CHROMOSOME–NUCLEAR MEMBRANE–CYTOPLASMIC INTERRELATIONS IN DROSOPHILA*

By HELEN GAY, Ph.D.

(From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island)

Plates 135 to 137

In the course of a study of the chromosomes and nuclei of salivary-gland cells of *Drosophila melanogaster* larvae (1), electron micrographs were obtained in which details of cellular organization suggested a mechanism for exchange of materials between nucleus and cytoplasm. Studies in many fields of experimental biology had demonstrated the importance of the nucleus in control of cellular function and at times had implied an actual transfer of substances from nucleus to cytosome. The method of transfer of these materials, however, whether as large visible particles or submicroscopic aggregates, had not been determined. The observations on *Drosophila* salivary-gland cells presented below supplement the earlier reports and hypotheses of nucleocytoplasmic interrelations by demonstrating a possible structural mechanism for transfer to the cytoplasm of nuclear materials originating at specific regions of chromosomes and their subsequent role in the formation of cytoplasmic organelles. The original reports of these findings have been published elsewhere (1–3) and will merely be summarized here as a background for presentation of more recent observations.

Materials and Methods

The tissues used in these studies were salivary glands of larvae of *Drosophila melanogaster*, preserved in a variety of fixatives, most of them containing osmium tetroxide (4, 5), and imbedded in *n*-butyl methacrylate. Serial sections were examined in the electron microscope. Cytochemical tests, using enzymes (prepared and purified in this laboratory) and staining reactions, were made on methacrylate-imbedded tissues and also on paraffin-imbedded material that served as a control.

OBSERVATIONS

The structural evidence for nucleocytoplasmic interchange was detected in salivary-gland cells of the third larval instar. These cells are set aside in definitive number in early embryonic stages and do not divide thereafter although they continue to grow as the larva ages. Development in *D. melanogaster* proceeds through three instars, of which the third or last is best known to

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geneticists because cells at this stage afford the giant chromosomes used so extensively in cytogenetic research. An ultrathin section of a midthird instar salivary-gland cell is shown in Fig. 2. The cytosome of these cells contains the usual discrete cytoplasmic structures such as mitochondria and endoplasmic reticulum. In addition, there are many spherical secretion granules whose number and size increase from the middle of the third instar period until pupation. The giant chromosomes observed within the nucleus have generally been regarded as polytene or composed of many closely appressed strands. Since the chromosomes and nuclear membrane of the midthird instar salivary-gland cell are involved in the nucleocytoplasmic phenomenon to be discussed below, a more detailed description of their structure as revealed by the electron microscope will be inserted here.

Chromosomes.—Electron micrographs obtained in an earlier study of ultrathin sections of smears and intact nuclei of *D. melanogaster* fixed in a variety of fixatives (1) indicated that the chromosomes are composed of numerous closely appressed chromonemata with no visible limiting membranes although the boundaries of the chromosomes can readily be distinguished. The absence of a chromosomal membrane should facilitate nucleocytoplasmic exchanges of the type that are under consideration here. Each of the chromonemata is divided into chromomeric (denser material) and non-chromomeric (less dense) regions. Lying side by side the chromomeres constitute the bands; the non-chromomeric intervals form the interband regions. It has been found that the difference in density between band and interband material revealed in electron micrographs of these giant chromosomes may vary considerably depending upon the state of the cell (its age or stage of secretory activity), the kind of fixative used, or the thickness of the section. However, 1 μ "control" sections obtained from the same glands that afforded the ultrathin sections used in electron microscopy have always revealed chromosomes with Feulgen-positive banded regions separated by Feulgen-negative intervals. This finding indicates that buffered osmium-tetroxide fixation preserves a band sequence resembling that which has been mapped and studied by cytogeneticists using aceto-carmine or acetic-orcein smear preparations. Fig. 4 shows a longitudinal section of a chromosome from a salivary-gland cell fixed in buffered osmium tetroxide. Examples of other types of fixation may be seen in Fig. 6 *a* and in previous publications (1, 6). It has been found that the component chromonemal strands (arrows, Fig. 4) range in different preparations between 200 and 500 A in diameter (the difference may be due to inability to distinguish in the photographs between the chromonemata and associated non-chromonemal material). Accepting 500 A as the diameter of the chromonema, it has been calculated that the number of component strands of the salivary-gland chromosomes of the midthird instar cell lies between 1000 and 2000 (probably either 2^{10} or 2^{11}). This is in excellent agreement with spectrophotometric measurements

