

# Variations in Sulfhydryl Concentration During Microsporocyte Meiosis in the Anthers of *Lilium* and *Trillium*\*

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## ABSTRACT

Anthers of lily and trillium were followed with respect to variations in protein and soluble sulfhydryls during meiosis and mitosis of the sporogenous tissue. In lily, the meiotic and mitotic cycles are each preceded by a rise in soluble —SH; in trillium there is only one rise which precedes meiosis. During division there is a marked drop in soluble —SH and a rise in soluble disulfides. Protein —SH remains approximately constant until diakinesis or metaphase when it falls briefly.

## INTRODUCTION

The purpose of this communication is to report on certain correlations between the occurrence of nuclear divisions and the concentration of thiol groups in the anthers of *Lilium longiflorum* (var. Croft) and *Trillium erectum*. Comparatively little is known about the variations sulfhydryls undergo during meiosis and mitosis, even though the presence of high sulfhydryl concentrations in proliferating tissues has long been recognized (1). In the present study, advantage has been taken of the extended period of microsporocyte meiosis in liliaceous plants, especially in trillium, to plot the variations in —SH content of the anthers during that interval. The correlation between the cytology of the microsporocyte or microspore and the chemical composition of the anther is, of course, not a direct one. Nevertheless, the major changes in thiol concentration probably have a real basis in the behaviour of the dividing cells, since they occur at times when meiosis and mitosis are the principal and probably the only cyclical events in the anther.

The results to be reported confirm an earlier conclusion that a high soluble thiol concentration is associated with nuclear division (2). In addition, they point to a sharp drop in the concentration of soluble sulfhydryl compounds during meiosis at

the time of chromosome contraction and movement; this change is paralleled by an increase in soluble disulfides and a decrease in protein —SH.

## Materials and Methods

Bulbs of *Lilium longiflorum* (var. Croft) were grown in a greenhouse and buds of appropriate length were periodically removed for analysis. The anthers were weighed and dropped into a tube kept cold by an acetone-dry ice mixture. *Trillium erectum* corms were obtained early in September and stored in moist peat moss at a temperature ranging from 2–4°C. At weekly or smaller intervals a number of buds were opened, half an anther of each was smeared and stained with acetocarmine for cytological observation. The remaining anthers were weighed and stored in a deepfreeze for subsequent study. Both lots of plants were purchased from commercial sources.

The frozen anthers were crushed with a stirring rod and enough cold ethanol added to make a paste, which was finely dispersed in 4 per cent sulfosalicylic acid. The homogenate was centrifuged, and the supernatant analyzed for soluble sulfhydryl content. The residue was washed with sulfosalicylic acid to remove remaining soluble compounds. Since no more than 5 per cent of the total soluble —SH was found in the six washes analyzed, measurements were restricted to the original extracts. The washed residue was dispersed by homogenization in a 1 per cent solution of tween 20 and a portion of this diluted fivefold with 0.5 per cent lauryl sulfate. An appropriate volume of the protein suspension was immediately titrated for approximate —SH content. The remainder of the suspension was treated

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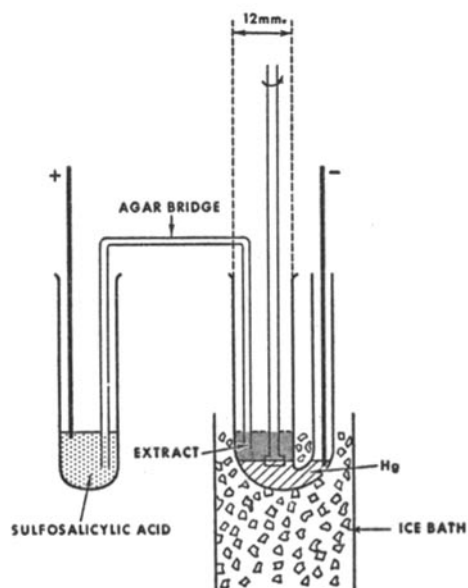


FIG. 1. Arrangement for electrolytic reduction of disulfides. The main cell is made from a test tube cut to appropriate length and fitted with a side arm. The stirrer is flattened at one end to provide adequate agitation. The electrodes are platinum, and the sulfosalicylic acid is a 4 per cent solution.

with an amount of  $\text{AgNO}_3$  sufficient to neutralize its  $-\text{SH}$  groups, and then stored under nitrogen in the cold overnight. The protein thus treated was assayed for  $-\text{SH}$  content the following day.

