

of a <sup>3</sup>H-TdR precursor pool. This pool effect is reflected by a parallel pattern in the percentage of labeled nuclei in the elongation region during the chase. It was also observed by SAMPSON and DAVIES<sup>3</sup> in *Vicia faba*, that the elongation region contains more heavily labeled nuclei than does the meristematic region. This is in agreement with the studies of PELC and LA COUR<sup>4</sup>, who found intense labeling in approximately 25% of the cells at a distance of 3–5 mm behind the meristem. SAMPSON and DAVIES<sup>3</sup>, also found that after a 4 h label of <sup>3</sup>H-TdR followed by a 48 h chase treatment, the amount of incorporated isotope increased even after the external source of isotope was removed. This could be due to a possible use of a precursor pool of <sup>3</sup>H-TdR in the cells of both regions formed during the preceding S period. In support of this idea, it was observed that in *Tetrahymena pyriformis* a pool of TdR derivatives is formed only during the S phase, and when DNA synthesis is completed, remains in the nucleus until the next round of DNA synthesis occurs after cell division<sup>2,5</sup>. This was also confirmed in the grasshopper neuroblast<sup>6</sup>.

DNA synthesis also occurs in the elongation region although there are no mitotic figures observed, and consequently, there is no grain count dilution. The data in Table II, show an increase in grain counts in the nuclei of the elongation region, and hence an increase in DNA synthesis during the chase period. This suggests the

existence of a <sup>3</sup>H-TdR precursor pool in the cells of the elongation region. This finding is in agreement with the autoradiographic data of WOODARD et al.<sup>7</sup> on the roots of *Vicia faba*, who also found this grain count elevation in the elongation region during the chase period after pulsing with <sup>3</sup>H-TdR. They also observed in this root a large <sup>3</sup>H-TdR precursor pool in the elongation region.

In accordance with the aforementioned authors<sup>2,5-7</sup>, the conclusion seems justified that there is a large <sup>3</sup>H-TdR precursor pool in the elongation region as seen by the higher grain counts in this region (Table II).

A similar pool of phosphorylated derivatives of <sup>3</sup>H-TdR has been detected by means of autoradiographic methods in cells of the mouse bone marrow<sup>8</sup>, in *Tetrahymena*<sup>9</sup> and, in mouse cells in culture<sup>10,11</sup>.

**Résumé.** Une certaine quantité de phosphates <sup>3</sup>H-TdR se forme dans les cellules du méristème et les parties allongées de la racine d'*Allium cepa* durant une exposition ininterrompue de 24 h. Au cours des périodes d'observation subséquentes, le contenu de cette réserve est utilisé comme source de précurseurs pour la synthèse du DNA.

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Table II. Average grain count per square over the nuclei of the meristem region compared to that of the elongation region after exposure to <sup>3</sup>H-TdR for 24 h, and subsequent exposures to unlabeled medium

Amount of time in unlabeled medium (h)	Average grain count	
	Meristem	Elongation
0	3.9 ± 0.4 *	6.9 ± 0.5
1	9.5 ± 1.0	11.0 ± 0.9
8	6.9 ± 0.8	8.1 ± 0.6
20	2.6 ± 0.3	3.6 ± 0.5

\* Mean ± standard error.

