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Enzyme Activity in *Lilium* Anthers[1)]

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According to our histochemical study of *Lilium* anthers (SINKE, IIJIMA and HIRAOKA, 1947), a remarkable metabolic which change is expected to occur in anther tissue in the course of development from premeiosis to meiosis. In the present paper, the activity change of some enzymes responsible for cellular oxidation is to be reported in the hope of contributing something to the knowledge about the metabolic change at issue.

Results

I. *Peroxidase:* Anthers of *Lilium speciosum* which contain sporogenous cells in premeiotic mitosis and those which contain pollen mother cells in pachytene stage were used as the present material. The former anthers may briefly be called "premeiotic anthers" and the latter "meiotic anthers". A uniform gruel, prepared by grinding fresh anthers with a buffer solution in a mortar, was used as an enzyme preparation. Peroxidase activity was measured by the amount of purpurogallin produced by the enzyme preparation for 10 minutes at 30°C using pyrogallol as a substrate in the presence of hydrogen peroxide (CHODAT, 1925). Table 1 represents the main results obtained which are given in terms of mg purpurogallin produced by 1 g fresh anthers. The enzyme is active at pH 5.6 and decreases its activity with increasing acidity of the enzyme system. It is found from this table that premeiotic anthers are inferior to meiotic anthers in peroxidase activity irrespective of concentration of hydrogen ions or of hydrogen peroxide in the system. The ratio of peroxidase activity in premeiotic anthers to that in meiotic anthers was 100 : 139 in nearly optimal concentration of hydrogen peroxide at pH 5.6.

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1) Preliminary note to "Studies of mitosis and meiosis in comparison VIII".

Table 1. Peroxidase activity

Constitution of enzyme system in cc				pH	Purpurogallin produced	
1% pyro	0.005 M H_2O_2	Buffer	Enzyme		premeiotic	meiotic
20	5	25	10	3.0	8.75	15.76
20	15	15	10		6.98	14.25
20	30		10		2.61	3.63
20	1	29	10	4.0	4.85	5.82
20	5	25	10		12.42	22.56
20	10	20	10		9.68	21.85
20	15	15	10		6.51	18.97
20	30		10		3.61	9.48
20	5	25	10	4.8	13.45	21.42
20	15	15	10		12.22	28.19
20	30		10		4.44	13.96
20	5	25	10	5.6	16.72	21.38
20	10	20	10		18.13	21.21
20	15	15	10		28.59	39.62
20	20	10	10		12.23	41.14
20	30		10		6.17	19.78

II. ***Catalase:*** Catalase activity was measured by examining the amount of oxygen gas liberated by the enzyme or by examining the amount of hydrogen peroxide remaining undecomposed using a 0.09 M hydrogen peroxide solution as a substrate at 2°C. The catalase activity is given in terms of cc oxygen gas liberated by 1 g fresh anthers. The main results, given in Table 2, show that the catalase activity is stronger in rather alkaline milieu than in acidic milieu and becomes

Table 2. Catalase activity (0°C, 760 mm)

(Constitution of system: 180 parts of H_2O_2 solution plus 10 parts of buffer solution plus 10 parts of enzyme preparation)

pH	Time in min.	H_2O_2 decomposed		Activity ratio	
		premeiotic	meiotic	premeiotic	meiotic
3.6	10	3.3	7.6	43	100
5.6	10	28.8	58.0	50	100
	10	29.9	51.2	58	100
	10	21.5	42.6	50	100
	20	46.3	82.0	56	100
8.0	10	46.9	88.3	53	100
	10	53.3	90.4	59	100
	30	61.3	102.5	60	100

nearly null at pH 3.6. An interesting fact observable in this table is that meiotic anthers are more active in catalase activity than premeiotic anthers.

III. ***Indophenol oxidase:*** A mixture of a 1% aqueous solution of a-naphthol and a 0.75% aqueous solution of p-phenylenediamine or a p-phenylenediamine solution alone was used as a substrate for the oxidase (BATTELLI und STERN, 1912). The oxidase activity was measured by examining the amount of coloured product produced by the enzyme preparation under constant aeration of oxygen gas for 10 minutes at 30°C. The action of the oxidase is active at pH 5.6, weak at pH 8.0 and almost null at pH 3.0. Therefore, the measurements of the oxidase activity were mainly carried out at pH 5.6, at the hydrogen ion concentration near the original one of anther slime (YAMAHA und ISHII, 1932). The results, summerized in Table 3, show clearly that premeiotic anthers are superior in the oxidase activity to meiotic anthers.

Table 3. Indophenol oxidase

(Constitution of system: 1 part of a-naphthol solution plus 1 part of paramine solution plus 7 parts of buffer solution plus 1 part of enzyme preparation)

Relative activity of indophenol oxidase			
premeiotic	meiotic	premeiotic	meiotic
100	66	100	47
100	79	100	47
100	81	100	60
100	79	100	56
100	85	mean 100	67

This relation holds good when p-phenylenediamine alone is used as a substrate, the mean activity ratio being 100 : 53.

