A CORRELATED LIGHT AND ELECTRON MICROSCOPE STUDY OF THE NUCLEOLAR MATERIAL DURING MITOSIS IN *VICIA FABA*

J. G. LAFONTAINE, Ph.D., and L. A. CHOUINARD, Ph.D.

From the Departments of Pathology and Anatomy of Laval University Medical School, Quebec, Canada

ABSTRACT

Root meristematic cells of *Vicia faba* were examined, with both light and electron microscopes, in order to study the behaviour of the nucleolar material during the mitotic process. Under light microscopy, the preprophase nucleolus is seen to consist of a densely stained material in which are embedded several unstained vacuole-like structures of varying size. The electron microscope reveals that the dense nucleolar material is formed of two structurally distinct components, each segregated into irregularly shaped zones blending with one another. One of these components is represented by 150 A granules which, in places, are arranged into thread-like structures approximately 0.1 μ in diameter; the other component apparently consists of fibrils 60 to 100 A in diameter. The large and medium sized intranucleolar vacuoles contain loosely scattered granules and fibrils similar to those just described. The granular and fibrillar components of the denser portion of the nucleolus persist as such during prophase and disperse throughout the nuclear cavity at the time of nucleolar disintegration. After nuclear membrane breakdown, these granules and fibrils, as well as those of the nucleoplasm, mix freely with similar elements already present within the forming spindle. No evidence has been obtained that, during or after nucleolar disintegration, the structural components of the nucleolus become associated as such with the chromosomes to form an external or internal matrix. Our observations suggest the existence, of a matrix substance within late prophase, metaphase, and anaphase chromosomes, the fine structure of which bears strong resemblance to that of their constituent coiled chromonemata. Data are presented, moreover, that indicate that part of this matrix substance, presumably formed at some time during prophase, is released from the chromosomes during their anaphasic movement. A number of observations indicate that the main bulk of the next nucleolus is derived from a prenucleolar fibrillogranular material, arranged into thread-like structures some 0.1 μ in diameter, which collect in the interchromosomal spaces during early and midtelophase. Finally, our data would seem to favour the view that most of this prenucleolar material results from a resumption of the synthetic activity of the early and midtelophase chromosomes rather than from a mere shedding of a preexisting matrix substance.

INTRODUCTION

As is well known, the nucleolus, as a formed body, lacks continuity during mitosis; it usually disappears from view at late prophase and is formed anew at telophase. The missing link in the nucleolar cycle is, of course, the source of the material from which the new nucleolus arises at telophase.

Many earlier cytologists (survey of literature in Gates, 1), correlating the cyclical changes in the staining characteristics of the chromosomes during mitosis with the appearance and disappearance of the nucleolus, claimed that the nucleolar material at telophase originates from a chromosomal matrix, itself derived from the disintegrated prophase nucleolus.

In more recent years the nucleolus has also been thought by Estable and Sotelo (2, 3) to persist, at least in part, in the form of filamentous structures, the nucleolonemata. Rattenbury and Serra (4), on the other hand, have suggested that the nucleolus in *Vicia faba* is formed from a material first appearing as a superficial coating on the early telophase chromosomes. Such a prenucleolar material, they believe, is derived from the perichromosomal plasm rather than from a chromosomal matrix. Earlier electron microscope observations of Lafontaine (5) have confirmed the existence in *Vicia faba* of such a coating of prenucleolar material over the surface of early telophase chromosomes.

Quite a different viewpoint, however, has recently been held by Swift (6) who maintains that this so called prenucleolar material has no direct connection with the formation of the nucleolus. The telophase nucleolus, he suggests, is best considered as the result of the synthetic activity of the classical chromosomal nucleolar organizing sites.

The present investigation, using correlated light and electron microscope techniques, was undertaken in an attempt to throw additional light on the problem of the behaviour of the nucleolar material during mitosis, particular attention being paid to its fate during nucleolar dissolution at late prophase and its source during nucleolar reconstitution at telophase.

MATERIAL AND METHODS

For the present study, roots were obtained by germinating seeds of *Vicia faba* in damp vermiculite maintained at room temperature. After a week or so, root tips 0.5 to 1 mm long were excised from secondary roots and fixed for 1 hour in ice cold 1 per cent osmium tetroxide, buffered with Veronal acetate to pH 7.5, containing both sucrose (7) and $CaCl_2$ (8). The root tips were then rapidly dehydrated in an ascending series of ethanol concentrations and finally embedded in Epon 812 according to Luft (9). Transverse and longitudinal sections of the root tips were cut with a Cambridge-Huxley ultramicrotome.

For light microscopy, sections ranging from 0.25 to 1 μ in thickness were mounted on glass slides, stained according to the Feulgen procedure (20 minutes hydrolysis in N HCl at 60°C) and counterstained with methylene blue (1 per cent methylene blue in 1 per cent aqueous sodium borate) for 5 minutes at room temperature. After such a counterstaining the Feulgen-positive chromosomes take on an intense purplish-blue color which greatly facilitates their observation, especially in relatively thin sections. The nucleolar material, at prophase and telophase, exhibits under the same conditions a distinctive metachromatic cabbage-green color; the vacuolar component of the formed nucleoli, however, remains unstained. In order to increase their contrast, the stained 0.25 μ thick sections were examined under phase contrast microscopy.

For electron microscopy, ultrathin sections were mounted on copper grids and stained successively with solutions of uranyl acetate and lead hydroxide for varying periods of time (10). These sections were examined in a Siemens Elmiskop I electron microscope, using the double condenser, 80 kv and 50 μ molybdenum objective apertures.

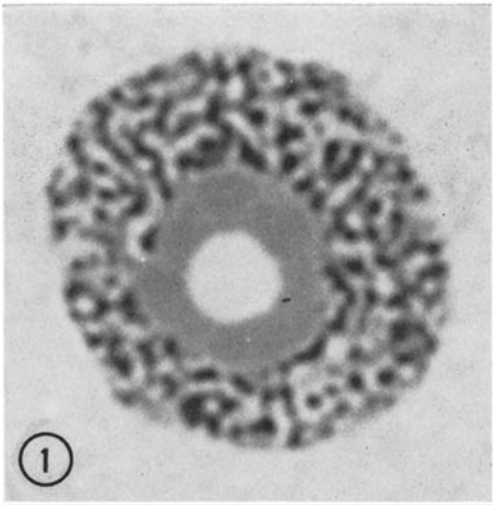
OBSERVATIONS

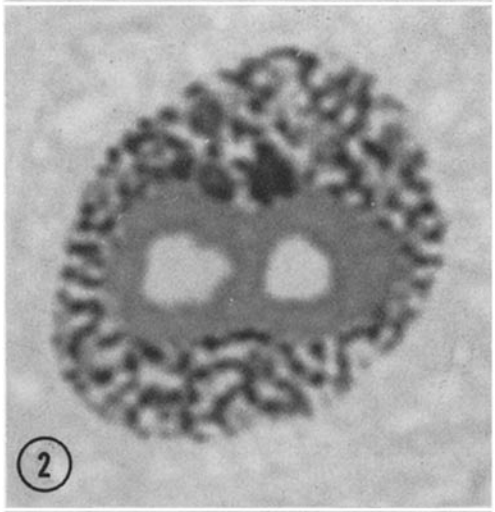
Preprophase

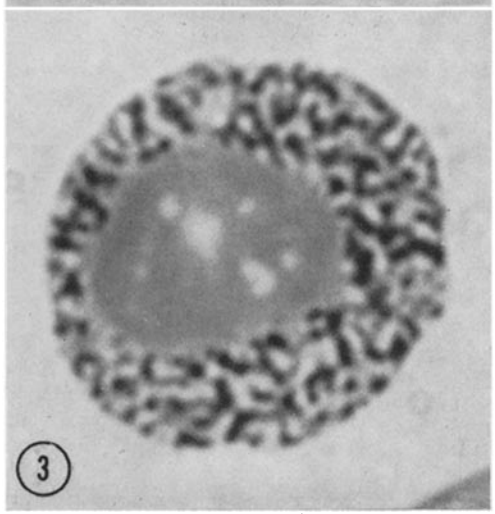
1. Light Microscopy

In *Vicia faba* the preprophase nucleus can be readily distinguished from the typical interphase nucleus by the fact that its chromosomes show an increased and a much more constant diameter ($\sim$0.4 μ) (11). At preprophase, moreover, very few heterochromatic masses are observed within the nucleus.

The preprophase nucleus usually contains a single, more or less centrally located, roundish nucleolus about 6 μ in diameter (Fig. 1). Occasionally, two separate but smaller nucleoli are seen within the nucleus; these two nucleoli may adhere to one another giving rise to a dumbbell type of structure (Fig. 2). The nucleolus, whether







FIGURES 1–3

single or double, contains a number of unstained vacuole-like structures of varying size and shape. Quite often one of these vacuoles is located more or less centrally and occupies a large portion of the nucleolar mass; most of the other vacuoles in such nucleoli are then small and barely recognizable even in relatively thin sections (Figs. 1 and 2). A smaller but sizable number of preprophase nucleoli contain no such large centrally located vacuole but instead several medium sized and small ones distributed at random within their mass (Fig. 3).

The perinucleolar halo, free of chromosomal material, described by Chayen *et al.* (12), has never been observed in the course of the present study. In favourable sections, a heterochromatin mass is seen adjacent to the surface of the nucleolus (Fig. 2).

2. Electron Microscopy

Under electron microscopy it is observed that the denser portion of the nucleolus is made up of two distinct components (Figs. 4 and 5). The peripheral portion of the nucleolus proper, the surface of the centrally located and larger vacuoles, as well as a number of zones of varying width extending more or less radially in between, consist predominantly of densely packed granules some 150 A in diameter. On closer examination a number of tiny, light, narrow spaces may be detected here and there within these granular zones, suggesting a thread-like arrangement of their constituent granules. The latter, however,

FIGURE 1

Light micrograph of a preprophase nucleus. The almost centrally located roundish nucleolus contains a large, central, unstained vacuole. A number of barely visible vacuole-like structures are also present within the denser portion of this nucleolus. × 4,000.

FIGURE 2

Light micrograph of a prophase nucleus with two nucleoli adhering to one another. Each nucleolus contains a large, unstained vacuole as well as a number of barely distinguishable vacuole-like structures located within its denser portion. A heterochromatic body is found on the surface of each nucleolus. × 4,000.

FIGURE 3

This preprophase nucleolus contains a number of medium sized and small unstained vacuoles distributed at random within its mass. × 4,000.

do not appear to be assembled more regularly within such coarse (0.1 μ) thread-like structures than elsewhere within the granular zones.

In addition to the granular zones just described, irregularly shaped patches of material of a different texture are consistently observed within the dense portion of the nucleolus. The material in question, because of its compactness, appears rather homogeneous and its fine structure is not readily analyzed (Fig. 4). However, since these patches normally contain most of the small, peripherally located vacuoles, grazing sections through the latter provide enough transparency to resolve the texture of this apparently homogeneous material into tightly packed convoluted fibrils, 60 to 100 A in diameter (Fig. 5). At the boundary of the patches just described, the densely packed fibrillar material blends with the surrounding granular component of the nucleolar mass.

The material within the large and medium sized intranucleolar vacuoles consists of loosely and uniformly distributed granules and fibrils, the size and density of which bear a strong resemblance to those described in the dense portion of the nucleolus (Figs. 4 and 5). However, the texture of the smaller, peripherally located vacuoles appears predominantly fibrillar, presumably, as assumed above, on account of grazing sectioning of the fibrillar zones within which these vacuoles are embedded.

As a result of thin sectioning, the preprophase chromosomes appear much less constant in diameter than in corresponding light micrographs (Fig. 6). Under electron microscopy these chromosomes are also much denser than the surrounding nucleoplasm and consist predominantly of rather tightly packed convoluted fibrils about 100 A in diameter as well as of a number of dense granules some of which reach a diameter of approximately 150 A. Whether or not the smaller granules do in fact represent kinks of the constituent fibrils cannot be decided from our micrographs.