made by Swift and Rasch (7) who reported degrees of polyploidy as high as 1024 N in nuclei of third instar larval glands. The arrangement of the chromonemata as determined from serial sections of these polytene chromosomes suggests that the chromonemata are coiled in pairs to form a hierarchy of coiled pairs constituting the coiled homologues (1).

Nuclear Membrane.—The nuclear membrane of the third instar salivary-gland cell of *Drosophila* is double layered when seen in cross-section and has a total thickness of about 250 A. In tangential sections, the nuclear membrane reveals a reticulate structural pattern (Fig. 3). Highly electron-scattering materials (apparently granular) form annuli which surround less electron-dense areas (the so called "pores" of some authors) which appear circular, hexagonal, or pentagonal in outline and are about 500 to 600 A in their greater diameter; they are spaced approximately 1000 A from center to center. Some of these areas show in the center a denser, dotlike structure (see arrow, Fig. 3). Whether the dotlike structures represent the presence of granules or fibers passing through the "pores," or components of an extremely thin supporting membrane across the "pores," remains to be determined. Although the "pores" as seen in cross-section of the nuclear membrane superficially appear as discontinuities, their interpretation as thorough-going holes which would confer sieve-like properties on the double layered membrane, must be accepted with due caution and full recognition of the physiological implications.

Transfer of Materials from Nucleus to Cytosome.—The salivary glands, as their name implies, are assumedly concerned with the production of a digestive fluid, but during middle to late third instar the larvae reduce their food intake preparatory to pupation. At this stage another intracellular synthetic process supersedes that of saliva production, and leads to the formation of secretion granules. Fraenkel and Brookes (8) have suggested that the secretion produced at this stage is subsequently disgorged from the oral cavity and serves to anchor the puparium to the substrate. The secretion granules contain a mucoprotein, giving positive tests for proteins when stained with acidic dyes in combination with pepsin digestion (9), and for polysaccharides when stained with the periodic acid–Schiff test (10). The production of these secretion granules begins during midthird instar. Early third instar glands do not reveal the PAS-positive granules (see Fig. 1).

At about the time that the granules first become apparent, the cells in which they are produced exhibit a blebbing of the nuclear membrane that is discernible at the level of resolution afforded by the electron microscope. Blebbing is either entirely absent or very rare during early stages of development. An analysis of the process suggests that during middle third instar a mechanism of nucleocytoplasmic interaction is brought into operation in salivary-gland cells that involves production of outpocketings of the nuclear membrane

and their detachment into the cytosome, where they could conceivably function in the formation of secretion granules.

In electron micrographs, the nuclear membrane of the third instar salivary-gland cells was found to be undulatory and frequently to protrude into the cytosome in the form of small outpocketings or blebs (see arrows, Fig. 2). Studies of many cells showing these blebs revealed that the chromosomal materials adjacent to the outpocketings were more highly electron-scattering than in other parts of the nucleus and frequently contained definite structural elements such as granules or vesicles (Figs. 2, 5, 8, of this paper and others in (1, 3, 6)). An analysis of serial sections which permitted tracing a bleb through its entirety, revealed that the differentiated highly electron-scattering material was always connected or confluent with a salivary-gland chromosome and was located adjacent to a nuclear membrane outpocketing. In some cases the connections between outpocketings and chromosomes were to single bands in intercalary regions and in others apparently to terminal chromatin. Occasionally the intercalary band was located in a "reverse repeat," some of which have been shown in cytogenetic studies to contain heterochromatin.

