaississement apical ectodermique et de la touffe ciliée apicale. L'archentéron n'est pas formé; quelques cellules mésenchymateuses sont situées au pôle végétal. Les embryons végétalisés sont caractérisés par une énorme vésicule entodermique à parois épaisses et par une petite vésicule ectodermique à parois minces. Des cellules mésenchymateuses et des cellules pigmentaires sont réparties dans le blastocèle.

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