Both protein and soluble thiols were titrated amperometrically in Tris buffer according to the method of Benesch, Lardy, and Benesch (3). A volume of 10 ml. was used in the electrode vessel. A solution of 0.0005  $N$   $\text{AgNO}_3$  was delivered by means of a 0.1 ml. pipette graduated to 0.001 ml. and fused to a syringe control. By using a rubicon galvanometer with a sensitivity of 0.005  $\mu\text{a.}/\text{mm.}$  in parallel with a resistance box,  $5 \times 10^{-8} \mu\text{M}$  of  $-\text{SH}$  could be measured with an error not exceeding 10 per cent. Generally, about three to five times that amount was taken for analysis and the error correspondingly reduced. The specificity of the titration was checked by adding *N*-ethyl maleimide to the extracts; under these conditions no  $\text{AgNO}_3$  was consumed.

The reliability of sulfosalicylic acid as an extraction medium was tested by determining the sulfhydryl content of the extract immediately, and  $1\frac{1}{2}$  hours after its preparation. Differences were of the order of 5 per cent or less, providing that the sulfosalicylic was freshly made. Addition of a chelating agent such as versene to the acid slightly improved the stability of the medium, but not enough to recommend its inclusion. Further

tests were made on recoveries of added amounts of oxidized or reduced glutathione; these, too, were satisfactory.

Disulfides were determined by prior electrolytic reduction according to the procedure of Dohan and Woodward (4). Minor modifications were introduced in order to accommodate volumes of the order of 0.5 ml.; these are illustrated in Fig. 1. Cooling of the reduction vessel stabilized the reduced sulfhydryl compounds. Each sample was treated for 10 minutes with a 7 milliampere current drawn from a DC power supply, and removed from the mercury immediately after termination of the treatment. The mercury surface was then twice rinsed with water and dried with filter paper. Upon completion of the day's runs, the mercury was washed in long columns of dilute nitric acid and distilled water. If this daily cleaning of the mercury reservoir was not followed, the reduction of disulfide became inconsistent.

Protein nitrogen was determined by digesting the acid-washed residue in a mixture of  $\text{H}_2\text{SO}_4:\text{H}_3\text{PO}_4:\text{CuSO}_4 \cdot 5\text{H}_2\text{O}:\text{H}_2\text{SeO}_3$  (75 ml.:25 ml.:1 gm.:1.2 gm.) and treating the digest with Nessler's solution in the presence of Rochelle salts.

#### RESULTS

The most pronounced change in the behaviour of sulfhydryl compounds during anther development is the rise in concentration of soluble thiols at or prior to the onset of nuclear division. In lily anthers there are two such rises; one preceding the zygotene of microsporocyte meiosis and the concurrent tapetal mitoses, the other preceding microspore mitosis (Fig. 2). In trillium anthers there is only one rise and it precedes microsporocyte meiosis; between the two sets of divisions there is only a temporary arrest of the thiol decline which begins in late microsporocyte meiosis (Fig. 3).

The above differences in thiol patterns may be related to corresponding differences in growth habits. In lily, the anthers develop during a period of active plant growth. By contrast, the anthers of trillium develop during an otherwise dormant period; in nature, the cycles of meiosis and mitosis often occur under a carpet of snow. The anthers of trillium plants stored in a refrigerator increase by about 50 per cent in fresh weight (and protein nitrogen) from September until the end of microspore mitosis. Such growth, however small compared to that of the lily anther, obviously must depend upon the presence of food reserves. It thus appears as though the major thiol requirements for cell division and possibly other processes were

