Intracellular Amounts of Nucleic Acids and Protein During Pollen Grain Growth in *Tradescantia**

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PLATE 210

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ABSTRACT

The rapid growth, large organelles, and synchronous development of *T. paludosa* pollen grains make them ideal subjects for cytochemical analysis. A microphotometric study of the nucleoli, chromosomes, and cytoplasm fixed at daily intervals during pollen grain maturation indicated that:

1. DNA (Feulgen) synthesis in the generative nucleus occurred during the first third of interphase, while the DNA content of the vegetative nucleus remained unchanged.

2. Throughout development, changes in RNA (azure B) content, in general, paralleled changes in protein (NYS[1], Millon) content in each organelle of the vegetative cell. Initially, the RNA and protein of all organelles increased up to mid interphase, when chromosomal and nucleolar fractions began to decline despite a continued increase in cytoplasmic RNA and protein. At least 24 hours before anthesis, the vegetative nucleolus had disappeared and chromosomal protein and RNA of the vegetative nucleus were apparently in rapid decline. Such a system offered an opportunity to study the role of the nucleus, especially the nucleolus, in RNA and protein metabolism in the cytoplasm, by noting what cytoplasmic processes could and could not continue at a time when nuclear mechanisms were absent or minimal. It was found that at least 2 fundamental processes continued during this period: both RNA and protein accumulated in the cytoplasm at a rapid rate.

It was concluded that the nucleus is not the sole source of cytoplasmic RNA, for the data suggest that there are at least 2 separate and independent, or remotely dependent synthesizing systems, one nuclear and the other cytoplasmic. It is evident that nuclear influence on cytoplasmic synthesis need be neither direct nor immediate.

One of the goals of microphotometry is to correlate morphological and cytochemical events in an attempt to elucidate function, not only of the organelle, but of the major substances of which it is composed. Thus a sequential history of intracellular amounts of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein during rapid biosynthesis might yield important information on chromosomal-nucleolar-cytoplasmic relationships.

Earlier cytochemical studies of such biosynthetic systems have indicated a participation of RNA in protein synthesis based on the concurrent increase of both substances within the cell (Brachet, 1942; Caspersson, 1950). More recent biochemical studies have not only confirmed involvement of RNA in protein synthesis (Gale and Folkes, 1955), but implicated the DNA molecule as well (Allfrey, 1954; Mirsky, Osawa, and Allfrey, 1956; Gale and Folkes, 1955). One line of research has dealt with the function of various cell organelles in RNA synthesis, particular attention being paid to the relationship between nuclear and cytoplasmic RNA (Brachet, 1955, 1957, for review). It has long been recognized that cytoplasmic growth is

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[1] NYS, naphthol yellow S.

accompanied by large nuclear and nucleolar size and high ribonucleoprotein content of the nucleolus. These coincident changes in the nucleus and cytoplasm have strongly suggested that nuclear components are somehow involved in cytoplasmic synthesis, although the precise nature of this relationship is not known. Much evidence has pointed to the nucleus as a source of cytoplasmic RNA. For instance, Goldstein and Plaut (1955) introduced nuclei, the RNA of which was labelled, into unlabelled cytoplasm of *Amoeba* and reported a transfer of label from the nucleus to the cytoplasm. This cytoplasmic label was removable with ribonuclease treatment. Moreover, Prescott (1957) has shown that while nucleated *Amoebae* are able to incorporate labelled uracil into cytoplasm, enucleated *Amoebae* are unable to do so. Although he concluded that "all the RNA found within the *Amoeba* cytoplasm and nucleus is elaborated within the nucleus," an alternative explanation might be that the presence of the nucleus is necessary in order to provide some substance or substances required for cytoplasmic RNA synthesis. Further observations on *Amoeba* by Rabinovitch and Plaut (1956) and Woodard (unpublished) indicate that there is a complete loss of nuclear RNA (azure B) at mitosis. If this RNA fraction is nucleolar, its loss during mitosis is not surprising, for nucleoli disappear at mitosis in most cells. While this loss could be interpreted as a transfer of nuclear RNA to the cytoplasm, it seems that an equally valid explanation might be that it results from the well known reduction in synthetic activity during mitosis. A reduction or cessation of RNA synthesis during a period of continued RNA breakdown could result in a net loss of RNA.