In order to establish with certainty that the nuclear membrane blebs were not attributable to fixation distortion, salivary-gland cells preserved by a variety of fixatives were examined. Fig. 5, for example, shows a bleb found in a cell preserved in Dalton's chrom-osmic fixative. Variation of the pH or ionic strength of any of the fixatives did not modify the appearance of the nuclear membrane outpocketings. All these studies have led to the conclusion that blebbing is not merely due to fixation distortion but is a characteristic feature of the salivary-gland cell at this stage of development. This conclusion is sustained by the observation that outpocketings do not appear during very early third instar as can be seen in Fig. 1. In a cell of the salivary gland at this stage of development the nucleus has a relatively smooth membrane and the cytoplasm contains few secretion granules.

A study of the changes that occur in the salivary-gland cell of *Drosophila melanogaster* from the end of second instar up to pupation revealed that secretion granules first appeared in the cytosome when about one-third of the period of third larval instar development had been completed, a time at which nuclear membrane blebs also first appeared. The number and size of secretion granules increased throughout the later hours of larval life as did the number of outpocketings. Just prior to puparium formation, however, fewer blebs were found. The salivary gland of *Drosophila* undergoes degradational changes at pupation. The question accordingly arose whether the production of nuclear membrane blebs represents the first stages of nuclear degeneration. Several lines of evidence which have been enumerated elsewhere (3) indicate that it does not. By all available criteria the salivary glands of the mid- to late third instar of *Drosophila* are composed of functionally active cells.

It is believed that the structural relationships just described reflect the functional activity of chromosomes in contributing materials to the cytoplasm. Further evidence about the materials involved in the transfer has been obtained by the use of the enzyme deoxyribonuclease on fixed salivary-gland cells. Light microscope examination of the results of nuclease digestion followed by the Feulgen and fast green staining reactions afforded proof that hydrolysis of deoxyribonucleic acid (DNA) in these cells had been effected. Electron micrographs of thin sections taken from these same tissues, which were treated with 0.1 mg./ml. deoxyribonuclease in 0.003 M $MgSO_4$, and of the controls, which were treated with 0.003 M $MgSO_4$, are shown in Fig. 6. Deoxyribonuclease has definitely reduced the electron-scattering capacity of the chromosomes. Fig. 7 shows the effect of deoxyribonuclease treatment in reducing the density of the material within an outpocketing of the nuclear membrane. This result indicates that the nuclear membrane bleb contains chromosomal material which most probably includes DNA. There is evidence that the highly differentiated chromosomal material which is always associated with the blebs (see Figs. 5 and 8) is not greatly reduced in density by deoxyribonuclease treatment; an analysis of this material is being pursued with other enzymes and cytochemical tests.

DISCUSSION

The intimate association observed between chromosomal materials and nuclear membrane outpocketings may be a manifestation of a mechanism for transport of materials of chromosomal origin to the cytosome. In this case the outpocketings from the nucleus would be detached and released into the cytosome. In several instances, a double membraned structure, similar to endoplasmic reticulum and having the form of a bleb, was observed adjacent to the nucleus but completely detached therefrom. The similarity in structure of the endoplasmic reticulum in cross-section and tangential view (Fig. 3) to that of the nuclear membrane suggests that the blebs freed from their connection with the nuclear membrane, become flattened to form the sac-like membranes of the so called endoplasmic reticulum. Under such circumstances, the contents of the outpocketing could also contribute to the production of cytoplasmic materials.

If the reticulum of the salivary-gland cells is associated with the formation of secretion granules (and the time sequence of events revealed in the present study, together with the suggestion in the electron micrographs of a close association of both large and small secretion granules with the open ends of the membranes, indicates such a relationship) an elaborate scheme could be formulated for the role of specific chromosomal regions in directing cytoplasmic functions. It is tempting to speculate that the endoplasmic reticulum in the young larval salivary-gland cells is the site of formation of saliva and that the