<sup>3</sup> M. SAMPSON and D. D. DAVIES, *Expl. Cell Res.* **43**, 669 (1966).  
<sup>4</sup> S. R. PELC and L. F. LA COUR, *Experientia* **15**, 131 (1959).  
<sup>5</sup> G. E. STONE, O. L. MILLER and D. M. PRESCOTT, *J. Cell Biol.* **25**, 171 (1965).  
<sup>6</sup> R. A. McGRATH, W. M. LEACH and J. G. CARLSON, *Expl. Cell Res.* **37**, 39 (1965).  
<sup>7</sup> J. WOODARD, E. RASCH and H. SWIFT, *J. Biophys. Biochem. Cytol.* **9**, 445 (1960).  
<sup>8</sup> L. E. FEINENDEGEN and V. P. BOND, *Expl. Cell Res.* **27**, 474 (1962).  
<sup>9</sup> O. L. MILLER, *J. Cell Biol.* **19**, 50A (1963).  
<sup>10</sup> J. E. CLEAVER and R. M. HOLFORD, *Biochim. biophys. Acta.* **103**, 654 (1965).  
<sup>11</sup> J. H. TAYLOR and R. D. McMASTER, *Chromosoma* **6**, 489 (1954).  
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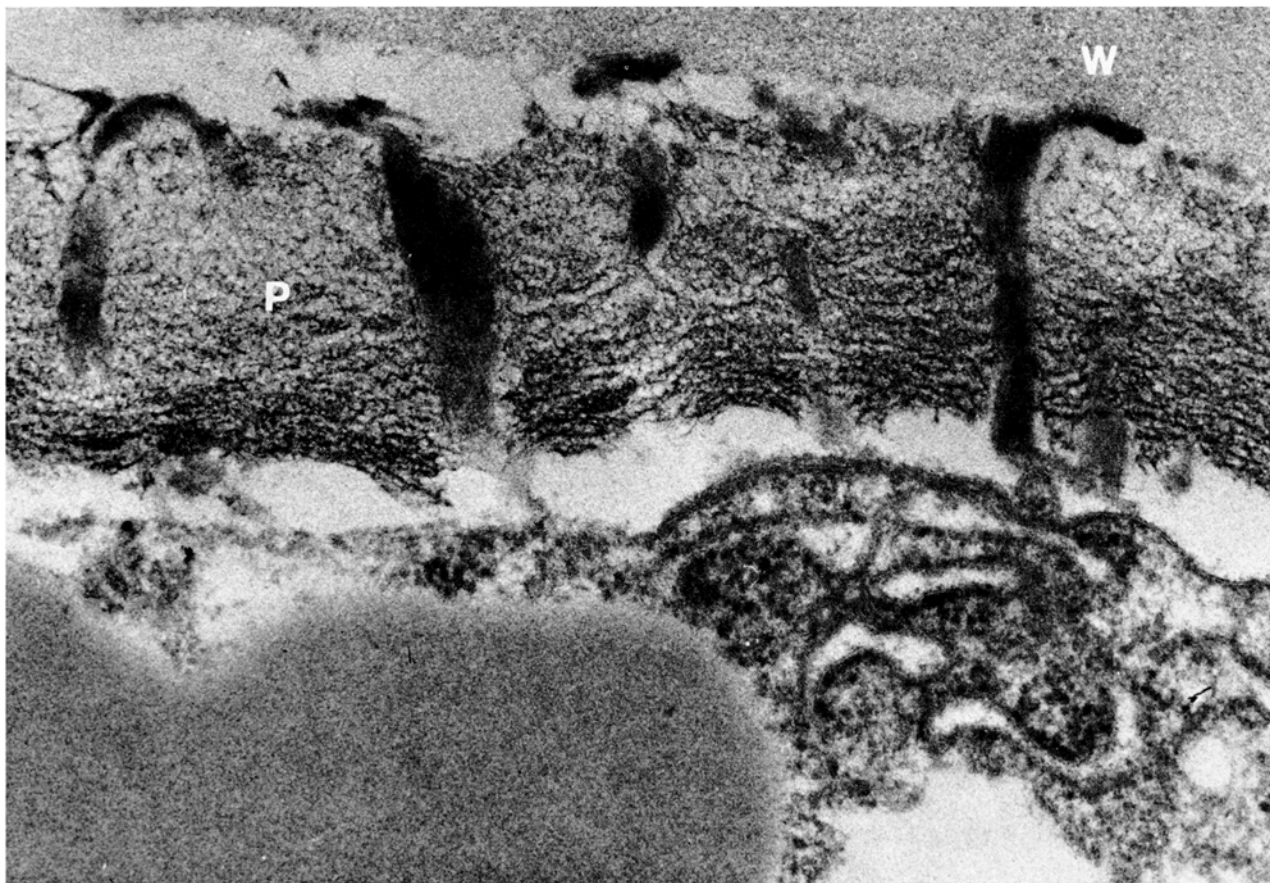
The Primexine of *Nelumbo nucifera*

The positions of apertures in the pollen wall are reportedly determined while the microspores are still enclosed within the special cell wall. In *Silene*<sup>1</sup>, *Zea*<sup>2</sup>, and *Helleborus*<sup>3</sup>, for example, the early apertures are characterized by the absence of primexine and the apposition of endoplasmic reticulum to the plasma membrane. Where the primexine fails to develop, the exine fails to form later in ontogeny. During our study of pollen development of *Nelumbo*, we found evidence which indicates a more involved method of determining apertures.