Additional, although circumstantial, evidence supporting the concept of nuclear origin of cytoplasmic RNA comes from the demonstration by many workers that nuclear RNA has a much greater rate of isotope incorporation than cytoplasmic RNA (Marshak, 1948). This high rate of incorporation in nuclei can probably be attributed to nucleolar uptake, for recent autoradiographic studies on *Drosophila* salivary glands and gastric caeca have shown that although the label appears first in nucleolar associated chromatin, the nucleolus subsequently has a far greater specific activity than either the cytoplasm or the chromosomes (Taylor, 1953). Ficq (1955) has also reported that in rapidly growing oocytes uptake of label by RNA of the nucleolus can be 100 times that of other cell constituents.

The above studies have suggested that cytoplasmic RNA may originate in the nucleus, and the order of appearance of labelled RNA, *i.e.* chromosomes, nucleoli, cytoplasm, has further suggested the hypothesis that RNA synthesized by the chromosomes is transferred to the cytoplasm, at least in part, via the nucleolus (Taylor *et al.*, 1955).

If cytoplasmic RNA were derived solely and directly by diffusion from the nucleus, one might expect changes in amounts of nuclear RNA to precede and parallel changes in amounts of cytoplasmic RNA. If, on the other hand, the correlation between nuclear and cytoplasmic changes in RNA content is only slight or absent, one might suspect that cytoplasmic RNA synthesis is independent of, or only remotely dependent on, the nucleus.

A microphotometric analysis of these relationships would require a rapidly growing, homogeneous tissue undergoing synchronous development, from which one could take periodic samples. Since pollen grain growth in *Tradescantia paludosa* offers these advantages, it was selected for this study of the sequence of changes of nucleic acid and protein amounts in the chromosomes, nucleoli, and cytoplasm.

Methods and Materials

Over a 5 day period, one anther was removed daily from the same bud, which, remaining on the plant, was protected from insects and desiccation by enclosing the spike in a sheet wax (parafilm) bag. The anthers were fixed in 3:1 ethanol-acetic acid, embedded in paraffin, and sectioned at varying thicknesses. Twenty micron sections were stained for DNA by the Feulgen method. Six micron sections were prepared for RNA measurements by 1 hour digestion in 0.001 per cent deoxyribonuclease (crystalline, Worthington) at pH 6.5, and staining in 0.025 per cent azure B buffered at pH 4 according to the method of Flax and Himes (1952). Upon completion of the azure B measurements, these slides were destained in water adjusted to pH 2 with HCl and restained using the naphthol yellow S (NYS) procedure for proteins (Deitch, 1955), *i.e.*, staining in 1 per cent NYS in 1 per cent acetic acid, and differentiating 15 hours in 1 per cent acetic acid. However, the NYS-stained cytoplasm of the 87 hour sample unaccountably contained large amounts of a refractile, granular material which scattered heavily at the wave length used for measurement. Since measurements of this preparation would have been overestimated, companion slides were stained by the Millon method for tyrosine (Rasch and Swift, 1953). The Millon-stained cytoplasm was free of non-specifically absorbing materials, and measurements were carried

TABLE I

Stains and Wave Lengths Used for Microphotometric Measurement

	Azure B	Millon	NYS	Feulgen
	A	*A*	*A*	*A*
Nucleoli.....	5000		4700	
Chromosomes..	6000		4700	6100 (5000 and 5500)*
Cytoplasm.....	(4400 and 4780)*	5000	4700	

* Two wave length method.

out at all stages in which the distribution of chromophore was sufficiently homogeneous.