As for the nucleoplasm, it is made up of loosely and uniformly distributed convoluted fibrils which can hardly be distinguished from those observed within the chromosomes. This resemblance is specially obvious in places where grazing sections of the chromosomes are observed (Fig. 6). Here and there, the nucleoplasm also contains dense granules, the diameter of which varies from approximately 150 to 300 A; the larger granules are often seen to be grouped into small clusters.

Prophase

1. Light Microscopy

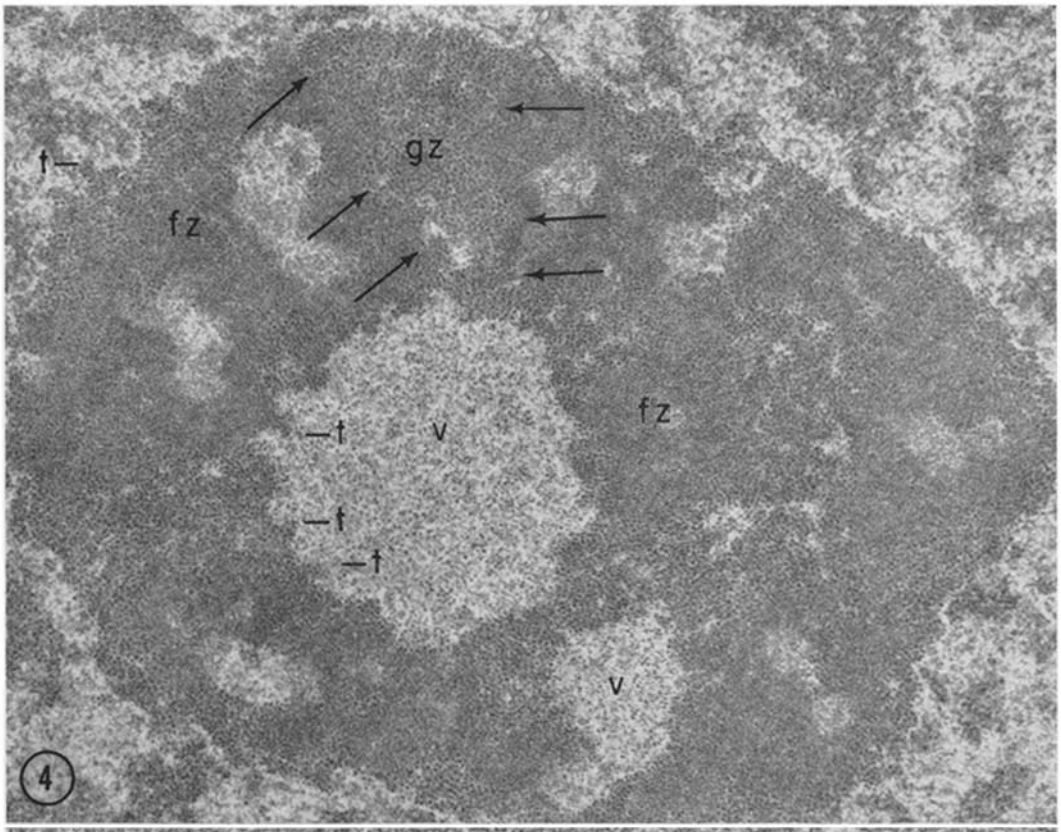
At early prophase (Fig. 7), the nucleolus has become slightly more irregular in outline and usually still shows a large, more or less centrally located vacuole as well as a number of much smaller ones

FIGURE 4

Electron micrograph of a preprophase nucleolus. Two types of structural components, one granular, the other fibrillar, segregated into distinct zones are found within the denser portion of the nucleolus. Some of the granular zones (*gz*) extend radially (arrows) from the surface of the nucleolus to that of the centrally located vacuole (*v*). Tiny narrow light spaces within certain of these zones suggest a thread-like arrangement of the granules. Such threads (*t*) are more easily recognizable on the surface of the nucleolus as well as on that of the large, centrally located vacuole. The remainder of the denser portion of the nucleolus consists of irregularly shaped fibrillar zones (*fz*) within which are located most of the smaller vacuole-like structures. The large central vacuole as well as the medium sized ones contain loosely scattered granules and fibrils similar to those found within the denser portion of the nucleolus; the contents of the smallest vacuoles appear to be predominantly fibrillar in texture. × 25,000.

FIGURE 5

Higher magnification of portion of the nucleolus shown in Fig. 4. The granular zones (*gz*) consist of 150 A particles apparently embedded in a ground substance which is not easily analyzed. The fibrillar zones (*fz*) on the other hand are made up of quite densely packed fibrils, 60 to 100 A in diameter. These fibrils are more easily resolved in grazing sections (arrows) of the peripherally located vacuoles present within such fibrillar zones. × 70,000.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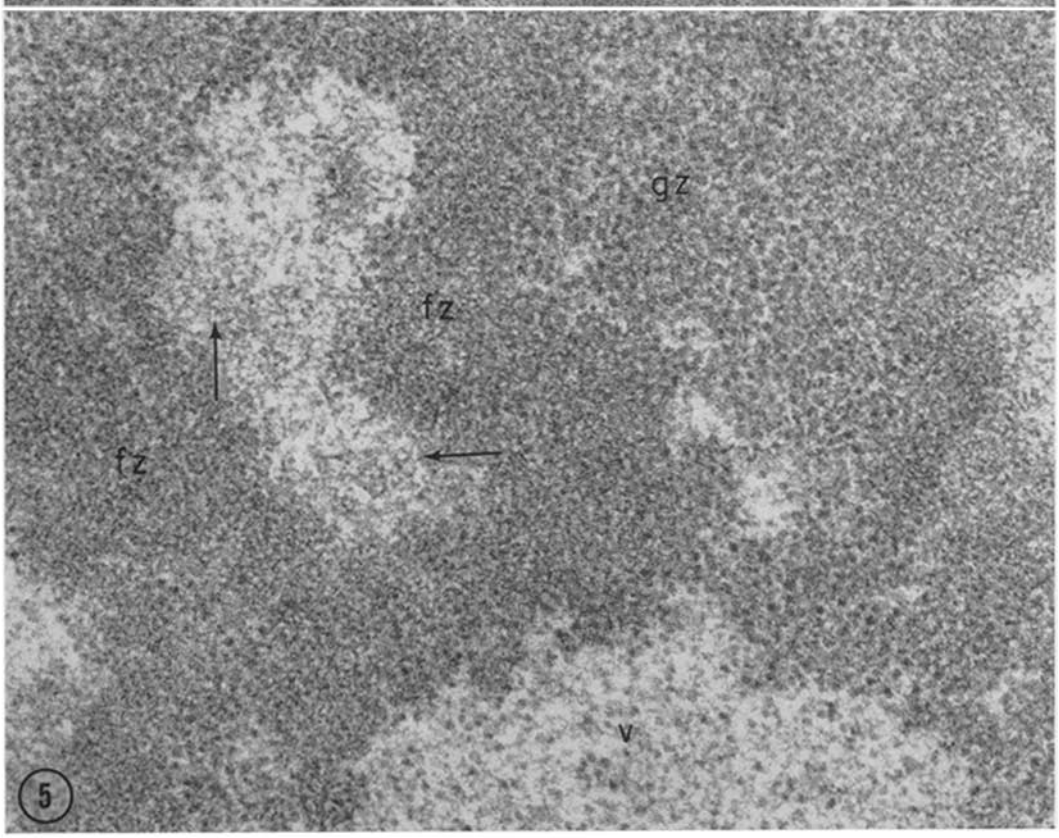
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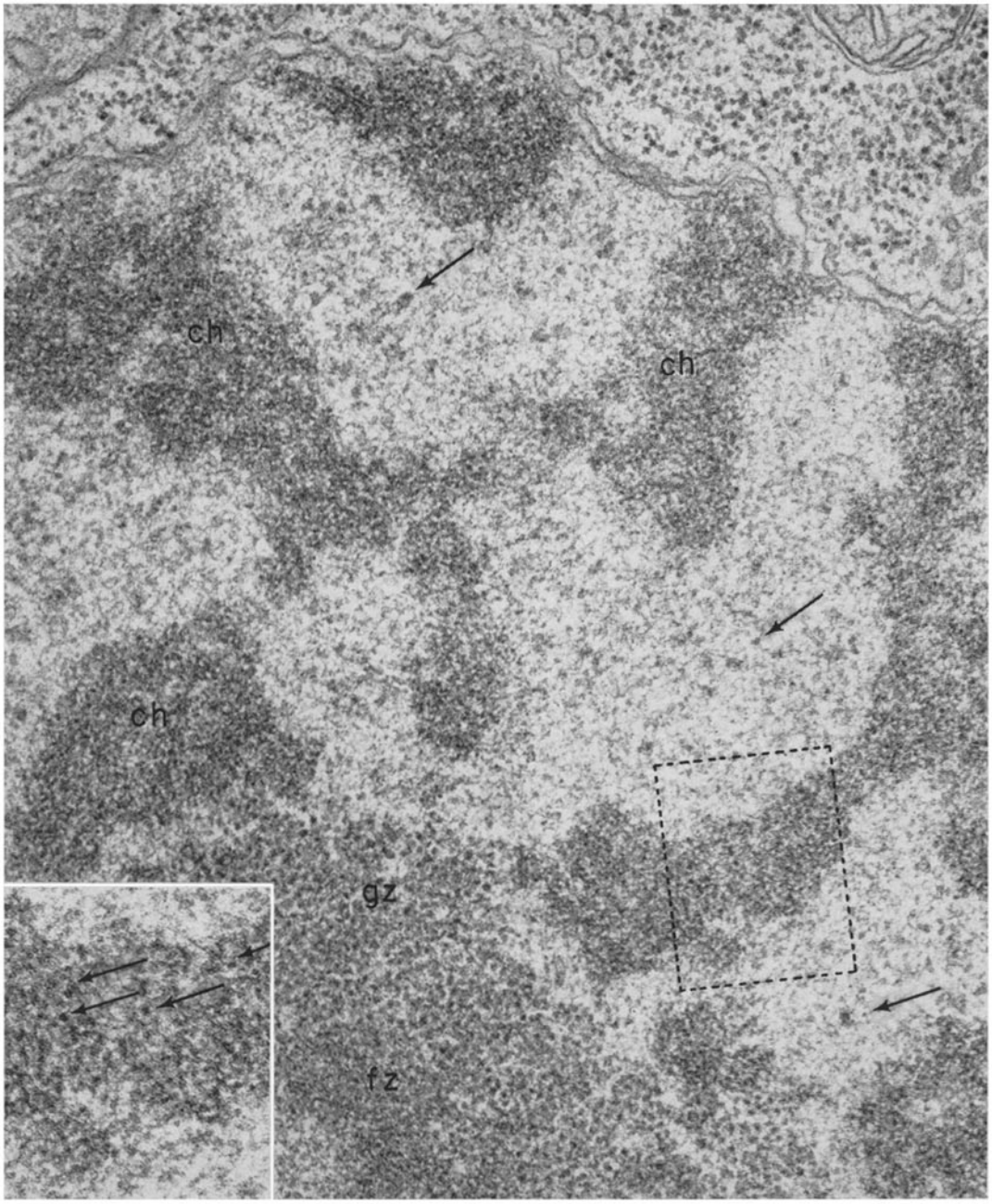


FIGURE 6

Electron micrograph of portion of a preprophase nucleus. Several segments of chromosomes (*ch*) of varying width, due to thin sectioning, are seen within the nucleoplasm. These segments consist of closely packed convoluted fibrils approximately 100 A in diameter as well as a number of dense granules, some 150 A in diameter. The nucleoplasm is made up predominantly of loosely distributed convoluted fibrils, and of granules (arrows) the diameter of which varies from 150 to 300 A. The portion of the nucleolus depicted in this micrograph shows an outer granular zone (*gz*) and an inner fibrillar zone (*fz*). × 47,000.