new function of forming a cementing substance may be attributed to the double layered lamellae produced late in larval life. This inference is supported by several lines of evidence. In addition to those enumerated above, it may be noted that the material between the secretion granules shows a pronounced basophilia; electron micrographs have shown that the endoplasmic reticulum is located in these regions. The capacity of ribonucleic acid (RNA) to facilitate protein synthesis has been reported by Caspersson (11), Brachet (12), Gale and Folkes (13), and many others. According to Caspersson, Schultz (14), and Brachet, RNA accumulates on the cytoplasmic side of the nuclear membrane. This situation might be explicable on the basis of accumulation of detached blebs which had become converted to layers of the endoplasmic reticulum with their associated RNA. Palade and Siekevitz (15) and Novikoff (16), moreover, have demonstrated that the microsomal RNA may be derived from the granular portion of the endoplasmic reticulum in tissue homogenates. These are all circumstantial lines of evidence but they present an array of related facts that cannot be ignored.

The condition responsible for blebbing during third instar may be the high degree of polyteny attained by the chromosomes that permits accumulation of a favorable level of some essential chromosomal product. Perhaps of more interest is the fact that some of the blebs seem to be associated with chromosomal regions that are heterochromatic. The phenomenon may therefore reflect some general property of heterochromatin and this may in turn account for the fact that many blebs are present in a cell since all chromosomes of *D. melanogaster* contain heterochromatin.

The concept of the nuclear membrane as an actively functioning structural component of the cell has not been considered in earlier theoretical discussions of nucleocytoplasmic interrelations. Schultz (17) suggested that the properties of the nuclear membrane might vary according to the chromosomes involved in its production, and that this variation might afford a basis for differential cell function and cell differentiation. The present findings in *Drosophila* salivary-gland cells sustain this point of view and extend the concept to include a nuclear membrane which is capable of continuous displacement and reformation. Swift (18) and Rebhun (19) have demonstrated the active participation of the nuclear membrane of oocytes of *Spisula* and *Otala* in formation of basophilic cytoplasmic structures, which in turn may be involved in protein synthesis. Although the mechanism by which the end result is attained may be different, the structures involved seem to be similar. In the present discussion the outpocketings have been considered as swellings of the existing membrane, but there is an alternative possibility concerning their method of origination, namely that a new membrane is formed as a result of passage of materials of chromosomal origin through a small opening in the membrane. In either case, the production of a new segment of the nuclear

membrane is involved, either as an abscission layer between the contents of the nuclear bleb and the nucleus, or as a boundary formed between the cytoplasm and the nuclear materials being transferred to the cytosome.

It would be hazardous to imply that the proposed mechanism is anything more than one of a possible series of alternative methods by which nucleocytoplasmic transfer of materials may be mediated. The blebbing phenomenon in *Drosophila* salivary-gland cells, even though it may be unique in nature, offers one mechanism whereby nucleocytoplasmic exchanges involving specific chromosomal regions may be effected. The possible intermediation of a nuclear membrane in gene-controlled reactions thus affords a new perspective in consideration of problems of gene action and development.

SUMMARY

The structural evidence for nucleocytoplasmic interrelationships observed in electron micrographs of salivary-gland cells of third instar larvae of *Drosophila melanogaster* has been reviewed. It has been found that the characteristic nuclear membrane outpocketings with their adjacent highly differentiated chromosomal materials occur at one stage of larval development at a time when a new cellular function is being initiated. Preliminary cytochemical studies to characterize the materials transferred from nucleus to cytoplasm indicate that deoxyribonucleic acid occurs within the blebs. Observations on chromosome and nuclear membrane structure are also presented.

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BIBLIOGRAPHY

1. Gay, H., *Dissertation Abstr.*, 1955, **15,** 899.
2. Gay, H., *Anat. Rec.*, 1954, **120,** 728.
3. Gay, H., *Proc. Nat. Acad. Sc.*, 1955, **41,** 370.
4. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 285.
5. Dalton, A. J., *Anat. Rec.*, 1955, **121,** 281.
6. Gay, H., *Carnegie Institution of Washington, Yearbook No. 54*, 1955, 255.
7. Swift, H., and Rasch, E. M., abstr. in *J. Histochem. and Cytochem.*, 1954, **2,** 456. More extensive data in Alfert, M., *Internat. Rev. Cytol.*, 1954, **3,** 131.
8. Fraenkel, G., and Brookes, V. J., *Biol. Bull.*, 1953, **105,** 442.
9. Kaufmann, B. P., McDonald, M. R., and Gay, H., *J. Cell. and Comp. Physiol.*, 1951, **38,** suppl. 1, 71.
10. Hotchkiss, R. D., *Arch. Biochem.*, 1948, **16,** 131.
11. Caspersson, T. O., Cell Growth and Cell Function. A Cytochemical Study, New York, W. W. Norton and Co., Inc., 1950.