In order to minimize error, photometric measurements were made at wave lengths best suited to the intensity of the stain. The wave lengths used are summarized in Table I. Extinctions of azure-stained nucleoli were corrected to a wave length of 6000 A to make nucleolar and chromosomal[2] values comparable. It should be mentioned that interpretations of data on azure-stained chromosomes are necessarily tentative, because basic dye binding by chromosomes can be inhibited by protein interference (Flax and Himes, 1952; Swift, 1953). Obviously, then, it is the shape of the chromosomal RNA curve, rather than its relative height that is significant in Text-fig. 2.

For photometric determinations of dye amounts, the "plug" method (Swift and Rasch, 1956) was used throughout except in the case of Feulgen-stained generative nuclei at 89 hours, and azure-stained cytoplasm. Since the stain was too irregularly distributed for "plug" measurements in these instances, the two wave length method of Ornstein (1952) and Patau (1952) was used. The amount of azure in individual pollen grains was estimated by summating the two wave length values of sections of single pollen grains. Although the RNA amounts so derived are actually the sum of chromosomal, nucleolar, and cytoplasmic amounts, the combined contribution of the nucleolus and chromosomes never exceeds 2 per cent of the total; such values will, therefore, be referred to as cytoplasmic RNA. The two wave length measurements were equated with the "plug" measurements, using the method of Swift and Rasch (1956).

Correction for the light absorption of NYS-stained chromosomes above and below the nucleoli was made using the formula $E_c = E_s - (1 - p/d)E_m$, in which E_c is the extinction of the nucleolus, E_s is the extinction of the nucleolus and the material above and below it, p is the mean optical path through the sphere, d is section thickness, and E_m is the extinction of chromosomes adjacent to the nucleolus (Swift and Rasch, 1956).

In dealing with sections of nuclei, total dye content was calculated by using the formula EV/d, in which E is the extinction of a section of the nucleus, V is the volume of the nucleus, and d is section thickness.

As is well known, the 2-celled pollen grain is composed largely of vegetative cytoplasm, the generative cytoplasm comprising only a small percentage of the total cytoplasmic mass (Sharp, 1943). Pollen grain growth can, therefore, be considered primarily vegetative cell growth. Any involvement of the generative nucleus in cytoplasmic RNA synthesis must be brief, for it is obvious from visual microscopic examination that both nucleolar and chromosomal RNA amounts decline rapidly in early interphase. It, therefore, seems likely that it is principally the constituents of the vegetative cell that are involved in cytoplasmic synthesis in the pollen grain. For this reason, except for the Feulgen data on generative nuclei, all microphotometric data on nucleoli and chromosomes were taken from the vegetative cell. Cytoplasmic RNA and protein measurements will necessarily include not only vegetative cytoplasm, but the small sheath of generative cytoplasm surrounding the generative nucleus.

RESULTS

As Text-fig. 1 and Table II indicate, the DNA content of both the early vegetative and generative nuclei were approximately equal. While the amount of DNA in the vegetative nuclei remained unchanged throughout the period analyzed, DNA synthesis occurred in early interphase of the generative nuclei. Twenty hours after the pollen grain mitosis, the mean DNA amount of the generative nuclei had increased about 50 per cent. A sample taken 44 hours after mitosis showed that the generative nuclei had doubled their DNA amounts. This amount was then maintained until anthesis (Swift, 1950; Woodard, 1956).

It is noteworthy that DNA synthesis takes place at different times in different tissues. In *Tradescantia* it has been shown that the premeiotic DNA synthesis extends into leptotene, and that DNA synthesis preceding the pollen grain division takes place just previous to mitosis (Swift, 1950; Moses and Taylor, 1955). The present data indicate that DNA synthesis following this division is completed during the first third of interphase. On the other hand, a plot of DNA amounts against nuclear

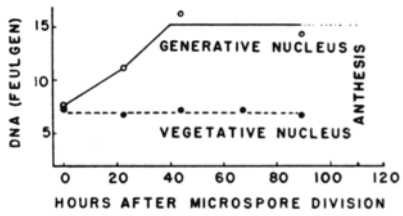
[2] For convenience, the protein and RNA amounts of the nucleus exclusive of the nucleolus will be termed chromosomal protein and chromosomal RNA. What percentage of these measured amounts is actually nuclear sap protein and RNA is not known.