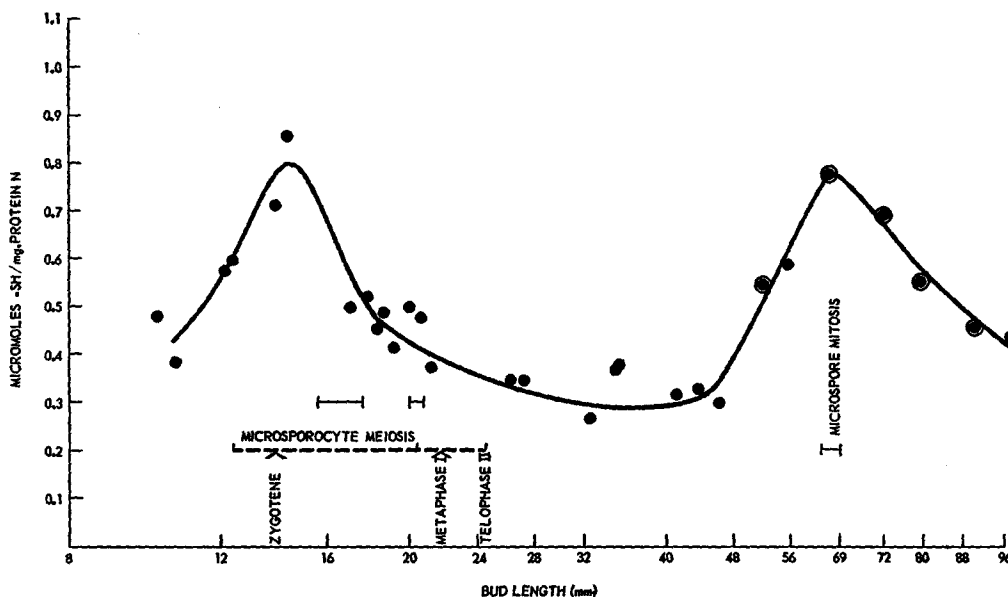


FIG. 2. Soluble sulfhydryl concentration during development of lily anther. The small bars over microsporoctete meiosis indicate the intervals of tapetal cell mitosis (7). Each dot represents the average of values obtained from two groups of anthers. The circled dots are drawn from analyses already published (2).

anticipated before the onset of winter. This explanation is consistent with the fact that in trillium the peak sulfhydryl concentration preceding meiosis is, on a protein nitrogen basis, four times that of lily. (The protein nitrogen of trillium anthers is approximately 1 per cent of the fresh weight, and this value changes little during the period studied; lily anthers on the other hand have a protein content equal to 0.3 per cent of the fresh weight. Fresh weight comparisons would, therefore, exaggerate the difference between the two species).

During the premeiotic rise in sulfhydryl, the content of disulfides remains low. This result is identical with the one obtained in lily anthers (2), thus lending further support to the conclusion that the rise in sulfhydryl concentration is due to a *de novo* synthesis and not, as Rapkin<sup>1</sup> had speculated, to the reduction of endogenous stores of oxidized glutathione. Approximately at the time of diakinesis, anthers begin to show variable, but increased amounts of soluble disulfides. In terms of total thiol concentration the increase in disulfide is all the more marked because of the decrease in sulfhydryl titers. The disulfides formed do not entirely account for the observed drop in sulfhydryl

concentration, but the pattern suggests a dehydrogenation rather than a disappearance of thiol compounds, as the principal change during meiosis.

A final point of interest is in the behavior of protein —SH during the latter phase of the meiotic cycle. It may be recalled that within the interval surrounding the mitosis of lily microspores there was comparatively little net change in protein —SH concentration (2). (Evidence for changes during active mitosis are difficult to obtain because microspores are not synchronous with respect to mitotic stage). The protein —SH levels at premeiosis and at tetrad formation are approximately equal, and in this respect resemble the behaviour of lily anther at the time of microspore mitosis. But, the active phases of meiosis are associated with a fall in protein —SH which is steepest around diakinesis and metaphase. This phase of the meiotic cycle is generally associated with marked changes in the sulfhydryl composition of the anther. The drop in protein —SH occurs simultaneously with a rise in soluble disulfides and a fall in soluble sulfhydryls. Just how the events in the soluble fraction are related to those in the protein is not indicated by the data. It is apparent, however, that at the conclusion of meiosis both protein —SH and soluble —SH begin a decline

<sup>1</sup> Rapkin, cited by Brachet (1).