The insert illustrates more clearly the granules (arrows) present within a chromosomal segment. × 80,000.

in its peripheral portion. As prophase progresses, the nucleolus becomes more irregular in contours (Fig. 8) and, by midprophase (Fig. 9), large pointed nucleolar projections are seen extending between the neighbouring chromosomes. At that time the larger vacuoles seen in earlier stages usually have completely disappeared from view and only medium sized and smaller vacuoles are found scattered within the nucleolar mass. In the slightly older midprophase nucleoli (Fig. 10), only small, barely recognizable vacuoles remain. During late prophase the contours of the nucleolar mass become increasingly difficult to delineate and, eventually, only diffused areas of variable staining intensity, merging imperceptibly with the surrounding nucleoplasm, can still be observed (Fig. 11). The distribution of the nucleolar material at that time is best studied in thin sections (0.25 μ) examined under phase contrast microscopy. Such preparations then reveal the presence of nucleolar material, but in lesser concentration, amongst the chromosomes in the peripheral portion of the nucleus (Fig. 12). Just before nuclear envelope breakdown, all remnants of the nucleolus as a formed body have disappeared; the nucleoplasm exhibits a weak, homogeneous, bluish-green staining reaction matching that of the spindle forming outside the nucleus.

During prophase, as is well known, the chromonemata undergo part of their characteristic coiling cycle. Under light microscopy and in relatively thin sections (0.25 to 1 μ), these chromonemata remain distinctly visible until about the onset of nucleolar disintegration. From then on, the chromatids appear homogeneously dense and their constituent chromonemata become rapidly lost from view. Sections, 1 μ in thickness, were also stained according to the Feulgen procedure alone in order to determine to what extent the compactness of the chromatids, from midprophase on, might be due to staining with methylene blue of a Feulgen-negative interchromonemal matrix material. Unfortunately, such preparations did not provide the expected information due to lack of contrast even under phase microscopy.

2. Electron Microscopy

At early prophase the fine structure of the nucleolus appears similar to that described at preprophase; the somewhat more conspicuous indentations which now characterize its surface follow quite closely the bends and twists of the neighbouring segments of chromosomes. By midprophase, these angular nucleolar projections have greatly increased in size; they are as compact in texture as the more centrally located nucleolar zones and likewise consist of both granular and fibrillar components (Fig. 13). As in earlier stages, the peripheral granular portion of the nucleolus extends in places more or less deeply within the nucleolar mass and blends with more centrally located patches of fibrillar material. Most of the small vacuole-like structures, still present within midprophase nucleoli, are located within such fibrillar zones.

Slightly before the nucleolus begins to disintegrate, a general loosening up of its mass takes place (Fig. 14). As a result, the fibrillar and granular zones of the nucleolus appear much lighter and the previously described thread-like arrangement of the granules is nowhere recognizable. In the course of the disintegration of the nucleolus at late prophase, its remnants are represented by heterogeneous masses consisting of intermingled and ill-defined granular and fibrillar zones (Fig. 15). These masses blend imperceptibly with the surrounding nucleoplasm already containing a large number of dispersed granules and fibrils of nucleolar origin. Since the nucleoplasm is already richly provided with fibrillar elements before (Figs. 6, 13, and 14) nucleolar disintegration, the eventual fate of the fibrillar component of nucleolar origin could not be followed further.

Slightly before the nuclear envelope begins to break down, all remnants of the nucleolus as a formed body have disappeared from view; its constituent granules and fibrils now are loosely and uniformly distributed within the entire nuclear cavity (Fig. 16). At that time, the granules present throughout the nucleoplasm show an increased density but they are still somewhat lighter than the free ribosomes in the cytoplasm.

During prophase, because of thin sectioning, only short segments of chromosomes, cut at various angles, are observed under electron microscopy. From early to midprophase each such chromosome segment shows portions of convoluted chromonemata of varying width between which are observed irregular light spaces; the density and fine structure of these spaces match those of the surrounding nucleoplasm (Figs. 13 and 14). As prophase progresses, the light spaces in ques-

tion decrease both in size and number, presumably in part at least, as a result of the closer and closer approximation of the successive chromonemal coils in each chromatid. By late prophase the chromatids have become quite compact and only a very few light zones are still observed within their mass (Fig. 15). At high enough magnification such zones still show a material similar to that observed within them at earlier stages. In favourable sections (Fig. 15), other light zones, which clearly correspond to spaces between the chromatids, contain loosely scattered granular and fibrillar elements of the type found elsewhere within the nucleoplasm.

As suggested by our light microscope observations, therefore, the electron microscope also reveals that the mid- and late prophase chromatids become progressively more compact in texture and that eventually their constituent chromonemata become hardly recognizable. The only structural elements that can be resolved within the condensed late prophase chromatids (Fig. 16) are similar to those observed within the preprophase chromonemata, namely, densely packed convoluted fibrils and a number of granules. If, as would appear, an interchromonemal matrix material is responsible for the increased compactness of the late prophase chromatids, its fine structure must then be very similar to that of the chromonemata themselves. The electron microscope, moreover, has failed to furnish evidence for the existence of a coating of matrix material of distinctive fine structure over the surface of the late prophase chromosomes.

Prometaphase, Metaphase and Anaphase

1. Light Microscopy

From prometaphase on, the spindle is homogeneously light blue and exhibits only slight indication of the presence of oriented fiber-like structures along its main axis.

In longitudinal sections, the condensed prometaphase and metaphase chromosomes as well as the anaphase daughter chromosomes show wavy contours; such an appearance is consistent with the generally held view that they are formed of helically disposed chromonemata (13). Grazing sections of anaphase chromosomes exhibit this feature quite distinctly (Fig. 19) and, moreover,

Figure 7

In this early prophase nucleus the nucleolus is roundish in contours and contains a large central unstained vacuole. × 4,000.

Figure 8

Slightly older prophase nucleus. The nucleolus shows irregular contours and a heterochromatic body on its surface. × 4,000.

Figure 9

Midprophase nucleus. The nucleolus is very irregularly shaped with large pointed projections extending between the neighbouring chromosomes. A number of medium sized and smaller vacuoles are present within the nucleolar mass. × 4,000.

Figure 10

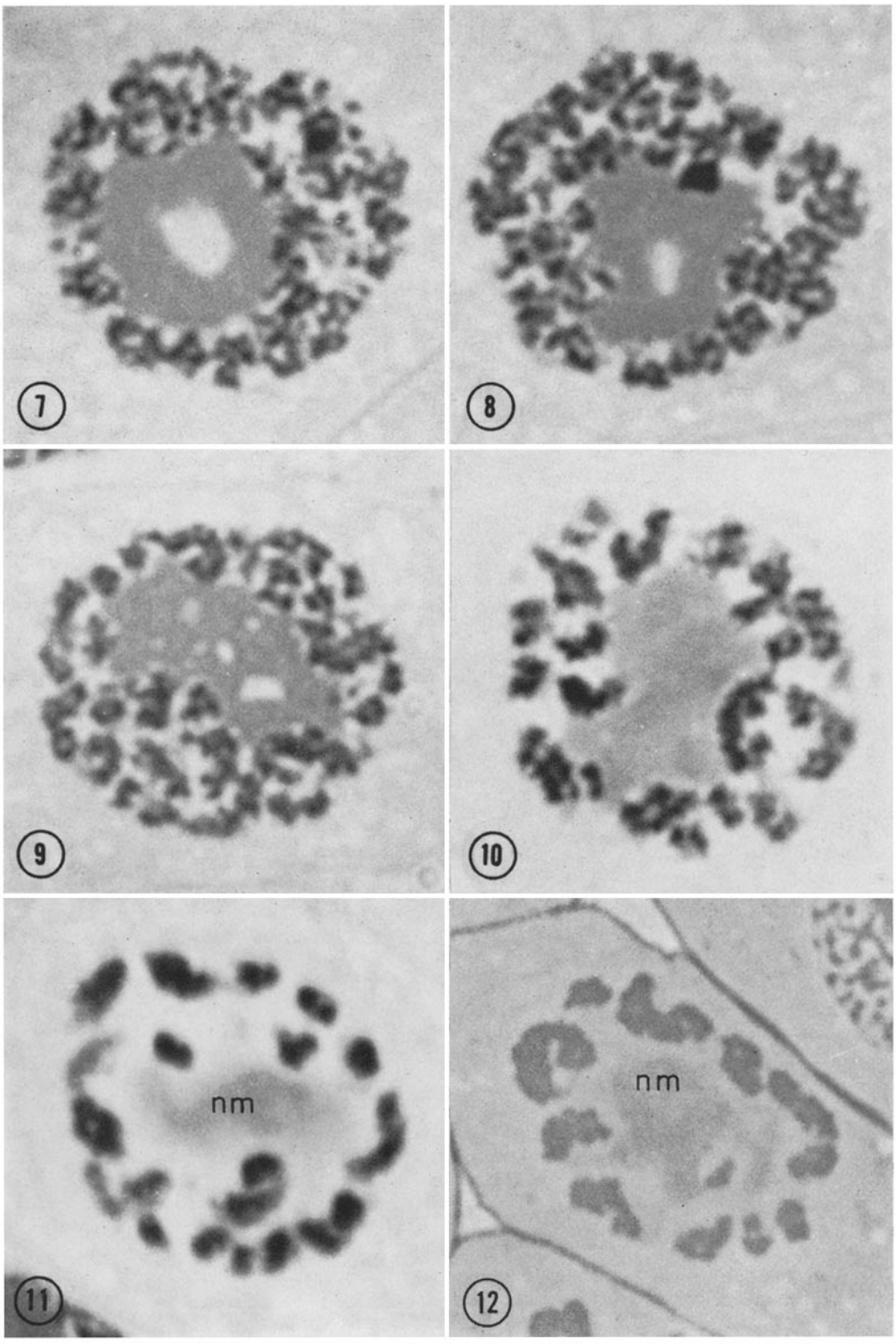
In this slightly older midprophase nucleus only barely visible vacuoles remain within the nucleolus. × 3,500.

Figure 11

Late prophase nucleus showing diffused areas of nucleolar material (*nm*) which merge imperceptibly with the surrounding nucleoplasm. × 3,500.