TABLE II

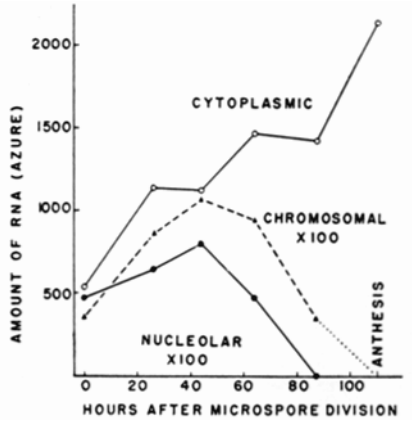
Amount of DNA (Feulgen) in Arbitrary Units in Individual Nuclei, Standard Error, and Number of Variates (n)

The "plug" method was used throughout except for generative nuclei of the 89 hour sample, which were measured with the two wave length method.

	Hrs. after mitosis					
	Very early interphase	22	44	67	89	110
Vegetative nucleus	7.2 ± 0.28 n = 10	6.8 ± 0.22 n = 10	7.3 ± 0.17 n = 10	7.3 ± 0.16 n = 10	6.8 ± 0.24 n = 10	—
Generative nucleus	7.5 ± 0.18 n = 10	11.2 ± 0.67 n = 21	16.2 ± 0.60 n = 13	—	14.3 ± 0.96 n = 6	—



TEXT-FIG. 1. Means of DNA (Feulgen) values (in arbitrary units) are plotted against time.



TEXT-FIG. 2. Means of RNA (azure B) values (in arbitrary units) of the various cell fractions are plotted against time. Since chromosomal RNA is unmeasureable at anthesis, the dotted part of the curve between 87 and 110 hours is an estimate made from visual microscopic inspection. (A preliminary report of these data was published in Swift *et al.* (1956)).

volume in *Tradescantia* root meristems shows that DNA synthesis occurs at approximately mid-interphase (Swift, 1953). These data lend additional support to the concept originally suggested by Swift (1953) that although there seem to be somewhat definite restrictions in the time of DNA synthesis within a tissue, within the organism, the time of synthesis may vary from tissue to tissue.

Changes in RNA amounts may be followed by referring to Text-fig. 2 and Table III. A photomicrograph of each of the stages studied is shown in Figs. 1 to 7. It may be seen that there was a postmitotic increase of RNA in chromosomes, nucleolus, and cytoplasm of the vegetative cell. This increase sharply contrasted with the rapid diminution in RNA of both nucleolus, (Figs. 1 and 2), and chromosomes in the generative nucleus during early interphase. At approximately 43 hours after the mitosis, RNA of the vegetative nucleolus

TABLE III

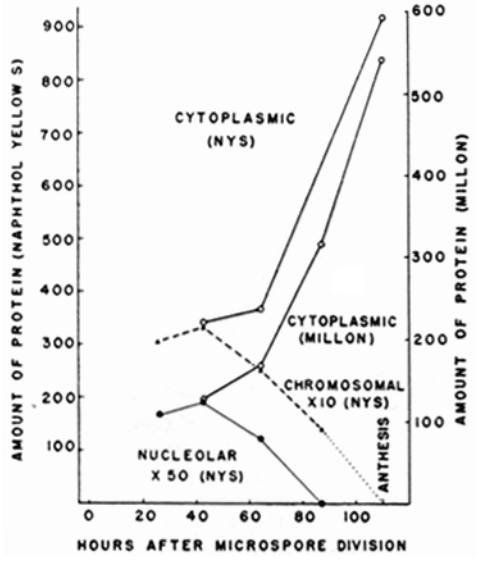
Intracellular Amounts of RNA (Azure B) in Arbitrary Units, Standard Error, and Number of Variates (n) in the Vegetative Cell