12. Brachet, J., Chemical Embryology, New York, Interscience Publishers, Inc., 1950.
13. Gale, E. F., and Folkes, J. P., *Nature*, 1954, **173**, 1223.
14. Schultz, J., *Cold Spring Harbor Symp. Quant. Biol.*, 1941, **9**, 55.
15. Palade, G. E., and Siekevitz, P., *Anat. Rec.*, 1955, **121**, 347.
16. Novikoff, A., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 65.
17. Schultz, J., *Exp. Cell Research*, 1952, suppl. 2, 17.
18. Swift, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 415.
19. Rebhun, L. I., *Rec. Genetics Soc. Am.*, 1955, **24**, 591.

EXPLANATION OF PLATES

PLATE 135

All figures except Fig. 1 are electron micrographs of sections of salivary-gland cells of midthird instar larvae of *Drosophila melanogaster*. Fig. 1 is a section of an *early* third instar salivary-gland cell.

FIG. 1. Section of an early third instar cell fixed in 1 per cent buffered osmium tetroxide, pH 6.5. The nuclear membrane has no blebs and the cytoplasm contains endoplasmic reticulum (*E.R.*) and mitochondria (*M*), but no dense secretion granules. × 4,000.

FIG. 2.[1] Section of a salivary-gland cell fixed in 1 per cent buffered osmium tetroxide, pH 6.5. Arrows indicate nuclear membrane outpocketings and/or differentiated chromosomal material. Numerous secretion granules (*S.G.*) are in the cytosome. Chromosomes indicated by CHR. × 4,000.

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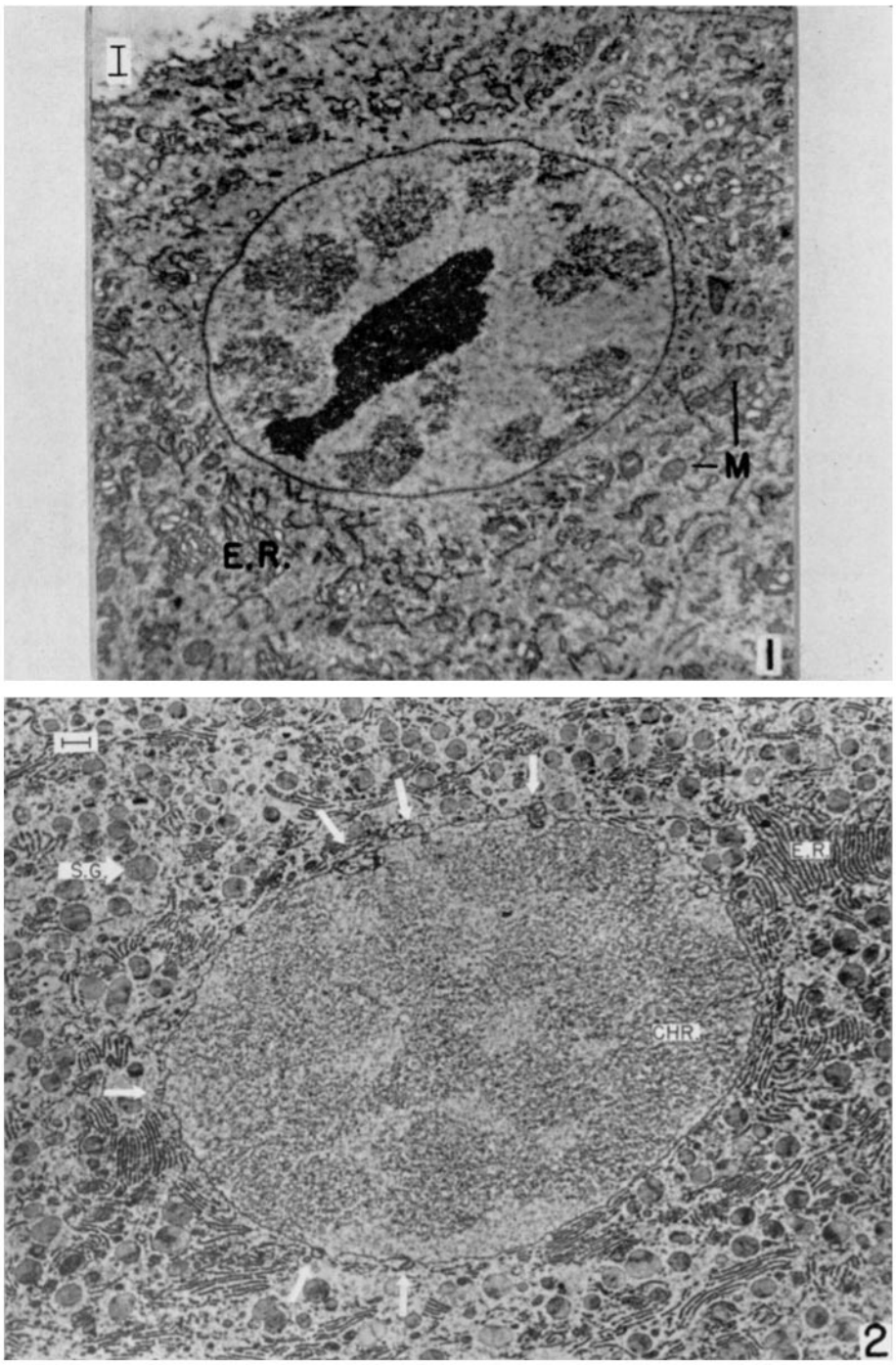
[1] Reprinted from the article by Gay (3), with the permission of The University of Chicago Press.



