*ENDOMITOSIS AND POLYRIBOSOME FORMATION**

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It has recently been shown[1, 2] that prior to secretory activity the gland cells which secrete royal jelly in the young worker bee undergo a number of endomitotic division cycles and during each cycle both the chromosomes and nucleoli undergo morphological changes which closely parallel the behavior of these nuclear components in normal mitosis. In the following study, attention will be centered on changes in nucleoli, which, as is now well known, release ribosomes into the nuclear sap, and these emerge, on passage through the nuclear pores, as ribosome aggregates or polyribosomes.

In bee glands, at a stage which corresponds to the interphase of mitosis, nucleoli are very prominent and the chromosomes are so extended that they cannot be identified with either a light or an electron microscope. But as nuclei enter the prophase stage of endomitosis and the chromosomes contract so that they can be recognized, the nucleoli undergo a process of fragmentation and release into the nuclear sap hordes of ribosomes and other proteins, both of which ultimately pass into the cytoplasm. At the following interphase of endomitosis, nucleoli reappear and the cycle is repeated. In this way, there is made available a myriad of polyribosomes needed for the secretory activity of gland cells.

The behavior of nucleoli in bee glands raises the question of the behavior of nucleoli in other types of endopolyploid cells, especially in cells showing polytene chromosomes. Three types of endopolyploid cells have been examined with both light and electron microscopes, all taken from *Drosophila virilis*. Nurse cells, contained in the egg follicles of *Drosophila* ovaries, as one of us showed in 1939,[3] undergo some six or eight endomitotic division cycles. It is now recognized that a main function of nurse cells is to synthesize polyribosomes, which pass into the oöcyte cytoplasm via protoplasmic bridges and are used for protein synthesis during the initial cleavage stages of the egg. (See Telfer[4] for an excellent review of the evidence.) Salivary glands possess a highly developed endoplasmic reticulum and require polyribosomes for the synthesis of proteins. The same is true of fat cells to a lesser degree.

Nurse Cells.—Ovaries were removed from young virgin females and for electron study were initially preserved either in osmic acid buffered at pH 6.0, or in glutaraldehyde, and subsequently treated in various ways, as indicated in the figure legends. Figure 1 shows the nucleus of a large nurse cell which, we estimate, has undergone some six endomitotic division cycles, resulting in a large number of nucleolar organizers and large masses of nucleolar material. In this figure some of the nucleoli have begun to fragment, but chromosomes cannot yet be identified so it represents a late interphase stage. Each nucleolus shows along the edge the usual deeply staining nucleolar units or granules, many of which contain ribosomes. The nuclear envelope is crowded with pores and, on close inspection, shows ribosome aggregates, presumably polysomes, emerging from the pores. The cytoplasm contains very little endoplasmic reticulum and consists mostly of ribosome aggregates and mitochondria (Fig. 2). As the chromosomes become visible,

all the nucleoli undergo fragmentation, and by the time the chromosome condensation has reached its maximum, only a few nucleolar fragments can be seen. Often, at this stage, amorphous masses of faintly fibrous material may be seen in the cytoplasm; King[5] interprets these as due to the extrusion of nonribosomal protein.

It is clear that the nucleoli of nurse cells show the same sequence of changes found in the bee gland cells.

Salivary Gland and Fat Cells.—Both these types of cells show polytene chromosomes and since the morphology and behavior of the single nucleolus is essentially the same in both types of cells, the figures presented are of salivary cells only.