	Hrs. after mitosis					
	Post-telophase	26	43	64	87	110
Chromosomal RNA	3.6 ± 0.45 n = 6	8.7 ± 0.66 n = 10	10.8 ± 0.86 n = 11	9.4 ± 0.95 n = 10	3.5 ± 0.26 n = 6	—
Nucleolar RNA	4.7 ± 0.29 n = 10	6.4 ± 0.29 n = 10	8.0 ± 0.42 n = 12	4.7 ± 0.30 n = 10	0	0
Cytoplasmic RNA	535 ± 53.2 n = 9	1149 ± 38.2 n = 6	1121 ± 89.8 n = 5	1465 ± 35.4 n = 5	1420 ± 44.1 n = 5	2137 ± 79.5 n = 5

TABLE IV

Intracellular Amounts of Protein (naphthol yellow S, Millon) in Arbitrary Units, Standard Error, and Number of Variates (n) in the Vegetative Cell

	Hrs. after mitosis					
	Post-telophase	26	43	64	87	110
Chromosomal protein (NYS)	—	31.3 ± 3.4 n = 11	33.5 ± 2.3 n = 11	25.5 ± 2.1 n = 10	14.2 ± 0.68 n = 10	—
Nucleolar protein (NYS)	—	3.4 ± 0.15 n = 10	3.8 ± 0.18 n = 11	2.4 ± 0.13 n = 11	0	0
Cytoplasmic protein (NYS)	—	—	339 ± 20.9 n = 10	368 ± 23.0 n = 10	—	920 ± 61.4 n = 10
Cytoplasmic protein (Millon)	—	—	123 ± 14.8 n = 10	167 ± 22.9 n = 10	315 ± 20.5 n = 10	541 ± 30.6 n = 10



TEXT-FIG. 3. Means of protein (Millon, naphthol yellow S) amounts (in arbitrary units) in the various cell fractions are plotted against time. Since chromosomal protein is unmeasureable at anthesis, that part of the curve (dotted line) between 87 and 110 hours is an estimate made from visual microscopic inspection.

and chromosomes began to decrease, although the cytoplasmic fraction continued to increase. The decrease in RNA content of the nucleolus persisted, the latter eventually disappearing at 87 hours (Fig. 5). At this time, the generative nucleolus is absent or minute (0.5 μ or less in diameter), and the chromosomes of the generative nucleus are azure-negative. Because of its irregular shape the vegetative nucleus at anthesis was unmeasurable photometrically, but visual microscopic inspection indicated that there remained little, if any, azure stain. In the absence of the nucleolus and during the final decline of chromosomal RNA, cytoplasmic RNA increased 50 per cent. That an increase in cytoplasmic RNA did occur was verified in a confirmatory experiment in which cytoplasmic RNA showed a 33 per cent increase between the disappearance of the nucleolus and anthesis.

It is worth noting that, during the last half of pollen grain development, a great deal of the cytoplasmic RNA was in the form of heavily basophilic structures, as seen at the light microscope level (Fig. 5). At the electron microscope level they appeared as groups of lamellae (Fig. 6), strongly resembling the ergastoplasmic lamellae of animals, that have recently received much attention because of their possible involvement in protein synthesis (Palade and Siekevitz, 1956) and the transmission of chromosomal specificity (Rebhun, 1956; Swift, 1956; Gay, 1955).

As to protein changes (Table IV) during pollen grain maturation, Text-fig. 3 indicates that the curve of protein content of the chromosomes, nucleoli, and cytoplasm approximated, in general, that of RNA in these organelles. Following the disappearance of the nucleolus and during the rapid decrease in chromosomal protein, cytoplasmic protein (Millon) has increased 72 per cent. The occurrence of an increase during this period was confirmed by an additional experiment which showed a 120 per cent increase in Millon protein after the disappearance of the nucleolus.