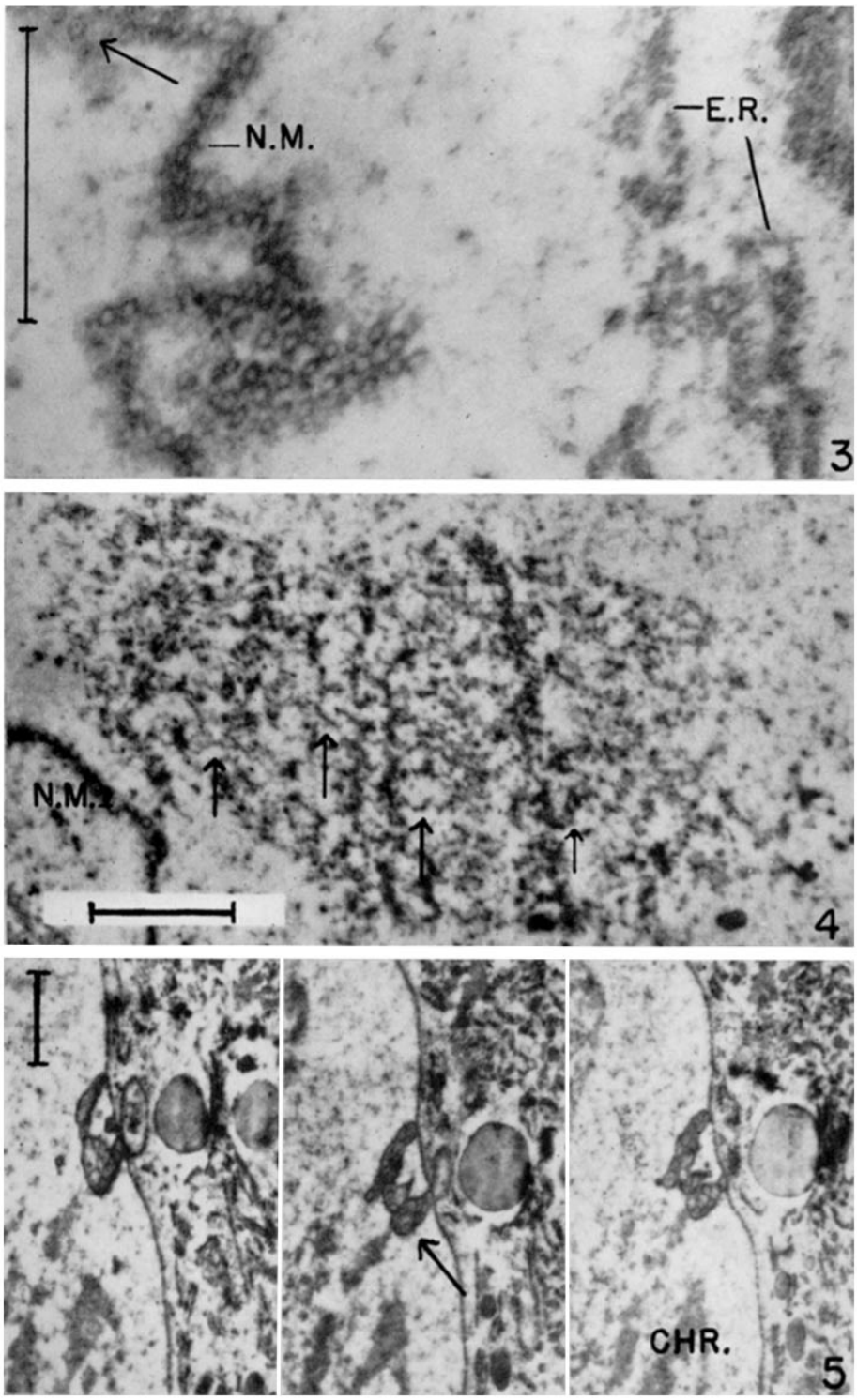
(Gay: Chromosome-nuclear membrane-cytoplasmic interrelations)