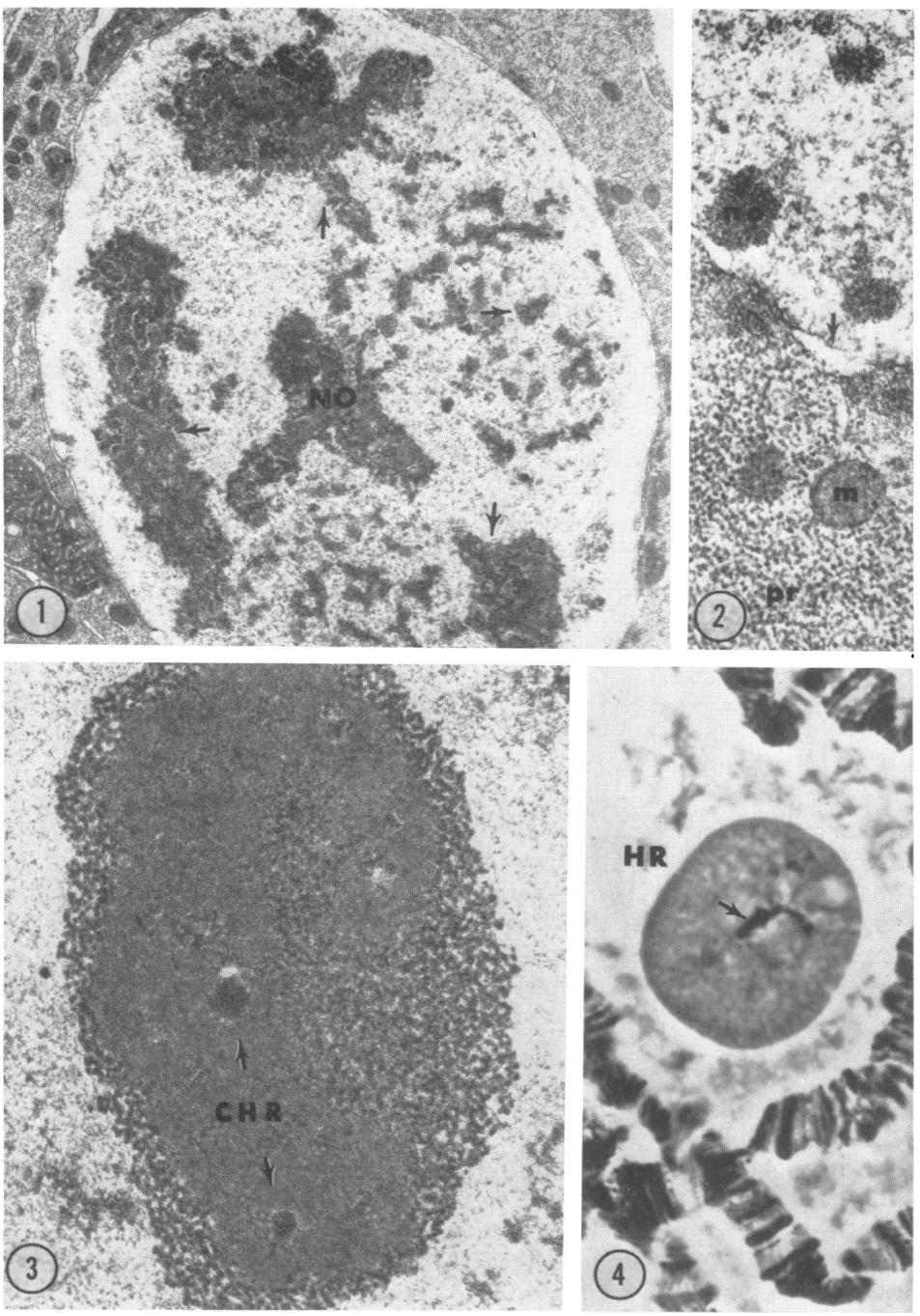
A number of investigators have noted a great variation in the morphology of nucleoli found in salivary gland cells. Two recent papers deal, respectively, with salivary gland cells of *Chironomus tentans*[6] and *Sciara coprophila*.[7] In the latter species some of the cells are reported as being entirely devoid of nucleoli. In order to verify this report, whole mounts were made of salivary glands of *D. virilis* taken from third-instar larvae, stained with lactic-acetic orcein and fast green, and slightly crushed so as not to scatter the nuclear contents. In a total of 72 nuclei, four failed to show any recognizable nucleoli, when viewed with a light microscope, and 68 nuclei showed nucleoli which differed widely in size and in the degree of vacuolization. Figure 4 is a photomicrograph of a nucleolus of maximum size. It appears to be vacuolated but, as will be shown below, this appearance is due to a fringe of ribosome-bearing processes (see Fig. 6). Of especial interest is the presence of a considerable amount of chromatin within the nucleolus which represents the nucleolar organizer region which has undergone a number of endomitotic replications. The nucleoli of other cells show a gradation in size down to tiny masses of material staining deeply with fast green.

For a study of the fine structure of nucleoli, salivary glands were taken from second-instar larvae. Figure 3 shows a nucleolus of near maximum size and is interpreted as being in the equivalent of a mitotic interphase. Around the outside there are tassel-like processes carrying numerous ribosomes (see Fig. 6 for details). The interior of the nucleolus consists of vaguely fibrous material which is irregularly honeycombed, with ribosome-bearing tassels filling the spaces.

In Figure 6 it will be noted that the bases of the tassels are more or less continuous with the fibrous material, indicating that the latter is the site of ribosome formation.

Figures 7 and 8 show a very large nucleolus,[8] and at the same magnification the smallest found,[7] respectively. It is at once apparent that in Figure 7 the ribosome-bearing processes have all been shed, leaving a residue of vacuolated fibrous material. Our electron micrographs show many cells in which, through fragmentation, the ribosome-bearing processes are breaking away from the main mass of the single nucleolus (Fig. 5), thus setting free the ribosomes in the nuclear sap. The nuclear membrane, as in other endomitotic cells, is crowded with pores, and polysomes can be observed emerging from these pores.