DISCUSSION

The photometric data presented above are not compatible with the hypothesis that the nucleus is

the sole source of cytoplasmic RNA. If RNA were synthesized only in the nucleus and transferred to the cytoplasm, one would not expect the RNA of the recipient cytoplasm to show a marked increase during and after the decline of nuclear constituents. There is evidence for cytoplasmic synthesis of RNA from other lines of investigation. For instance, if cytoplasmic RNA were derived by simple diffusion from nuclear RNA, these RNA's might be expected to be identical. However, it has been reported by many workers that they differ in several respects (Brachet, 1955, for review): they vary in both molar composition and nucleotide sequence. A mathematical analysis of time and rate of incorporation of P^{32} into nuclear and cytoplasmic RNA has indicated that nuclear RNA is probably not a precursor of cytoplasmic RNA (Barnum, Huseby, and Vermund, 1953). However, Taylor *et al.* (1955), using a similar analysis on autoradiographic data on salivary glands of *Drosophila*, concludes that the data fit both: (1) the concept of nucleolar RNA acting as a precursor of cytoplasmic RNA, and (2) the concept of a common precursor for both nucleolar and cytoplasmic RNA, *i.e.*, a cytoplasmic synthesis of RNA. Further evidence for the high degree of independence of cytoplasmic RNA synthesis is found in the report that enucleated *Acetabularia* is capable of a net synthesis of RNA up to 15 days after enucleation (Brachet *et al.*, 1955). The RNA data presented herein cannot test the possibility that during early interphase, when nuclear organelles are robust, the nucleus may synthesize and transfer RNA precursors to the cytoplasm. These precursors may assemble to form RNA molecules after the apparent degeneration of nuclear mechanisms, at which time, but not before, they will bind azure B.

Because changes in protein content parallel those in RNA content in each organelle, interpretations of the RNA data may likewise apply to protein. It would thus appear that, as in the case of RNA, there is no simple relationship between nuclear, and cytoplasmic amounts of protein. In this connection, Brachet *et al.* (1955) have reported a net increase in protein amounts in *Acetabularia* 15 days after enucleation. They have concluded that although the presence of the nucleus is required for prolonged maintenance of cytoplasmic synthesis, the relationship between the nucleus and cytoplasm must be remote.

Until the kinetics of RNA and protein metabolism are better understood, possibly through the combined use of autoradiography and microphotometry, interpretations of either microphotometric or autoradiographic data alone are somewhat limited in scope. Nevertheless if, as some workers suspect, the carrier of genetic specificity from nucleus to cytoplasm is RNA (Dounce, 1953; Rich and Watson, 1954) or protein (Casperson, 1950; Brachet and Chantrenne, 1957), it is tempting to suppose that the large amount of chromosomal and nucleolar RNA and protein during early development of the pollen grain is an expression of active nuclear participation in synthesis and transfer of one or both of these materials to the cytoplasm during this period. In this way sites for nuclear specific synthesis may be established in the cytoplasm. One might further speculate that, as nuclear RNA and protein amounts diminish, beginning at 43 hours after the mitosis, secretion by the nucleus wanes as cytoplasmic synthesis increases, until finally, at the disappearance of the nucleolus, RNA and protein synthesis may be largely, if not entirely, a function of the cytoplasm.

CONCLUSION

It is concluded that RNA synthesis is not an exclusive function of the nucleus, for in the absence of the nucleolus and during the rapid decline of chromosomal RNA, cytoplasmic RNA has been seen to increase. The data further suggest that there are two separate, and at times independent, or only remotely dependent systems operating in the biosynthesis of RNA and protein, one nuclear and the other cytoplasmic. The evidence indicates that nuclear participation in these cytoplasmic processes need not be either direct or immediate.