PLATE 136

FIG. 3.[1] Tangential section of nuclear membrane (*N.M.*) and adjacent endoplasmic reticulum of a cell fixed in 1 per cent buffered osmium tetroxide, pH 6.5. Note "pores" in nuclear membrane and "rosette" pattern in endoplasmic reticulum. Arrow indicates "pores" with dot-like center granules. × 40,500.

FIG. 4. Longitudinal section of a polytene chromosome of a cell fixed in 1 per cent buffered osmium tetroxide, pH 6.5. Arrows indicate short lengths of the continuous coiled longitudinal chromonemata. × 20,500.

FIG. 5. Three sections from a series of a cell from a gland preserved in Dalton's fixative, pH 7.5. These non-consecutive sections show a nuclear membrane bleb, highly differentiated chromosomal material (arrow), and the associated chromosome (*CHR.*). × 12,000.



(Gay: Chromosome–nuclear membrane–cytoplasmic interrelations)

PLATE 137

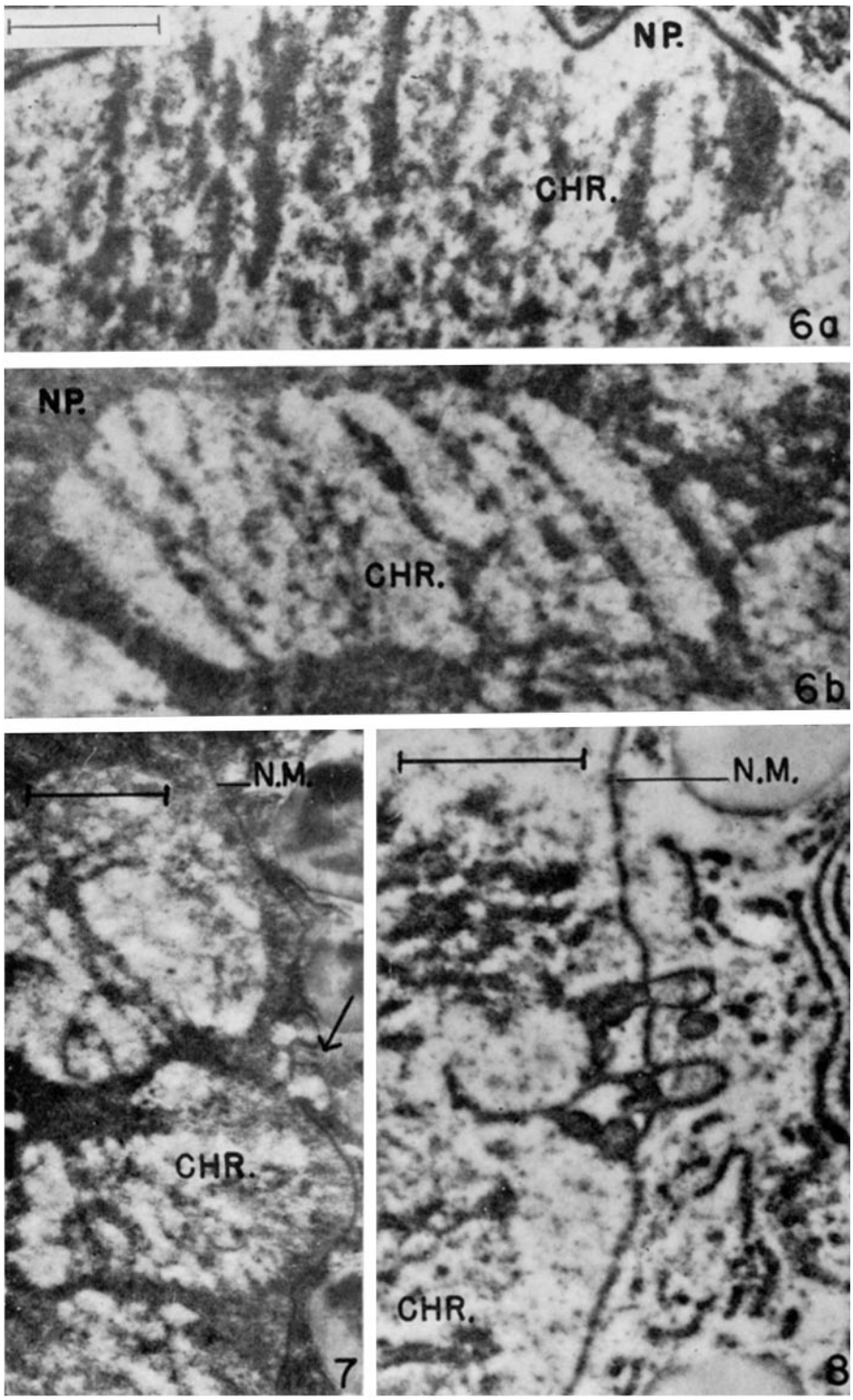
FIG. 6. Effect of deoxyribonuclease digestion on a cell fixed in Dalton's fluid, pH 6.5. × 21,000.

FIG. 6 *a*. Section of a chromosome of a control gland treated in 0.003 M $MgSO_4$ pH 6.0, for 4 hours at 37°C. Note dense bands of the chromosome (*CHR.*) and the similarity in density of material of chromosomal interband regions and nucleoplasm (*NP.*).

FIG. 6 *b*. Thicker section of a chromosome from a gland hydrolyzed in crystalline deoxyribonuclease 0.1 mg./ml. in 0.003 M $MgSO_4$, pH 6.0, for 4 hours at 37°C. Note reduction in density of chromosome in both band and interband regions, as compared with nucleoplasm. (The latter is much more dense in Fig. 6 *b* than in Fig. 6 *a* because of greater thickness of section.)

FIG. 7. Section of a Dalton-fixed salivary-gland cell treated with deoxyribonuclease showing a nuclear membrane outpocketing (see arrow). Note absence of electron-scattering material (DNA?). × 19,000.

FIG. 8. Section of a gland cell fixed in 1 per cent buffered osmium tetroxide, pH 7.5, showing an outpocketing of the nuclear membrane and the associated highly differentiated chromosomal material. × 26,000.



(Gay: Chromosome-nuclear membrane-cytoplasmic interrelations)