Figure 12

Section, 0.25 μ in thickness, of a late prophase nucleus photographed under phase contrast microscopy. The presence of diffused areas of nucleolar material (*nm*) is also revealed between the chromosomes in the peripheral portion of the nucleus. × 4,000.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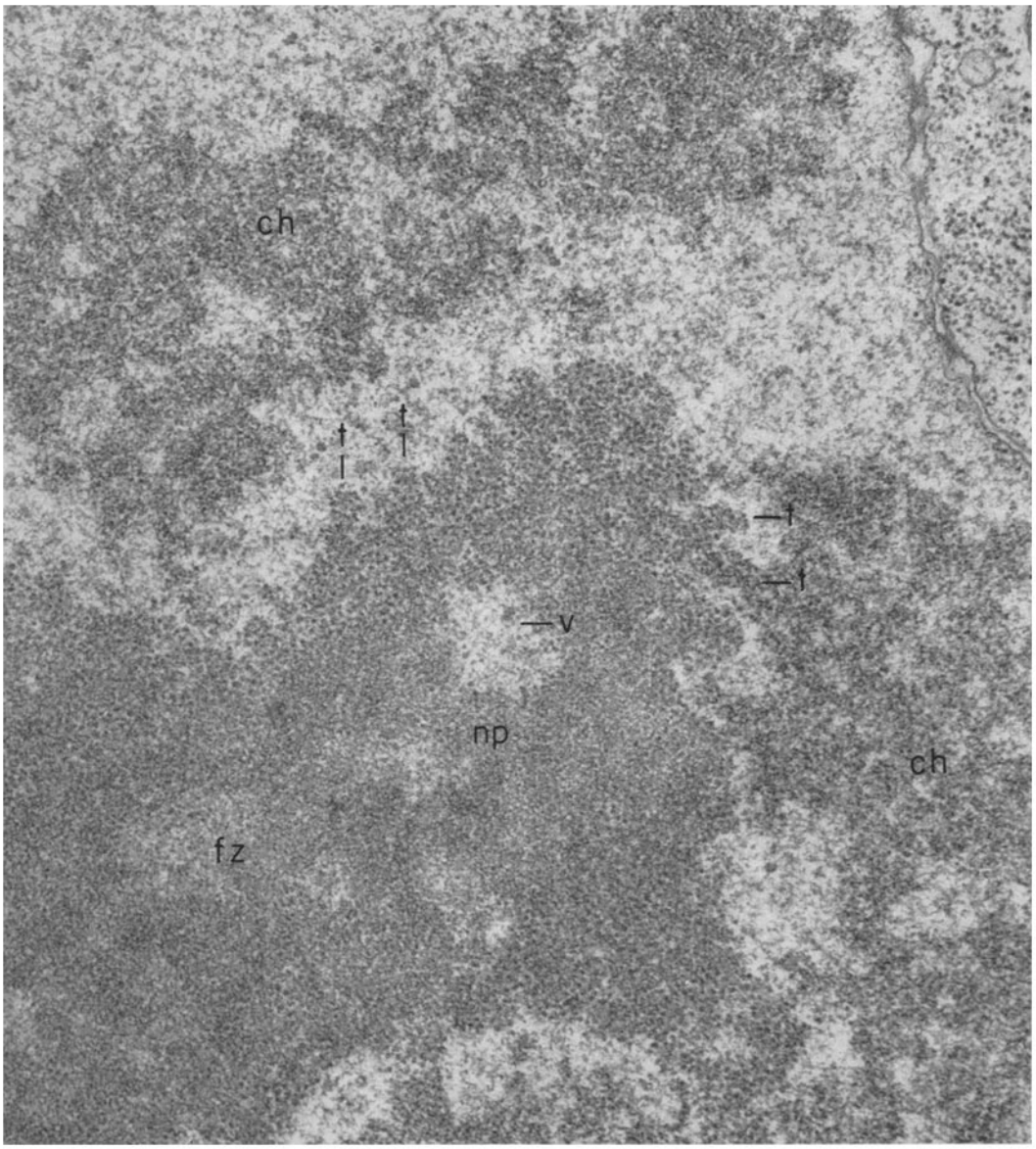


FIGURE 13

Electron micrograph of part of a midprophase nucleus illustrating a nucleolar projection (*np*) which extends between segments of neighbouring chromosomes. This projection, except for a few small vacuole-like (*v*) structures, is quite compact in texture and consists of a central fibrillar zone (*fz*) surrounded by a relatively thick layer of granular material. At the periphery of the nucleolar projection the 150 A granules are arranged in places into coarse, thread-like structures (*t*). The segments of chromosomes (*ch*) seen in this micrograph show coiled chromonemata cut at various angles. The light spaces located between these chromonemata contain fibrillar and granular elements similar to those found in the surrounding nucleoplasm. × 38,000.

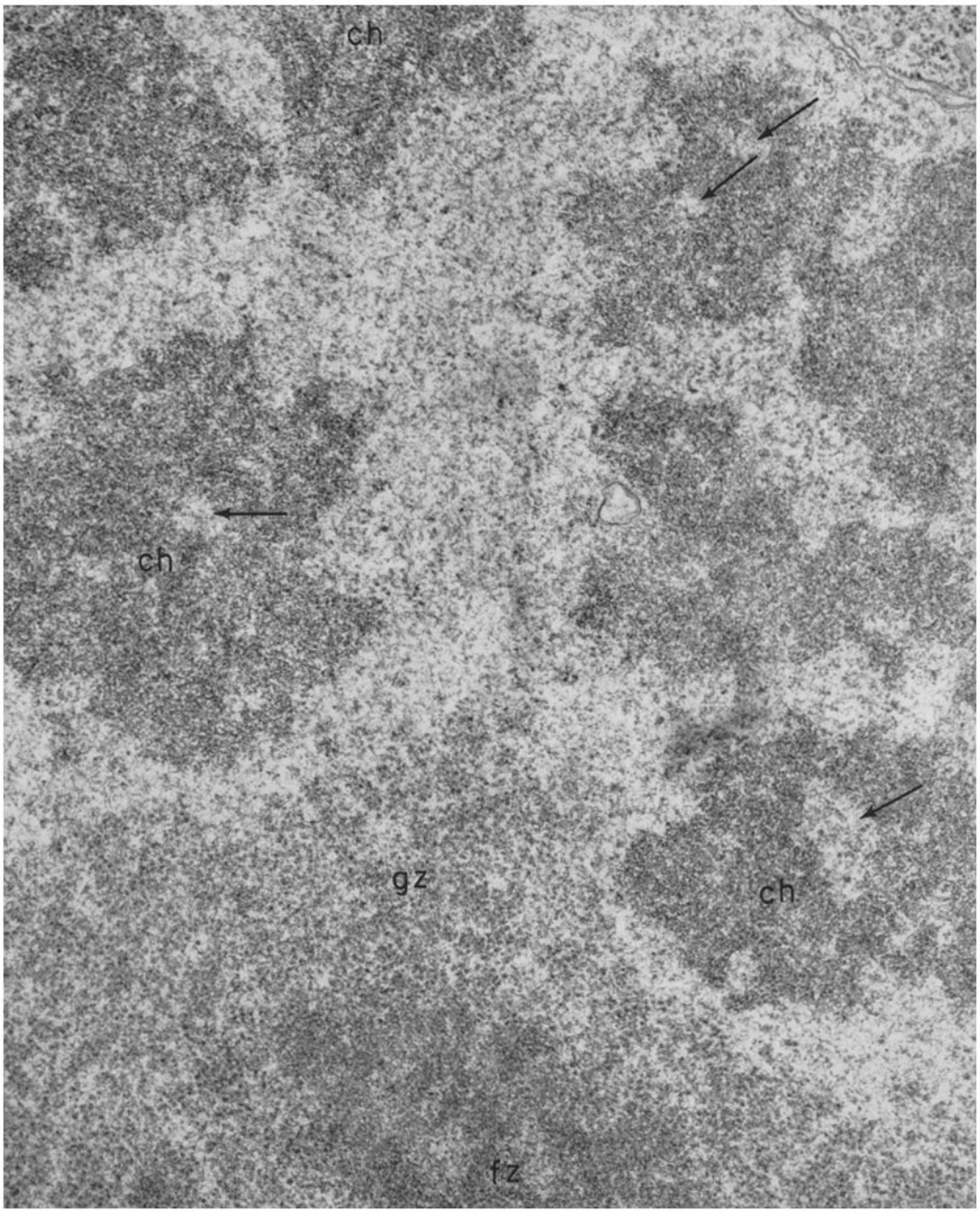


FIGURE 14

Electron micrograph of part of a midprophase nucleus illustrating the onset of nucleolar disintegration. A loosening up of both the granular (*gz*) and fibrillar (*fz*) nucleolar zones may be noted. A fibrillar component is now also observed pervading the granular zones. The few small interchromonemal light spaces (arrows) still observed within the chromosomes (*ch*) contain a material similar to that of the nucleoplasm. × 35,000.

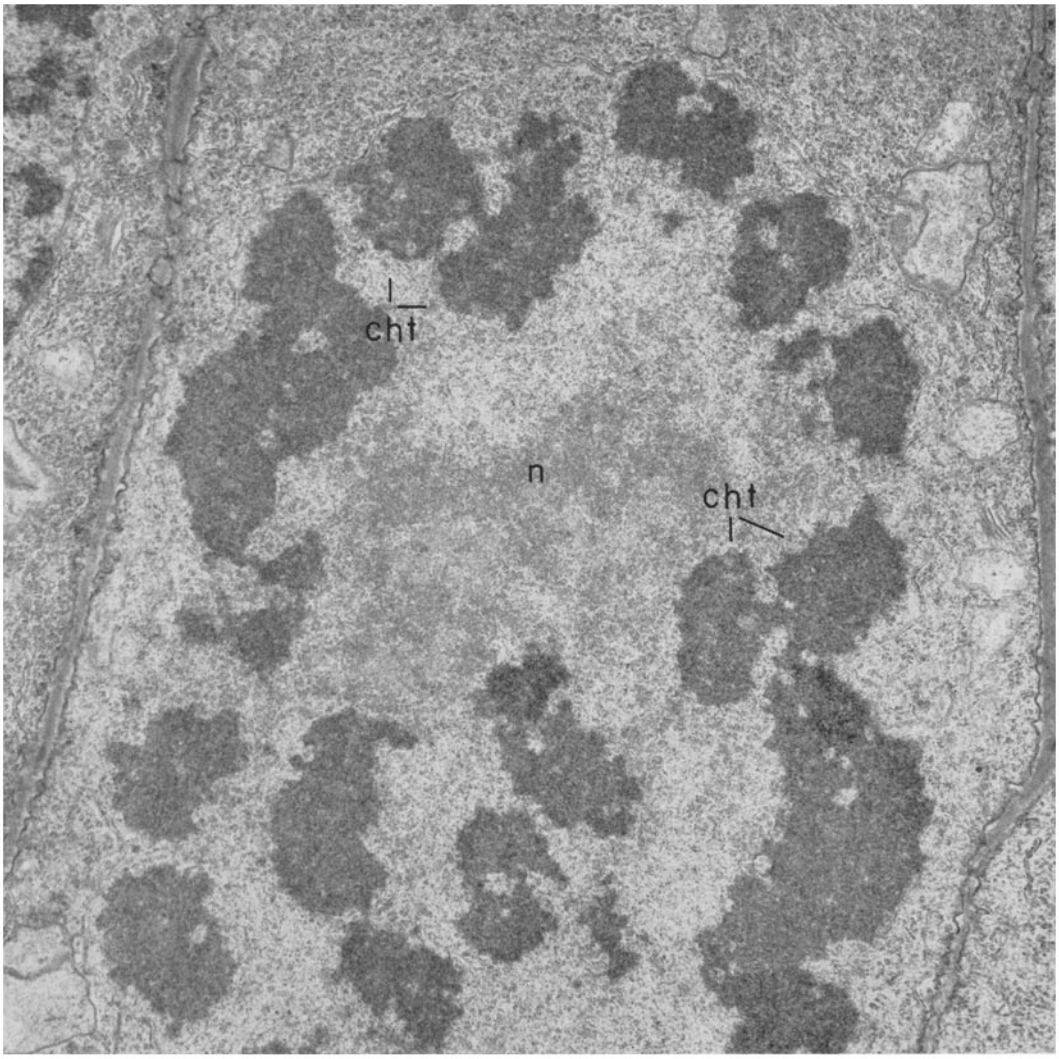


FIGURE 15

Electron micrograph of a late prophase nucleus. The nucleolus (*n*) has reached an advanced stage of disintegration and now appears as a heterogeneous mass consisting of intermingled and ill-defined zones of fibrillar and granular material. The density of the nucleoplasm itself is greatly increased as a result of the accumulation of granules and fibrils of nucleolar origin. The chromosomes in this nucleus are quite compact in structure except for a few lighter zones, the density of which matches that of the surrounding nucleoplasm. Chromatid, *cht.* × 13,000.

suggest the presence of a light blue Feulgen-negative material seemingly located in between the coiled chromonemata.