In none of the electron micrographs have we found the complete absence of nucleolar material, so we do not know whether fragmentation is complete or leaves a small residue of fibrous material. The relatively small size of the nucleolar residue in Figure 7—it has a diameter much less than the polytene chromosomes—probably might not be detected in light-microscope preparations.



All of the figures were made from material initially fixed in glutaraldehyde, except Figs. 4 and 5.

FIG. 1.—Nucleus of a nurse cell in the interphase of an endomitotic cycle. Many nucleoli are present (*NO*) and some have begun to fragment. 10,500×.

FIG. 2.—A portion of the nucleus with the fibrous remains of a fragmented nucleolus (*top*). At the arrow, the outer membrane of the nuclear envelope is forming an endoplasmic tubule. The cytoplasm is filled with polyribosome aggregates (*pr*), mitochondria (*m*), and some fibrous nucleolar material. 39,000×.

FIG. 3.—Nucleolus of a salivary gland cell at interphase. The surface is covered with ribosome-bearing processes (see Fig. 6) and a honeycombed fibrous interior with tassel-like processes occupying the cavities, and portions of the nucleolar organizer region of the X chromosome (*CHR*). 13,200×.

FIG. 4.—Photomicrograph of a nucleolus which appears vacuolated. The nucleolar portion of the X chromosome is visible. 1,560×.

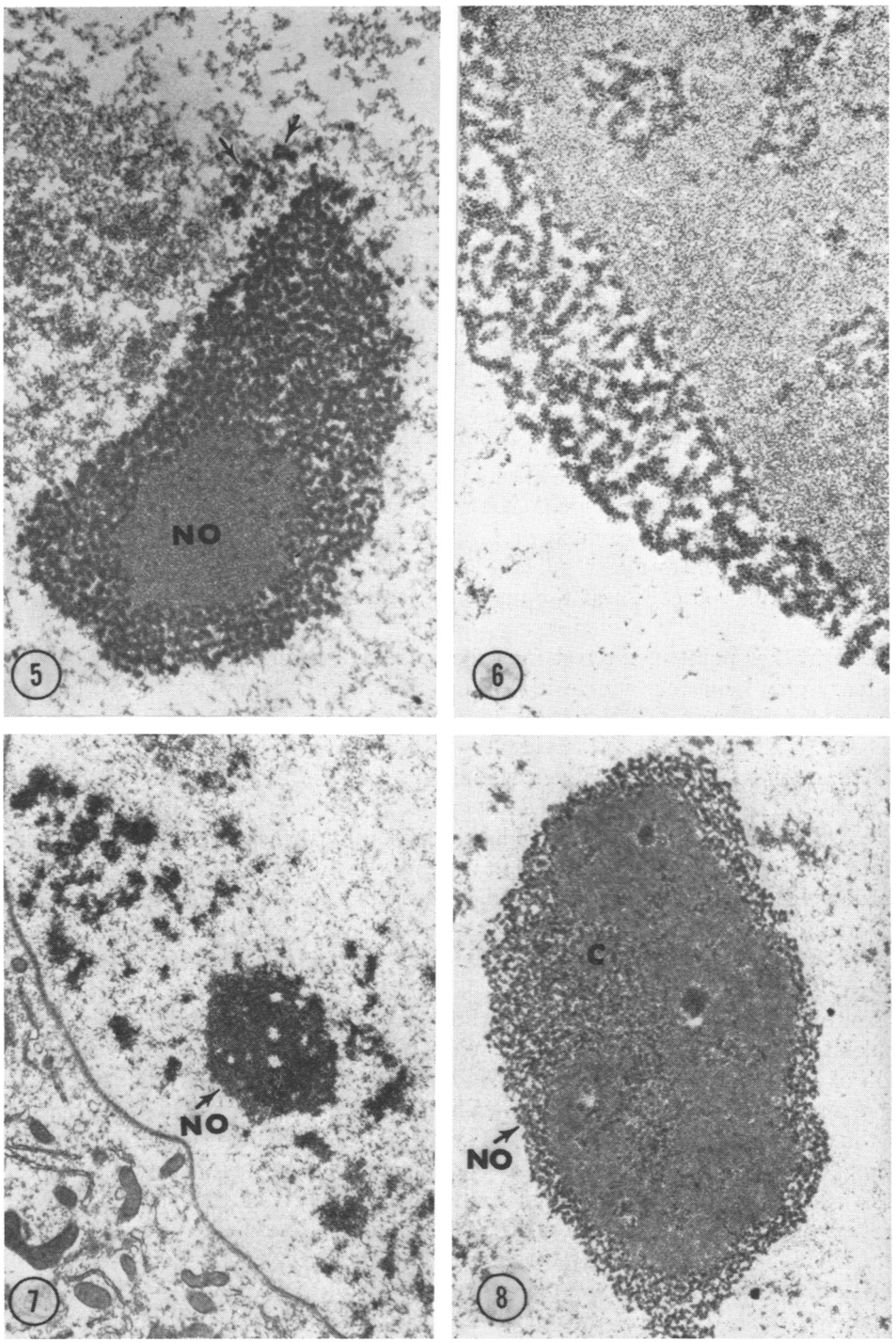


FIG. 5.—Taken from a cell initially preserved in osmic acid at pH 6.0. Ribosome-bearing processes are breaking away from the main mass of the nucleolus and are scattered about in the nuclear sap. This represents a prophase. 13,800×.

