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A Histochemical Study of *Lilium* Anthers

By

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KUWADA (1942) has put forward the view that the meiotic condition develops in a definite tissue or organ in the sporophyte, which brings the chromosomes into pairs. The present-day physiology teaches us next to nothing on this subject, but the studies of the meiosis and mitosis in comparison from the histophysiological angle may help us find some clue to the analysis of this condition.

In the present investigation, it is mainly intended to see histochemically the difference in the distribution of some biocatalysts between the sporogenous cells in the premeiotic stage and the pollen mother cells in meiosis.

Material and method: Young anthers of *Lilium speciosum* in different stages in development were cut and treated with chemical reagents which has usually been used in the histochemical investigation of some biocatalysts and other related substances.

Result and conclusion: The main result obtained is briefly tabulated in the following table.

The result of the present investigation given in this table shows that the cytoplasm of the sporogenous and the pollen mother cells oxidizes Nadi-reagent, p-phenylenediamine, and pyrogallol in the presence of H_2O_2. Among these reagents, Nadi has histochemically been used by several investigators, especially by RIES (1937) for indophenol blue oxidase and by AMANO (1939) for cytochrome c. The result of our spectrographical study of the pollen grains of some higher plants, bakers' yeast, and the thoracic muscles of some insects shows that the biocatalyst which is responsible for the oxidation of Nadi-reagent in these materials is thermolabile cytochrome c (SINKE, SIGENAGA and HIRAOKA)[2]. It is, therefore, highly probable that the sporogenous and

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1) Preliminary report to "Studies of Mitosis and Meiosis in Comparison VII".

2) Not yet published.

Table

	Sporogenous cells in late premeiotic stage	Pollen mother cells in early meiotic stage	Tapetal cells in late premeiotic stage	Tapetal cells in early meiotic stage
Nadi-reagent	blue	violet	blue	violet
p-phenylene diamine	+	+		
Pyrogallol+H_2O_2	dark brown	yellowish brown	yellowish brown	brown
o-cresol+H_2O_2	−	−	−	++
Benzidine+H_2O_2	−	−	±	+
2-6-dichlor-phenol-indo-phenol	++	++	++	++
Na-nitropresside	+	++	+	++
K-permanganate	+	++	+	+++
Schiff's reagent	−	−	±	++
Acidified $AgNO_3$	− or ±	− or ±	−	−
OsO_4	+	++	±	+

the pollen mother cells contain the cytochrome.

The sporogenous and the pollen mother cells more or less strongly oxidize pyrogallol in the presence of H_2O_2, showing the existence of pyrogallol-peroxidase in them. Contrary to pyrogallol, benzidine and o-cresol are not oxidized in the presence of H_2O_2.

It is seen in the table that the sporogenous and the pollen mother cells reduce 2-6-dichlor-phenol-indophenol, Na-nitroprusside and K-permanganate. This fact shows that ascorbic acid and gultathione are contained in these cells.

The tapetal cells have been regarded by several authors such as BONNET (1912) and TISCHLER (1915) as the secretory cells which supply nutrient substances to the sporogenous and the pollen mother cells. As can be seen in the table, while the tapetal cells oxidize Nadi-reagent, and reduce 2-6-dichlor-phenol-indophenol and Na-nitroprusside both in the premeiotic and meiotic stages, they oxidize benzidine and o-cresol in the presence of H_2O_2 and reduce Schiff's reagent in the latter stage. The tapetal cells in this stage, therefore, probably contain some bio-catalysts such as thermolabile cytochrome c, benzidine- and o-cresol-peroxidases, ascorbic acid and gultathione. The reduction of Schiff's reagent probably shows the existence of aldehyde-like substances in

these cells. From the fact mentioned above, it may be stated that the tapetal cells strikingly resemble the sporogenous and the pollen mother cells in their chemical inclusions.

As shown in the table, anthers of *Lilium* in the premeiotic stage differ from those in the meiotic stage in the following points:—

i) K-permanganate, Na-nitroprusside and OsO_4 are reduced more strongly in the pollen mother cells in the early meiotic stage than in the sporogenous cells in the premeiotic one. This fact shows that a marked increase in the reduction power takes place in these cells in the late premeiotic stage. The result of the staining reaction test undertaken by one of us (M.I.) that neutral violet extra and methylene blue are more strongly reduced in the pollen mother cells in the early meiotic stage than in the sporogenous cells in the premeiotic one is quite in accordance with that of our investigation.

ii) The sporogenous cells in the premeiotic stage are coloured dark brown while the pollen mother cells in the meiosis are yellowish brown in colour when the sections of anthers are treated with pyrogallol in the presence of H_2O_2. It is very probable, therefore, that a certain change in the activity of pyrogallol-peroxidase has been taking place in the late premeiotic stage. This fact is harmonious with the result of enzymological investigation of *Lilium* anthers carried out by HIRAOKA (1947) that activity of pyrogallol-peroxidase is markedly stronger in the meiotic stage than in the premeiotic one.

iii) The tapetal cells strongly oxidize benzidine and o-cresol in the presence of H_2O_2 in the meiotic stage while they hardly or only weakly oxidize these reagents in the late premeiotic stage. It seems very probable, therefore, that a marked change in the activity of the peroxidases takes place in the latter stage. According to the result of the investigation of one of us (M.I.), this phenomenon is widely found among many embryophyta.

From the facts mentioned above, it is highly probable that a marked change in the activity of some biocatalysts takes place in the sporogenous and the tapetal cells just before the beginning of the meiosis. The result of the respiratory physiological investigation carried out by one of us (N.S.)[1] that the respiratory activity is more strongly inhibited by KCN and NaN_3 in the meiotic stage than in the premeiotic one, supports this view.

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1) To be published later.

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