In apparently transverse sections each chromatid of metaphase chromosomes (Fig. 17 and insert) and each anaphase chromosome (Fig. 18 and insert) appear either as a densely and rather homogeneously stained roundish mass, slightly irregular in outline, or as a ring with its constituent chromonemata surrounding a central weakly staining core of varying diameter. The existence of such a core is also suggested, but to a lesser extent, in some metaphase or anaphase chromosomes viewed laterally (Fig. 19). In preparations stained according to the Feulgen procedure alone, this core, when visible, is Feulgen-negative

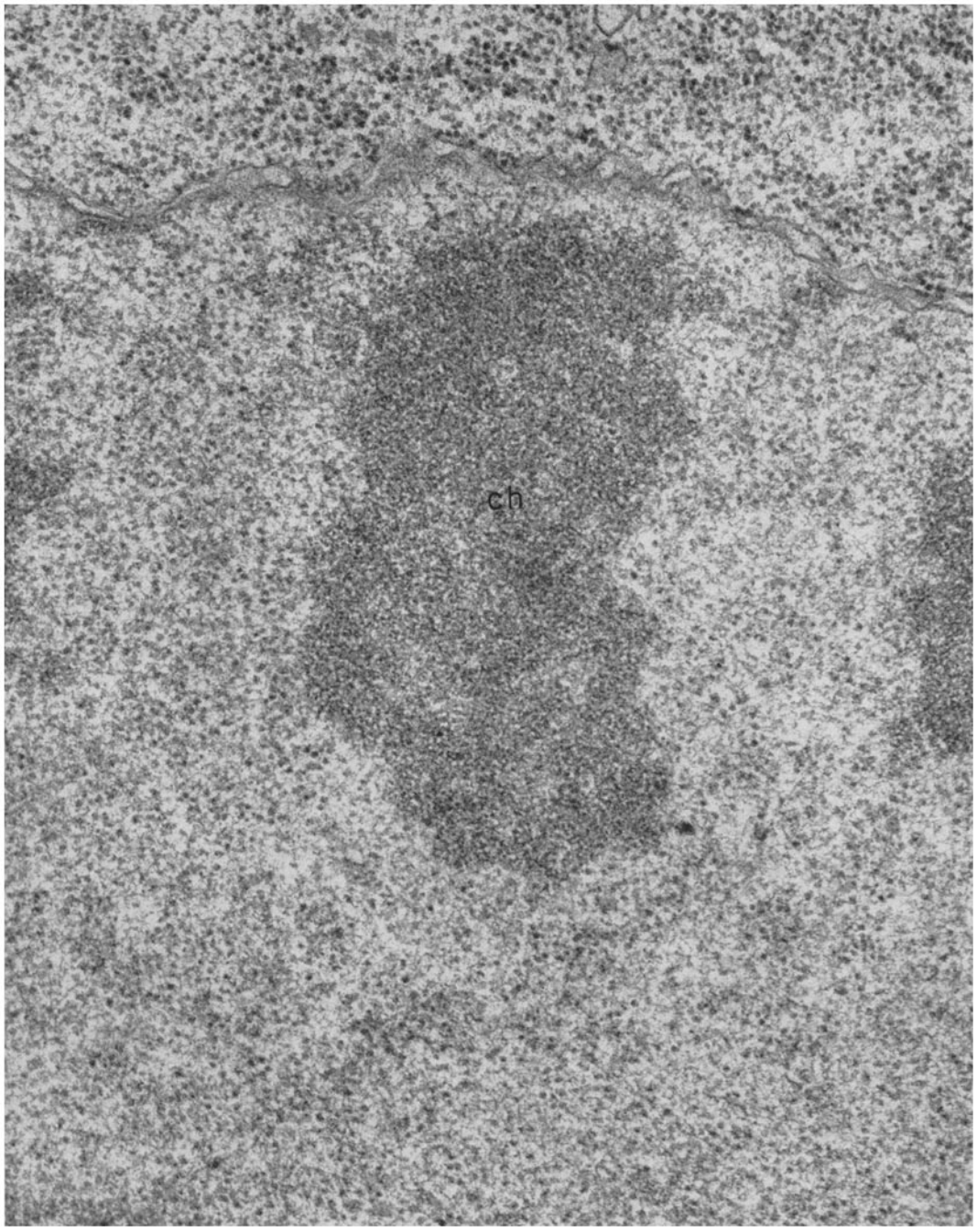
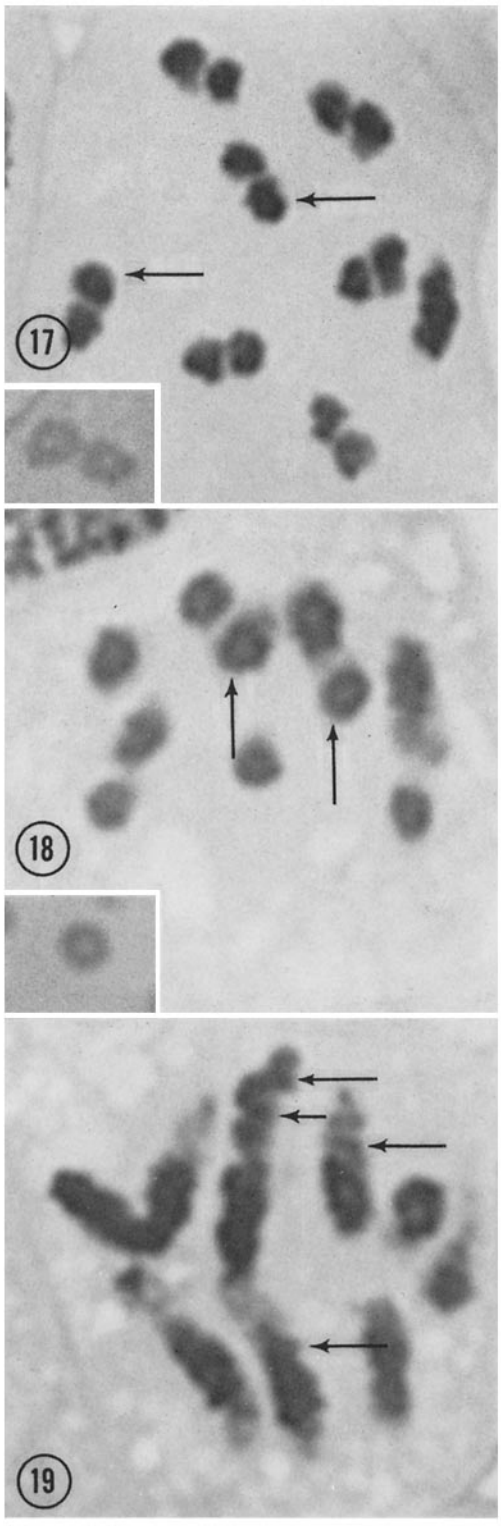


FIGURE 16

Electron micrograph of portion of a late prophase nucleus illustrating the dispersion of the granules and fibrils of nucleolar origin within the already fibrillar nucleoplasm. The chromosome (*ch*) appears as a rather compact and homogeneous mass consisting predominantly of fibrillar elements and of a number of granules indistinguishable from those observed within the nucleoplasm. × 50,000.



FIGURES 17–19

(Fig. 18 and insert). Transverse sections of both metaphase and anaphase chromosomes have failed to reveal the presence of a detectable coating of distinctive staining properties over their surface.

2. Electron Microscopy

At very early prometaphase, small breaks appear here and there in the now undulating nuclear envelope. As the latter becomes more disorganized (Fig. 20), its numerous fragments, consisting either of long flattened cisternae or of smooth roundish vesicles, circumscribe a more or less elliptical zone within which the chromosomes are located. The fine structure of the material present within this zone is still similar to that observed in the nucleoplasm at late prophase; it consists exclusively of loosely and uniformly distributed granules and fibrils. Eventually, during prometaphase all remnants of the nucleolar envelope disperse and the material which they formerly circumscribed becomes indistinguishable from that of the forming spindle.

At metaphase (Fig. 21) and anaphase, the spindle is made up of a fibrillar ground substance in which are embedded a large number of granules, some 150 A in diameter, often grouped into clusters

FIGURE 17

Light micrograph of a polar view of a metaphase figure. A number (arrows) of chromosomes have apparently been sectioned transversely and each chromatid appears as a roundish, homogeneously stained mass.

The insert shows a transverse section, 0.25 μ in thickness, of a metaphase chromosome photographed under phase contrast microscopy. A light core is clearly recognizable within each chromatid. × 4,000.

FIGURE 18

Light micrograph of a rather oblique section of an anaphase figure illustrating the presence of a light core in a number of chromosomes (arrows). × 4,000.

The insert represents a transverse section, 0.5 μ in thickness, of an anaphase chromosome stained according to the Feulgen procedure alone; the core is Feulgen-negative. × 4,000.

FIGURE 19

Light micrograph of anaphase chromosomes seen both laterally and in grazing sections. The chromonematic gyres of the obliquely sectioned chromosomes are clearly recognizable (arrows); a lighter staining material also appears to be present between these gyres. × 4,000.

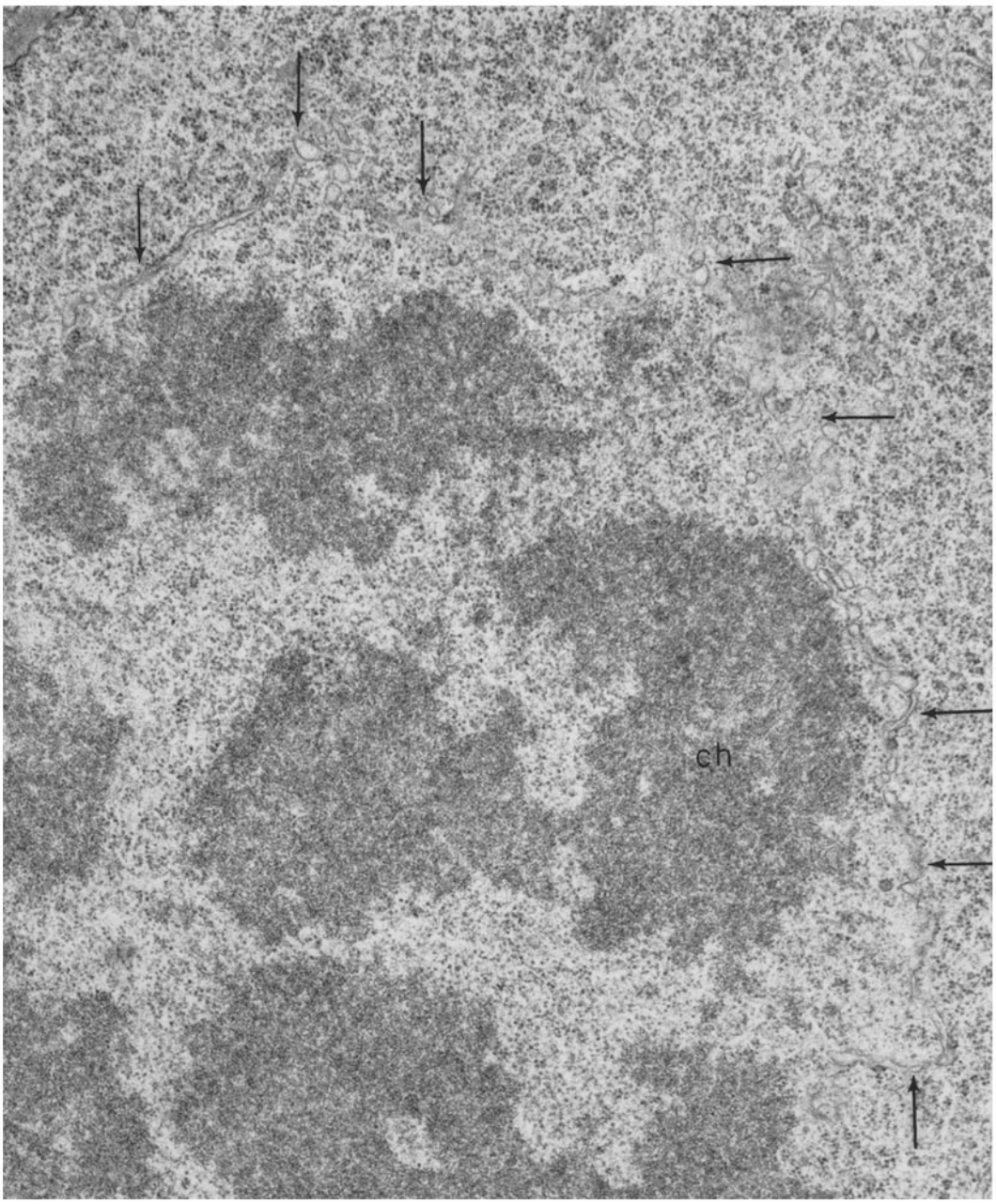


FIGURE 20

Electron micrograph of portion of an early prometaphase figure. The fragments of the disintegrating nuclear envelope (arrows) consist of flattened cisternae and of roundish membrane-bounded vesicles. The fine structure of the material present within the area of the cell delimited by these nuclear envelope fragments is similar to that observed in the nucleoplasm at late prophase. Chromosome, *ch.* × 27,000.