REFERENCES

Allfrey, V., *Proc. Nat. Acad. Sc.*, 1954, **40,** 881.
Barnum, C., Huseby, R., and Vermund, H., *Cancer Research*, 1953, **13,** 880.
Brachet, J., *Arch. Biol.*, 1942, **53,** 207.
Brachet, J., *in* The Nucleic Acids, (E. Chargaff and J. N. Davidson, editors), New York, Academic Press, Inc., **1,** 1955.
Brachet, J., Chantrenne, H., and Vanderaeghe, F. *Biochim. et Biophysica Acta*, 1955, **18,** 544.
Brachet, J., and Chantrenne, H., *Cold Spring Harbor Symp. Quant. Biol.*, 1957, **21,** 329.
Caspersson, T., Cell Growth and Cell Function, New York, Norton, 1950.
Deitch, A., *Lab. Inv.*, 1955, **4,** 324.
Dounce, A., *Nature*, 1953, **172,** 541.
Ficq, A., *Arch. Biol.*, 1955, **66,** 509.
Flax, M., and Himes, M., *Physiol. Zool.*, 1952, **25,** 297.
Gale, E., and Folkes, J., *Biochem. J.*, 1955, **59,** 66.

Gay, H., *Proc. Nat. Acad. Sc.*, 1955, **41**, 370.
Goldstein, L., and Plaut, W., *Proc. Nat. Acad. Sc.*, 1955, **49**, 874.
Marshak, A., *J. Cell. and Comp. Physiol.*, 1948, **32**, 381.
Mirsky, A., Osawa, S., and Allfrey, V., *Cold Spring Harbor Symp. Quant. Biol.*, 1957, **21**, 49.
Moses, M., and Taylor, J., *Exp. Cell Research*, 1955, **9**, 474.
Ornstein, L., *Lab. Inv.*, 1952, **1**, 250.
Palade, G., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 171.
Patau, K., *Chromosoma*, 1952, **5**, 341.
Prescott, D., *Exp. Cell Research*, 1957, **12**, 196.
Rabinovitch, M., and Plaut, W., *Exp. Cell Research*, 1956, **10**, 120.
Rasch, E., and Swift, H., *J. Histochem. and Cytochem.*, 1953, **1**, 392.
Rebhun, L., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 93.
Rich, A., and Watson, J., *Proc. Nat. Acad. Sc.*, 1954, **40**, 759.
Sharp, L., Fundamentals of Cytology, New York and London, McGraw-Hill Book Company, Inc., 1943.
Swift, H., *Proc. Nat. Acad. Sc.*, 1950, **36**, 643.
Swift, H., *in* International Review of Cytology, (G. H. Bourne and J. F. Danielli, editors), New York, Academic Press, Inc., 1953, **2**.
Swift, H., Rebhun, L., Rasch, E., and Woodard, J., Fourteenth Symposium of the Society for the Study of Development and Growth, Princeton, New Jersey, Princeton University Press, 1955.
Swift, H., and Rasch, E., *in* Physical Techniques in Biological Research, (G. Oster and A. W. Pollister, editors), New York, Academic Press, Inc., 1956, **3**.
Swift, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 415.
Taylor, J., *Science*, 1953, **118**, 555.
Taylor, J., McMaster, R., and Caluya, M., *Exp. Cell Research*, 1955, **9**, 460.
Woodard, J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 93.

EXPLANATION OF PLATE 210

FIGS. 1 to 7. Seriated stages of pollen grain development stained for RNA with azure B. Vegetative nuclei are designated by letter *v*. Magnification of light photomicrographs is 1350. Section thickness is 6 microns.

FIG. 1. Post-telophase. Arrow points to generative nucleolus.

FIG. 2. 26 hours after pollen grain mitosis. Note rapid diminution of generative nucleolus (arrow).