**Materials and Methods.** Flower buds of *Nelumbo nucifera* Gaertn. were collected from Pamplemousses Gardens, Mauritius and the anthers were dissected after 9 h and fixed in 0.2M glutaraldehyde (precipitated with barium carbonate) in 0.05M cacodylate-HCl buffer. The neutral fixative was computed to have an osmolality of 310 milliosmols<sup>4</sup>. The material was postfixed with 0.5% osmium tetroxide in 0.05M cacodylate-HCl buffer at

pH 7.6 brought to a calculated 230 milliosmols with glucose. The specimens were dehydrated in ethanol and embedded in Epon-Araldite<sup>5</sup> using propylene oxide as an intermediate solvent. Sections were cut with a diamond knife on a Porter-Blum MT-1 microtome. Thick sections were stained with toluidine blue and mounted in anisol for phase microscopy. Unsupported thin sections mounted on 3–400 mesh grids were stained with 1% aqueous uranyl acetate and/or lead hydroxide<sup>6</sup> or 1% aqueous phosphotungstic acid (pH 2.0) and examined with a Zeiss EM 9a.

**Results.** The primexine of *Nelumbo* consisted of fibrils or lamellae of matted fibrils aligned more or less parallel with the plasma membrane. Radially-directed posts, the probacules, penetrated from the cytoplasmic surface through the fibrillar layer to the inner surface of the special cell wall (Figure). The probacules and fibrillar matrix were always distributed over the entire microspore surface. We never saw any presumptive evidence for the



The entire microspore is surrounded by primexine (P) which is penetrated by darkly stained probacules. The special cell wall (W) surrounds the microspores.  $\times 44,000$ .

3 colpi of the mature grain even though we examined more than 1000 thin sections and numerous thick sections of *Nelumbo* tetrads cut from anthers collected from 3 buds.

**Discussion.** Our observation of a primexine stage in which no apertures are delimited demands that the 3 apertures of the mature grain form later. They might be formed in the way described for *Silene*, *Zea*, and *Helleborus*, if the primexine were degraded and subsequently resynthesized with the apertures delimited or if a focal degradation of the primexine distinguished the apertural regions from non-apertural regions. In *Nelumbo* the apertures might also be formed by another method entirely, perhaps at a much later stage. Since the foot layer of *Populus* decreased in thickness during development much more than could be accounted for by the increase in its area, ROWLEY and ERDTMAN<sup>7</sup> suggested that some lytic process could reduce the mass of the exine. The apertures of *Nelumbo* could form by a localized reduction in exine thickness. From our observation we cannot say how the apertures are determined only that they must be formed in some way other than that detailed in the standard model. We do not dispute the current model for the determination of apertures by the absence of primexine or that the primexine's absence is related to the apposition of endoplasmic reticulum to the plasma membrane. We only forward that the model is too limited to account for our observation in *Nelumbo*.

According to the prevailing model for aperture determination, primexine is not formed at sites of future

germinal apertures. However, in *Nelumbo* primexine occurs uniformly over the entire microspore surface although 3 colpi are present at maturity. Therefore, our evidence from *Nelumbo* demands extension of the model<sup>8</sup>.

**Zusammenfassung.** Im Gegensatz zur allgemein akzeptierten Theorie ist bei *Nelumbo* die gesamte Oberfläche der Mikrospore mit Primexine bedeckt; dennoch werden am murenen Pollen drei Colpi entwickelt.

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<sup>1</sup> J. HESLOP-HARRISON, *Grana palynol.* 4, 7 (1963).

<sup>2</sup> J. J. SKVARLA and D. A. LARSON, *Am. J. Bot.* 53, 1112 (1966).

<sup>3</sup> P. ECHLIN and H. GODWIN, *J. Cell Sci.* 3, 175 (1968).

<sup>4</sup> M. D. MASER, T. E. POWELL III and C. W. PHILPOTT, *Stain Technol.* 42, 175 (1967).

<sup>5</sup> H. H. MOLLENHAUER, *Stain Technol.* 39, 111 (1964).

<sup>6</sup> M. J. KARNOVSKY, *J. Cell Biol.* 11, 729 (1961).

<sup>7</sup> J. R. ROWLEY and G. ERDTMAN, *Grana palynol.* 7, 517 (1967).

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