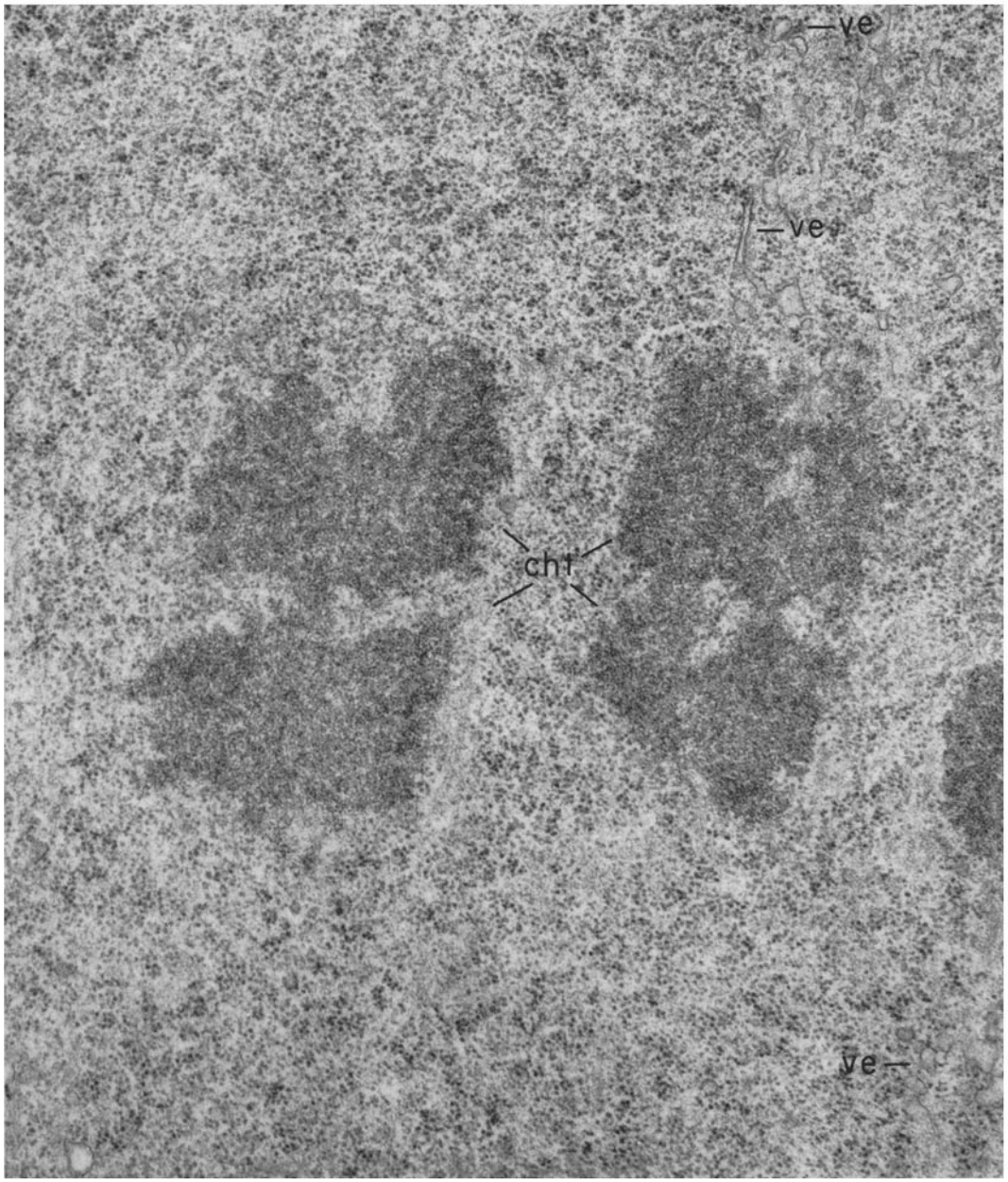


FIGURE 21

Portion of the spindle area with two chromosomes on the equatorial plate. The spindle consists predominantly of small clusters of 150 A granules distributed more or less uniformly within the fibrillar ground substance. Here and there groups of roundish and elongated vesicles (*ve*) are also observed. No noticeable concentration of material of distinctive fine structure is present on the surface of the chromosomes. Chromatid, *cht*. × 34,000.

of varying sizes, and a number of roundish and elongated membrane-bounded vesicles.

The chromatids of prometaphase (Fig. 20) and metaphase chromosomes (Figs. 21 and 22) as well as the daughter anaphase chromosomes are disclosed as compact masses much denser than the surrounding spindle material. The only structural elements that can be resolved within these masses, *i.e.* convoluted fibrils and granules, appear similar to those observed within the chromosomes during prophase. In the present study the chromosomal core, often observed under light microscopy, has never been seen in the electron microscope at metaphase, and only occasionally at anaphase. When distinguishable (Fig. 23), such a core is seen to contain loosely scattered fibrils and granules. Both longitudinal and transverse (Figs. 22 and 23) sections of either metaphase chromatids or anaphase chromosomes show no evidence of an existing coating of distinctive fine structure over their surface. The chromonemal gyres, clearly recognizable in grazing sections under light microscopy (Fig. 19), are not distinguishable as such in the electron microscope, even when their presence is strongly suggested by the rather wavy contours of the chromosomes at both metaphase and anaphase. If, as would appear, an internal matrix material is responsible for the masking of the gyres in question, its fine structure must therefore be comparable to that of the chromonemata themselves.

The chromatid segments, which, judging by their size and location at anaphase, correspond most likely to Heitz's nucleolar secondary constrictions, are seen under electron microscopy to consist of a low density fibrillar material devoid of any granular elements (Fig. 24).

Telophase

1. Light Microscopy

Just after having completed their anaphasic movement, the chromosomes gather at the poles and their arms form both parallel and V shaped figures. At that time already, a thin coating of metachromatic cabbage-green material is detectable on the surface of the chromosomes. Grazing sections of the chromosomes at that stage (Fig. 25) suggest that this material also pervades the spaces between the chromonemal gyres; this distribution of the material in question is still more apparent slightly later on when the chromosome arms are grouped in a more parallel fashion (Fig. 26). As telophase progresses, the nucleus as a whole noticeably increases in size and the enlarged interchromosomal spaces appear almost completely filled with a similar metachromatic material (Figs. 27 and 28).

By midtelophase the coiled chromonemata have become much more distinct and their interlacing aspect more pronounced; at this stage one or two small roundish masses of metachromatic material corresponding to the forming nucleoli (Fig. 29) can be recognized. It must be pointed out that, at this time, these nucleoli are barely distinguishable from the immediately surrounding interchromosomal material which has a similar staining intensity and metachromatic characteristics. During their subsequent growth the nucleoli, in many places on their surface, still show continuity with the surrounding metachromatic material. This gradual enlargement of the nucleoli is, moreover, concomitant with a corresponding progressive disappearance of the material in question (Fig. 30). By late telophase, the uncoiled chromonemata give rise to a complex network extending throughout the nuclear cavity (Fig. 31). A few small patches of metachromatic material extending from the nucleolar surface into the neighbouring nucleoplasm are usually still observed in such nuclei. At the end of our observation period, *i.e.* posttelophase, a few small light, vacuole-like zones have appeared within the nucleoli (Fig. 32).

From midtelophase on, as the metachromatic material gradually disappears from the interchromosomal spaces, a very light blue orthochromatic staining material, corresponding to the forming nucleoplasm, becomes recognizable throughout the nucleus (Figs. 30 and 31).

2. Electron Microscopy

At very early telophase, the electron microscope reveals the existence of a material, noticeably denser than that of the spindle and of distinctive fine structure, which forms a coating of variable thickness over the surface of the chromosomes (Fig. 33). In the upper portion of the telophasic figure, the coatings of neighbouring chromosomes merge, thus filling part of these interchromosomal spaces; elsewhere the chromosome arms are farther apart and separated by irregularly shaped zones of spindle material. As judged by its distribution, there can be little doubt that the coating just described corresponds to that revealed

under light microscopy after methylene blue staining (Fig. 25). Under electron microscopy this coating is seen to consist of loosely arranged fibrillar elements, 60 to 100 A in diameter, intermingled with dense 150 A granules indistinguishable from those observed in the neighbouring spindle material. The presence of the intervening fibrillar material rather than a closer packing of the granules seems to be responsible for the greater density of the fibrillogranular chromosomal coating as compared to that of the material in the spindle area. In places, moreover, the impression is gained that this fibrillogranular coating material is arranged into some sort of coarse, thread-like structures approximately 0.1 μ in diameter. As suggested by corresponding light micrographs (Figs. 25 and 26), this coating is found not only in intimate contact with the wavy contours of the chromosomes but apparently also pervades some of the light areas seen within the chromosome mass (Fig. 33).

As in metaphase (Fig. 24) and anaphase, the early telophase chromosomal segments, corresponding to the nucleolar secondary constrictions, exhibit a fibrillar texture of low density devoid of any granular elements (Fig. 33). At very early telophase, at least, such chromosomal segments are not coated with the fibrillogranular material present elsewhere on the surface of the chromosomes.

As the chromosomes assemble closely during early telophase ("polar clumping" of the classical authors), the fibrillogranular material, previously observed on their surfaces, now appears as thin layers squeezed between them (Fig. 34). From then on, this material noticeably increases in amount within the enlarging interchromosomal spaces (Fig. 35).

The youngest telophase nucleoli observed already show under electron microscopy many of the structural features of mature nucleoli. Their central portion appears quite dense and, except for a few very small lighter areas, consists of granular and fibrillar material similar to that observed in preprophase and prophase nucleoli (Fig. 37). These two types of material, furthermore, are already segregated into irregularly shaped zones blending with one another. The more peripheral portions of these small nucleoli, however, exhibit a fluffy appearance due to the rather loose arrangement of their constituent thread-like structures, some 0.1 μ in diameter. It should be noted that the nucleolar surface is always continuous, in places, with fibrillogranular material, equally fluffy in appearance, located in the interchromosomal spaces. Besides being grouped into fluffy patches these thread-like structures are also observed intermingling with the unraveling chromonemata throughout the nucleus, thus giving the latter a quite complex appearance at that stage (Fig. 37). During the subsequent growth of the nucleolus, there occurs a gradual decrease in the amount of the fibrillogranular material just described and, by late telophase and even posttelophase, only remnants of such a material are seen, located mostly on the surface or in the immediate vicinity of the nucleolar mass (Figs. 38 and 39).