FIG. 6.—Details of the ribosome-bearing tassels along the edge of the nucleolus as well as in cavities of the fibrous interior. 26,600×.

FIG. 7.—Smallest nucleolus found in salivary gland nuclei. Note that only fibrous material is seen along with vacuoles formerly occupied by ribosome-bearing processes. 9,500×.

FIG. 8.—Fully developed nucleolus magnified 10,200×. Note the beginning of fragmentation along the lower edge.

In summary it may be said that in all of the endomitotic cells examined, there is a fragmentation of the ribosome-bearing regions of nucleoli in early prophase stages.

It is interesting to note that in the royal-jelly glands of the bee and in nurse cells, as polyploidy increases by endomitosis, the nucleolar organizer regions are scattered about the nucleus. In contrast, in cells with polytene chromosomes the organizers on the X and Y chromosomes are held together and there results one enormous nucleolus. There arises now the question: Where does the material needed for the formation of new nucleoli come? The answer is that it comes from the same source as in mitotic cells. Long ago, McClintock, working with *Zea mays*,[8] reported that as the telophase chromosomes uncoiled there are exuded from their surfaces droplets of material which collect at the site of the nucleolar organizer and form the nucleolus of the next interphase period. From time to time since the introduction of electron microscopy, there have been reports of presumed nucleolar material associated with all the chromosomes. Most recently, Garcia and Kleinfeld[7] report that in *Sciara coprophila*, there are a great number of salivary chromosome bands which are capable of forming micronucleoli. These micronucleoli, seen by many others along salivary chromosomes, contain RNA. Presumably these micronucleoli collect at the nucleolar organizer to form, in the case of polytene chromosome cells, a single nucleolus.

One further point of interest must be made. Workers using pulse labeling of salivary gland chromosomes, with either tritiated thymidine or tritiated uridine, have noted the great variation in the uptake of these isotopes by a given band in the cells of an individual, or in individuals of the same age. The explanation for this phenomenon is simply that salivary gland chromosomes are undergoing endomitotic changes. The DNA is synthesized at one period in the growth cycle, and the RNA at other times so that the uptake of labeled RNA precursors, or a failure of uptake, in a given cell depends on the stage in the endomitotic cycle represented.

It has long been assumed that the nucleolus is the site of ribosome formation, and Figure 5 confirms this fact morphologically. Recent work using hybrid molecules of DNA complementary to ribosomal RNA[9–11] now affords definite proof that ribosomes are formed by nucleoli.

For biologists in general the greatest interest of the work reported here is a better understanding of endomitosis and the role it plays in cells specialized for the secretion of proteins. In normal mitosis following cytokinesis the two daughter cells have a minimum volume. In the so-called G_1 period of growth the normal volume of the daughter cells is restored by the synthesis of all the substances or organelles normally present in the cell under consideration. Such syntheses require the presence of polyribosomes derived from the breakdown of the nucleolus in the previous prophase of mitosis. This is the basic mechanism involved in rapidly growing and dividing cells. But when cells become specialized, for the synthesis of digestive enzymes for example, they cease to divide and, under genetic control, only a few kinds of protein are made. If there is need for more secretion than can be produced by a diploid cell, two solutions are possible. One solution is to increase the number of diploid cells; this seems to be the rule in vertebrates, as in the case of the pancreas of man. In larval organs of the invertebrates, a second solution has developed, and that is increasing the synthesizing power of a gland cell by endomitosis. In this connection, it should be remembered that gland cells

must have ready access to all the precursors needed for protein synthesis, either by bathing the cells directly in hemolymph, as in invertebrates, or by having such cells in direct contact with the basement membrane with its adjacent capillary network. The latter is usual in vertebrates. In addition to having a rich food supply, a gland cell must have an outlet for secretion provided by the gland duct. This topographical relationship applies to glands of all types. In the case of larval organs of insects, the number of gland cells does not change once the gland is formed. To do so would require doubling the length of the duct and soon back pressure would impede secretion flow. On the other hand, doubling the volume of a cell by endomitosis would involve little lengthening of the duct, since three dimensions are involved. Endomitosis provides an increase in synthesizing power and at the same time eliminates the delay caused by cell division.

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