IV. ***Polyphenol oxidases:*** a) *Guaiacol oxidase:* The enzyme activity was measured by examining the amount of tetraguaiac quinone produced by the enzyme for 24 hours at 30°C using guaiacol as a substrate. The enzyme is found to be active at pH 5.6, but inactive at pH 3.0 and 8.0. The results obtained clearly show that premeiotic anthers are more active in the oxidase activity than meiotic anthers, the mean activity ratio being 100 : 74.

b) *Pyrogallol oxidase:* The amount of purpurogallin produced by the enzyme for 6 hours at 30°C was examined using pyrogallol as a substrate to see the oxidase activity. The enzyme is active at pH 5.6

and weakly active at pH 3.6, but not at pH 8.0. The comparison of the oxidase activity at pH 5.6 in premeiotic anthers with that in meiotic anthers shows that premeiotic anthers are stronger in the oxidase activity than meiotic anthers, the mean activity ratio being 100 : 60.

c) *Pyrocathecol oxidase:* A mixture of a pyrocathecol solution and the enzyme preparation was kept at 30°C with occasional shakings for 3 hours, and a coloration of pulp was examined to infer the enzyme activity. The results obtained were represented in Table 4. The enzyme is weakly active at pH 3.0, and is active at pH 5.6 and 8.0. At pH 5.6, premeiotic anthers are stronger in the power of oxidizing pyrocathecol than meiotic anthers. The enzyme preparation alone also turns more or less brown. This coloration is stronger in premeiotic anthers than in meiotic anthers.

Table 4. Pyrocathecol oxidase

pH	test		blank test	
	premeiotic	meiotic	premeiotic	meiotic
3.0	dark brown	brown grey	yellow grey	yellow grey
5.6	reddish brown	brown	brown grey	yellow grey
8.0	reddish brown	reddish brown	brown grey	yellow grey

The enzyme preparation gave no coloured product using veratorol as a substrate within 24 hours at pH 5.6.

V. ***Monophenol oxidases:*** a) *p-cresol oxidase:* The enzyme activity was measured by examining the amount of coloured product, capable of being extracted by ether, produced by the enzyme for 24 hours at 30 °C using p-cresol as a substrate. The enzyme is active at pH 5.6, weak at pH 8.0 and at 3.6. The results obtained at pH 5.6 show that the oxidase activity in anthers decreases in the course of development from premeiosis to meiosis. The mean ratio of the oxidase activity in premeiotic anthers to that in meiotic anthers was 100 : 72.

b) *o-cresol oxidase:* The enzyme preparation oxidized o-cresol weaker than p-cresol to give only a faint yellow coloured product, capable of being extracted by ether, after 24 hours at 30°C. It was found that the oxidase activity in premeiotic anthers is superior to that in meiotic anthers, the mean ratio of the former to the latter being 100 : 81.

c) *Tyrosinase:* The enzyme preparation did not give any coloured product using tyrosine as a substrate.

VI. ***Ascorbic acid and its oxidase:*** Amount of ascorbic

acid and of total ascorbic acid were determined by 2-6-dichlorphenol indophenol method after FUJITA and EBIHARA (1937). The results obtained show that premeiotic anthers contain 40.3 mg% ascorbic and 58.0 mg% total ascorbic acid, while meiotic anthers only 31.1 mg% ascorbic and 46.2 mg% total ascorbic acid on an average. The activity of ascorbic acid oxidase was determined by examining the amount of ascorbic acid left unoxidized using ascorbic acid as a substrate at 30°C (TAUBER, 1937). The oxidase is not active in acidic milieu (near pH 3.0), and active at pH 5.6 and 8.0. It was shown that ascorbic acid consumed by 1 g fresh anthers in 10 minutes was 2.33 mg in premeiotic anthers and only 0.84 mg in meiotic anthers on an average at pH 5.6. Therefore, premeiotic anthers are stronger in their oxidase activity than meiotic anthers.

VII. ***Succinic acid oxidase:*** Oxidation of succinic acid was examined in aerobic condition after BUTKEWITSCH and FEDOROFF's method (1929). 1 g fresh premeiotic anthers produced 0.32 mg fumaric acid at pH 5.6 and 0.33 mg at pH 8.0, while meiotic anthers only 0.21 mg at pH 5.6 and 0.12 mg at pH 8.0 for 3 hours at 30°C on an average.

VIII. ***Dehydrogenase:*** Total dehydrogenase activity of premeiotic anthers and of meiotic anthers were determined by methylene blue method after THUNBERG (1920) and by dinitrobenzene method after LIPSCHITZ (1925) in anaerobic condition. The results obtained at pH 5.6 showed that the time required for decolorization of methylene blue was 13.0 minutes in premeiotic anthers, while 19.7 minutes in meiotic anthers on an average. The dehydrogenase activity is, therefore, stronger in premeiotic anthers than in meiotic anthers. Similar results were also obtained with dinitrobenzene method, premeiotic anthers produced much orange coloured product from m-dinitrobenzene than meiotic anthers.

Conclusion

Among the enzymes studied in the present investigation, there exist two groups keenly discriminating between them in the mode of activity change from premeiosis to meiosis. Peroxidase and catalase from the one group in which premeiotic anthers are inferior in their activity to meiotic anthers, while oxidases and dehydrogenase form the other group in which premeiotic anthers are superior in their activity to meiotic anthers. Whether hydrogen peroxide is involved in an

enzyme system seems to be a key point to characterize these two groups. These facts not only indicate the occurence of a remarkable change in metabolic activity in anther tissue when meiosis commences as demonstrated in our histochemical study (SINKE, IIJIMA and HIRAOKA, 1947), but also form, together with the results of studies in respiratory activity (SINKE and SIGENAGA[1], SINKE[2]), a piece of evidence to show how is the metabolic change at issue.

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1), 2) To be published later.