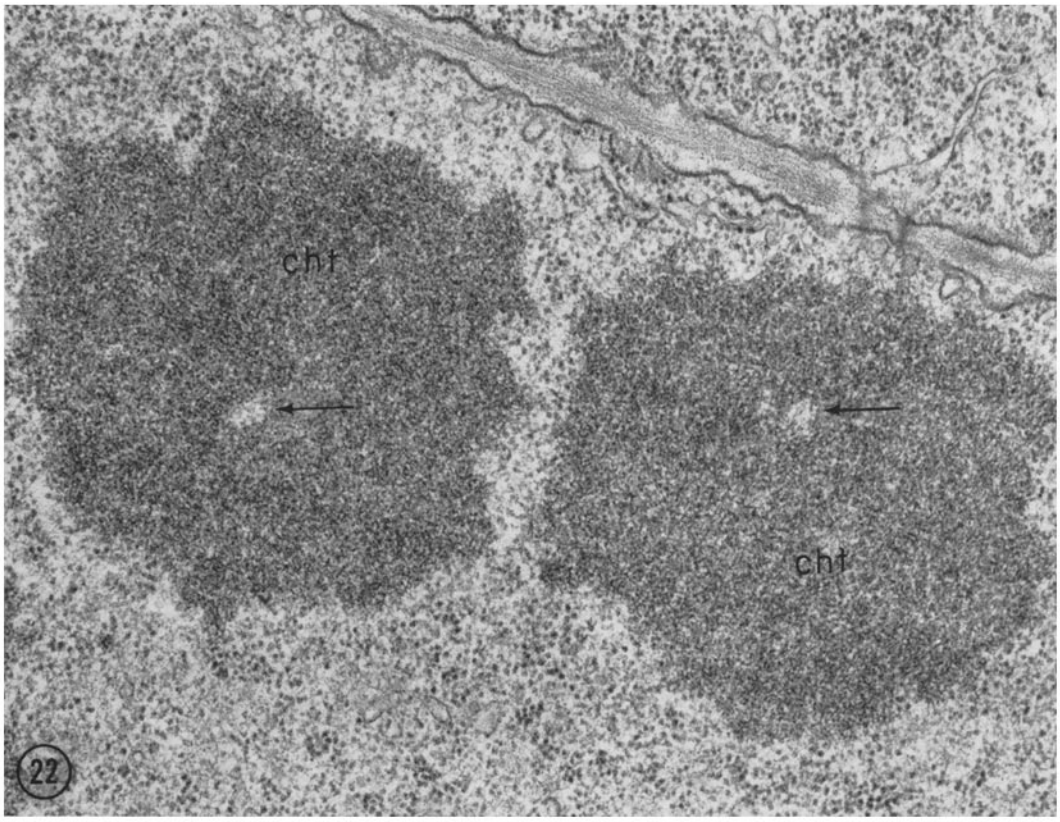
Concomitant with the disappearance of the fibrillogranular material from the interchromosomal spaces, a much lighter material, the nucleoplasm, may be observed in the same regions, consisting mostly of loosely scattered fibrils less than 100 A in diameter and of a number of dense granules the majority of which are indistinguish-

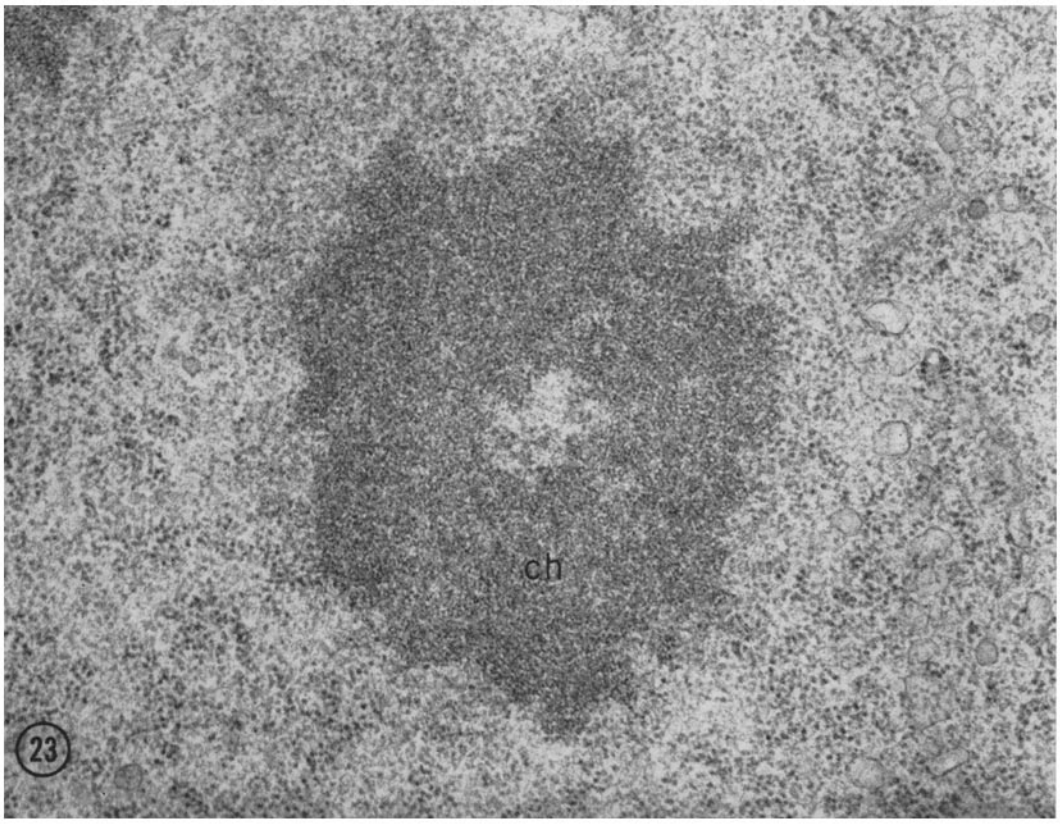
FIGURE 22

Metaphase chromosome in transverse section. Each chromatid (*cht*) shows serrated contours and, except for a tiny light zone (arrows), exhibits no recognizable core comparable to that often observed under light microscopy. The chromatids consist predominantly of a dense fibrillar material as well as of a few granules of varying diameter. × 36,000.

FIGURE 23

Transverse section of an anaphase chromosome (*ch*). This chromosome is seen to consist of a homogeneously dense outer zone surrounding a small light core. Both the surface of the chromosome and the boundary of its inner core are quite irregular in contours. The denser portion of the chromosome consists mostly of densely packed fibrils and also of a number of granules of varying diameter. × 35,000.


cht
cht
22


ch
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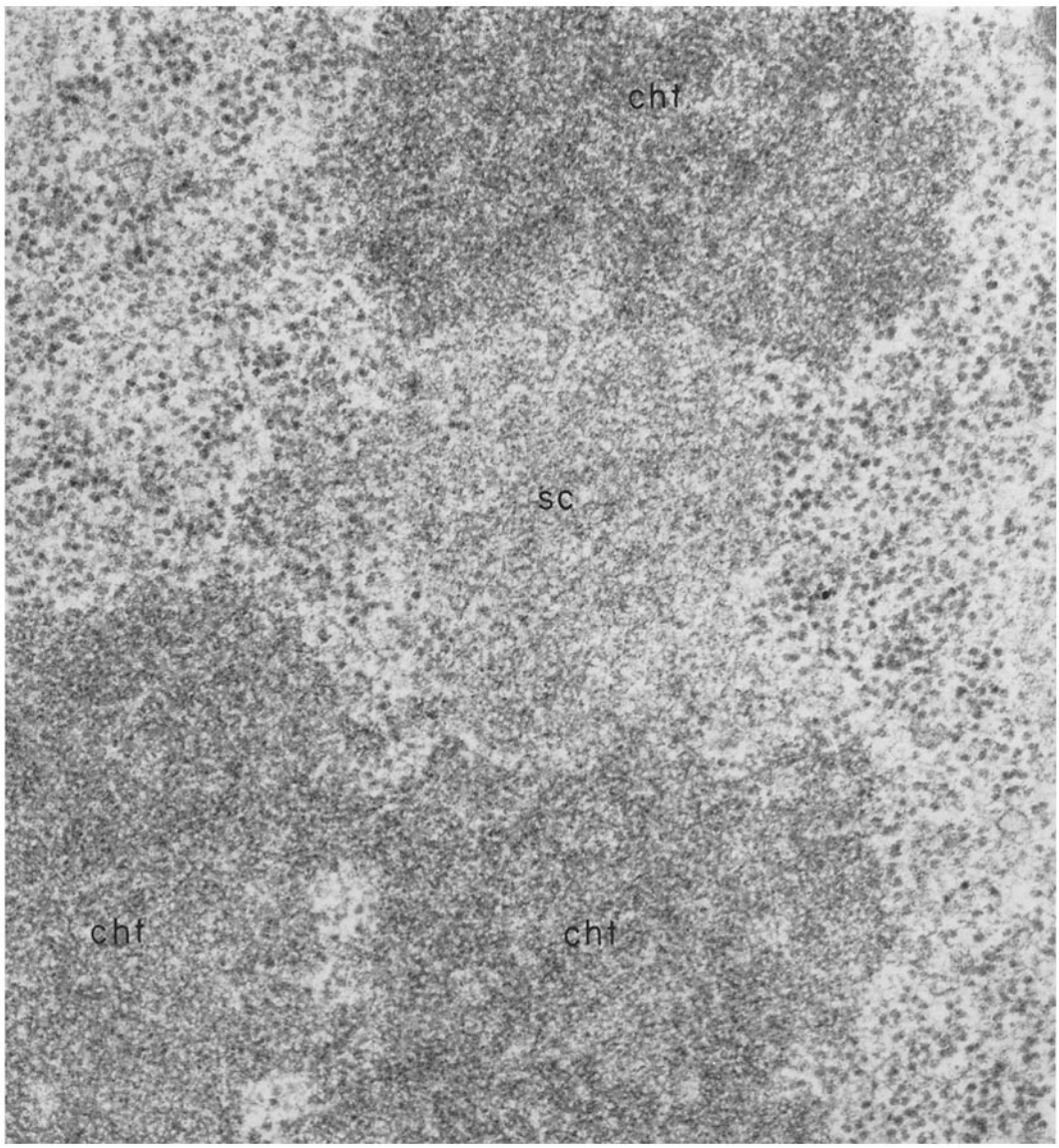


FIGURE 24

Electron micrograph of portion of two metaphase chromatids (*cht*) showing a nucleolar secondary constriction (*sc*). This constriction is filled with a material exclusively fibrillar in texture and of a lesser density than that of the adjacent chromatid segments. × 60,000.

able from those present within the nucleolus (Figs. 37 to 39).

Formation of the Nuclear Envelope and of the Spindle Remnants

During early telophase, as the chromosomes become more parallelly aligned, a nuclear envelope begins to form at the periphery of the telophase figure (Fig. 34). The forming nuclear envelope, then, consists of membrane-bounded vesicles and flattened cisternal profiles of varying length in the immediate vicinity of the peripherally located chromosomes. Soon thereafter these membranous elements assemble over the surface of the

telophase figure and fuse here and there to give rise to a more or less continuous double-layered envelope. By midtelophase, the formation of the nuclear envelope is about completed.

During the early stages of nuclear envelope reconstitution, zones containing both spindle material and occasionally cytoplasmic organelles become imprisoned within the telophase nucleus (Fig. 36). In all cases examined these zones were still circumscribed by a discontinuous, double-layered envelope. Such zones correspond to the light staining areas seen under light microscopy (Figs. 26 and 28).

DISCUSSION

Structural Components of the Nucleolus at Preprophase

Our electron microscopic observations provide evidence for at least two definable components, each segregated into distinct zones, within the dense portion of the preprophase nucleolus in *Vicia faba*. One of these components consists of closely packed 150 A granules (Figs. 4 and 5) similar to those observed by several investigators in nucleoli of a wide variety of both plant and animal cells (5, 15–20). In *Vicia faba* it is observed, moreover, that, in places within the nucleolar mass and especially within its peripheral portion, these 150 A granules are assembled into seemingly coarse, thread-like structures some 0.1 μ in diameter (Figs. 4 and 5). Elsewhere, it must be stressed, such an arrangement is not at all obvious. It is not clear whether or not this apparent absence of filamentous structures in certain nucleolar granular areas is due to the close packing of the elements in question or to the presence of a more amorphous intervening material as suggested by Bernhard (17). The occurrence of coarse thread-like structures has been demonstrated in nucleoli from very different sources and it has usually been assumed that they correspond to the filamentous "nucleolonemata" described by Estable and Sotelo (2, 3) under light microscopy. Such a direct correspondence cannot be so readily established in *Vicia faba* where the thread-like structures observed have a diameter ($\sim$0.1 μ) which is below the resolving power of the light microscope.

The second structural component of the dense portion of the preprophase nucleolus, in *Vicia faba*, consists of closely packed fibrils grouped into zones mostly located in its more central portion (Figs. 4 and 5). The existence of a second, non-granular component in the nucleolus has been reported in a number of previous electron microscope investigations of this organelle (5, 17, 19, 20) and has usually been described as being "amorphous" in nature (20) or "more finely divided" (19). After heavy metal staining this so called "amorphous substance" appears essentially fibrillar in texture (Figs. 5 and 6).