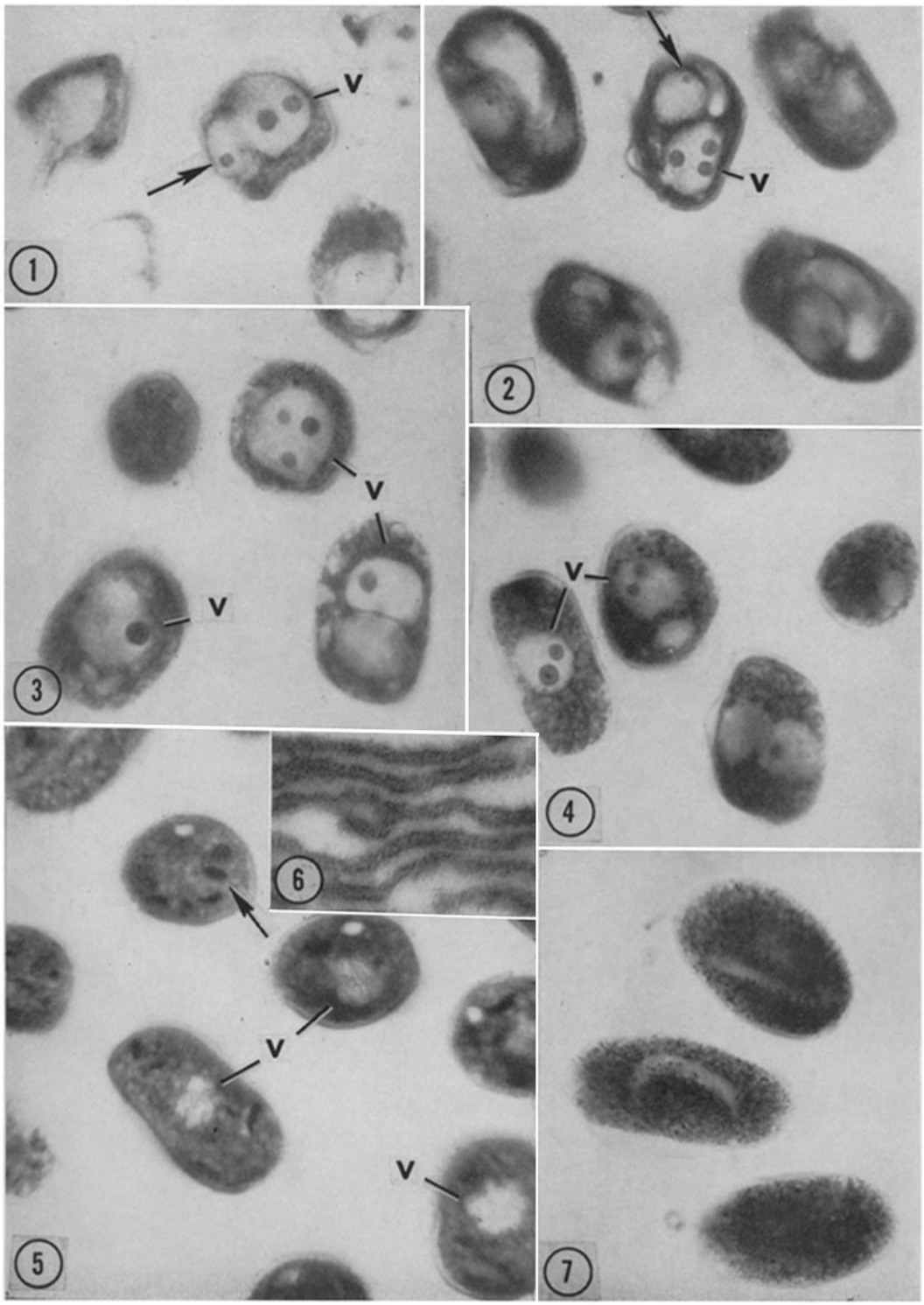
FIG. 3. 43 hours after pollen grain mitosis.

FIG. 4. 64 hours after pollen grain mitosis.

FIG. 5. 87 hours after pollen grain mitosis. Note the absence of the vegetative nucleolus.

FIG. 6. 87 hours after pollen grain mitosis. Electron micrograph of a section of pollen grain (fixed in OsO_4 and embedded in methacrylate), showing lamellar structure of the basophilic bodies in the cytoplasm (Fig. 5, arrow). × 36,000.

FIG. 7. Anthesis. 110 hours after pollen grain mitosis.



(Woodard: Nucleoproteins during growth)