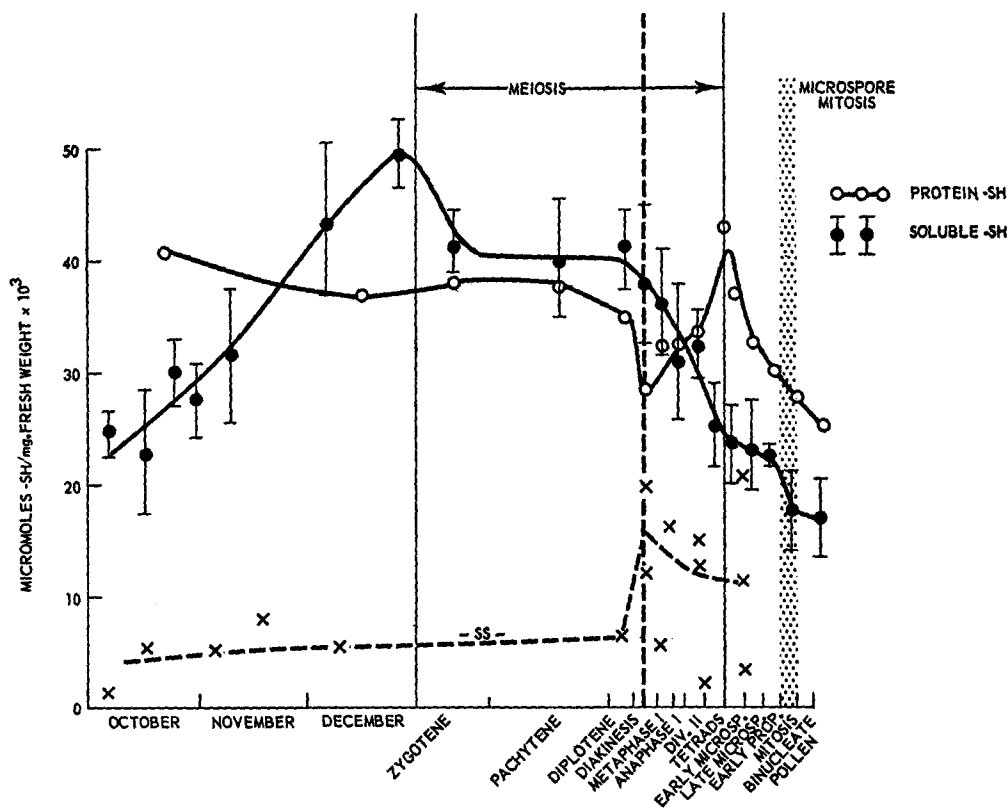


FIG. 3. Sulfhydryl changes during development of trillium anther. The abscissae from zygotene on are in rough proportion to the duration of meiotic and mitotic stages. The bars represent two standard deviations. The variation in protein —SH is of the same order as that of soluble —SH. The crosses represent individual determinations of soluble disulfide; the low number of these determinations is due to exhaustion of material.

which persists through and beyond microspore mitosis.

#### DISCUSSION

The principal conclusion to be drawn from these studies is that meiosis and mitosis probably have comparatively high requirements for soluble sulfhydryl compounds. Whether an actual rise in sulfhydryl concentration precedes active division appears to depend on physiological circumstance. A sufficient store of thiols may be laid down to cover more than one meiotic or mitotic cycle. This conclusion is consistent with the many repeated observations that growing and proliferating tissues are rich in nitroprusside reactivity (1).

The major component of the soluble sulfhydryls is probably glutathione. The conclusion is based on a series of analyses of lily anthers during microspore mitosis by means of the glyoxalase technique. This study has already been referred to (2), and details of it have been omitted because of

the identity in patterns obtained by the amperometric and enzymatic methods. A procedural point may, however, be of some interest. In using an acetone powder of baker's yeast as source of enzyme, a reducing component was found to be present in the powder which converted some of the oxidized glutathione to the reduced form, thus altering the original GSH/GSSG<sup>2</sup> ratios of the extracts. Treatment of the powder with an ether solution of peroxide effectively removed the interfering component. The glyoxalase technique, because of its laboriousness, was not applied to trillium anthers; it is only presumed that in these, too, glutathione is the major sulfhydryl component. It need not be supposed, however, that glutathione is the only compound capable of satisfying the sulfhydryl requirements of dividing cells. The thiol recently discovered in legumes may be just as effective (5).

<sup>2</sup> Reduced glutathione  
Oxidized glutathione

It may be inferred from the pattern of sulfhydryl variations during microsporocyte meiosis that an important phase in the utilization of sulfhydryls occurs beyond prophase and may extend to the conclusion of division. Evidence for this is difficult to find in microspore mitosis because of the comparatively brief duration of division. Even so, the existence of variations during mitosis of lily microspores has been noted (2), and in the case of trillium microspores the sharp decline of soluble —SH during mitosis is patent. In keeping with the idea that high sulfhydryl concentration is particularly important to the phases of chromosome condensation and separation is the fact that in both lily and trillium, the anthers do not reach their maximum sulfhydryl concentration until the zygotene stage. It is doubtful, however, that the sulfhydryls present are exclusively concerned with chromosome movement; there is ample evidence that glutathione influences growth processes in general (6). The particular point of interest in this study is that during chromosome separation glutathione function may take on a special significance.

The simultaneity of changes in the protein and soluble sulfhydryl fractions points to a direct relationship between them. This is consistent in a general way with the views expressed by Rapkine,<sup>1</sup> Brachet (1), and Mazia (8), but it would be difficult on the basis of the data to specify the kind of relationship involved.

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