According to Porter (19), the non-particulate zones in interphase nucleoli of *Allium cepa* are characterized moreover by the presence of the extremely dense particles noted previously (5) in interphase, prophase, and telophase nucleoli in this species as well as in *Vicia faba* and reported since then in nucleoli of other plant species (21). For reasons which are still not understood, such dense particles have not been observed in the present study within nucleoli at preprophase or for that matter at any of the other stages.

A segregation into distinct zones of the two structural components of the nucleolus has been reported in *Allium cepa* (19) and in *Drosophila* and *Sciara* salivary gland nucleoli (20). In *Vicia faba* nucleoli such a segregation is by no means exclusive; high magnification micrographs (Fig. 5) show indeed that a few 150 A granules are scattered within the fibrillar zones and that, likewise, an intervening material is apparently present in the granular zones. This intervening material is not readily analyzed in our micrographs but seems to consist of fibrils similar to those observed in the dense fibrillar zones.

The preprophase nucleolus in *Vicia faba* also shows, in addition to the two structurally distinct types of zones just referred to, vacuole-like structures of varying sizes which contain loosely and uniformly distributed granules and fibrils (Figs. 4 and 5) similar to those found within the denser portion of the nucleolus. It would therefore seem more likely that these vacuoles contain nucleolar material in a dispersed form rather than elements of the nuclear sap as previously suggested (20). Such fluid-filled intranucleolar vacuoles are thought to form as a result of the physiological activity of the nucleolar mass and to be extruded from time to time into the surrounding nucleoplasm (22–24).

Fate of the Structural Components of the Nucleolus during Prophase and Prometaphase

Our observations show that the nucleolus, roundish in outline at prepropase, gradually becomes highly irregular in shape during midprophase and, eventually, disintegrates in the course of late prophase. In connection with the actual fate of the nucleolar material during the disintegration process, several earlier cytologists (review of literature in Gates, 1) have claimed that part, if not all, of it accumulates as a matrix on the surface or within the late prophase chromosomes. For our present purpose the two important points to know are whether or not at the time of nucleolar disintegration the structural components of the nucleolus persist as such within the nucleus and, if so, to what extent they become associated with the chromosomes to form a matrix.

Concerning the first problem, our electron microscope observations show that the structural components of the disintegrating nucleolus do persist as such and mix freely with the surrounding nucleoplasm at late prophase. This conclusion is based on the following observations: (*a*) at the

FIGURE 25

Light micrograph of a group of chromosomes at very early telophase. In the upper half portion of the telophase figure a lighter staining, metachromatic, material (*mm*) can be seen on the surface of and in between the densely stained chromosomes. Grazing sectioning of segments (arrows) of two of these chromosomes suggests, moreover, that a similar material likewise pervades the space between the interchromonemal gyres. × 40,000.

FIGURE 26

Telophase nucleus slightly older than that shown in Fig. 25. The chromosomes are all in grazing sections and the metachromatic material (*mm*) is seen pervading both the interchromosomal and interchromonemal spaces. A number of small lighter zones of spindle remnant material (arrows) are also observed between the chromosomes. × 5,000.

FIGURE 27

Light micrograph of an oblique section of a midtelophasic figure illustrating the enlarged interchromosomal spaces filled with metachromatic material (*mm*). × 4,000.

FIGURE 28

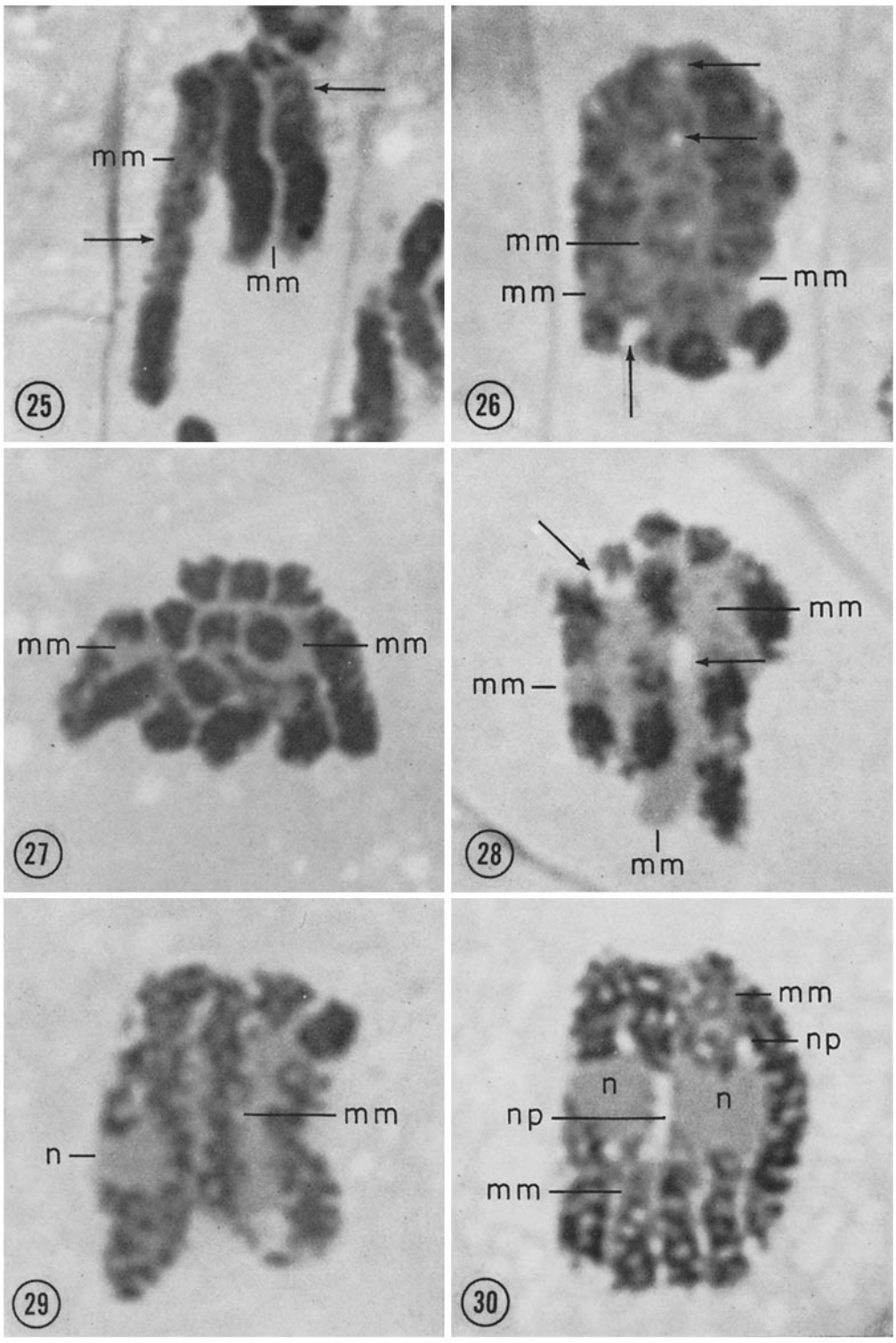
This slightly oblique section of a more advanced midtelophasic figure shows the large amount of metachromatic material (*mm*) which is present within the interchromosomal spaces at that time. Three unstained zones of spindle remnant material (arrows) are also clearly recognizable. × 4,000.

FIGURE 29

Midtelophase nucleus at the time when the nucleolus (*n*) is first recognized as a formed body. Note that this nucleolus exhibits a staining intensity similar to that of the interchromosomal and interchromonemal metachromatic material (*mm*). × 5,000.

FIGURE 30

In the present micrograph the two nucleoli (*n*) have reached a certain size and only a few patches of metachromatic material (*mm*) are still observed amongst the uncoiling chromonemata. Note that the surface of the nucleolus is continuous with one of these patches. The many interchromosomal and interchromonemal light spaces are occupied by a very light staining material corresponding to the forming nucleoplasm (*np*). × 4,000.


mm
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mm
30

onset of nucleolar disintegration the nucleolar mass is somewhat loosened (Fig. 14) but its constituent granules and fibrils are still similar to those observed within nucleoli at earlier stages; (*b*) during the gradual process of nucleolar disintegration, gradients of these two components (Fig. 15) are seen merging imperceptibly with the surrounding nucleoplasm; (*c*) finally, when all detectable cortical matrix of nucleolar origin over the chromosomes (Fig. 16). Furthermore, since no significant increase in the concentration of granular elements is detectable within the chromosomal masses at late prophase, it is reasonable to assume that the bulk of this particulate nucleolar component does not become incorporated as such in the form of an internal matrix substance. Be-

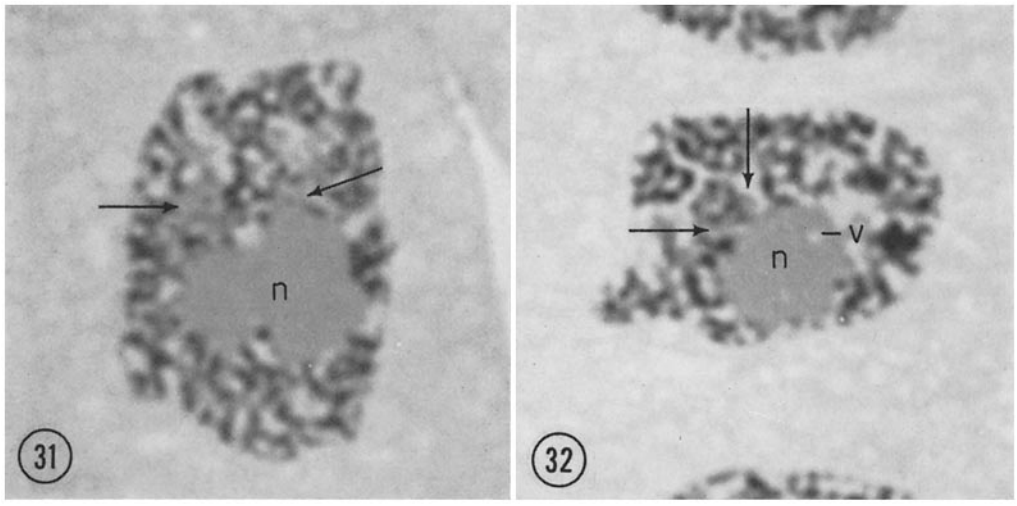


FIGURE 31

Late telophase nucleus illustrating two forming nucleoli (*n*) which have fused to give rise to a dumbbell-type structure. The uncoiled chromonemata form a complex network throughout the nuclear cavity. In places within this network small patches of metachromatic material (arrows) are observed, some of them being continuous with the surface of the nucleoli. × 4,000.

FIGURE 32

Light micrograph of a post-telophase nucleus. Only very small patches of metachromatic material (arrows) are observed within the nuclear cavity and all of these are located in the immediate vicinity or adjacent to the nucleolar surface. A few small, vacuole-like (*v*) structures are present within the otherwise homogeneously stained nucleolar mass (*n*). × 4,000.

formed remnants of the nucleolus have disappeared from view, the nucleoplasm appears homogeneously filled with a large number of granules (Fig. 16) similar to those previously observed within the formed nucleolar mass. At that time the nucleoplasm also shows a large concentration of fibrillar material part of which is undoubtedly of nucleolar origin. Unfortunately, these fibrils cannot be distinguished from similar elements already present in the nucleoplasm prior to the onset of nucleolar breakdown (Figs. 6, 13, and 14).

Concerning the second point, our observations have consistently failed to reveal the presence of a cause of the probable masking effect of the already fibrillar structure of the chromosomes, it is unfortunately not possible to decide whether or not part of the fibrils of nucleolar origin do contribute as such to the formation of an internal chromosomal matrix. Evidence for the existence of an internal matrix in late prophase, metaphase, and anaphase chromosomes will be presented later on in this discussion.

The electron microscope reveals at early prometaphase, that the broken-down fragments of the nuclear envelope circumscribe an elliptical area containing the chromosomes and a material

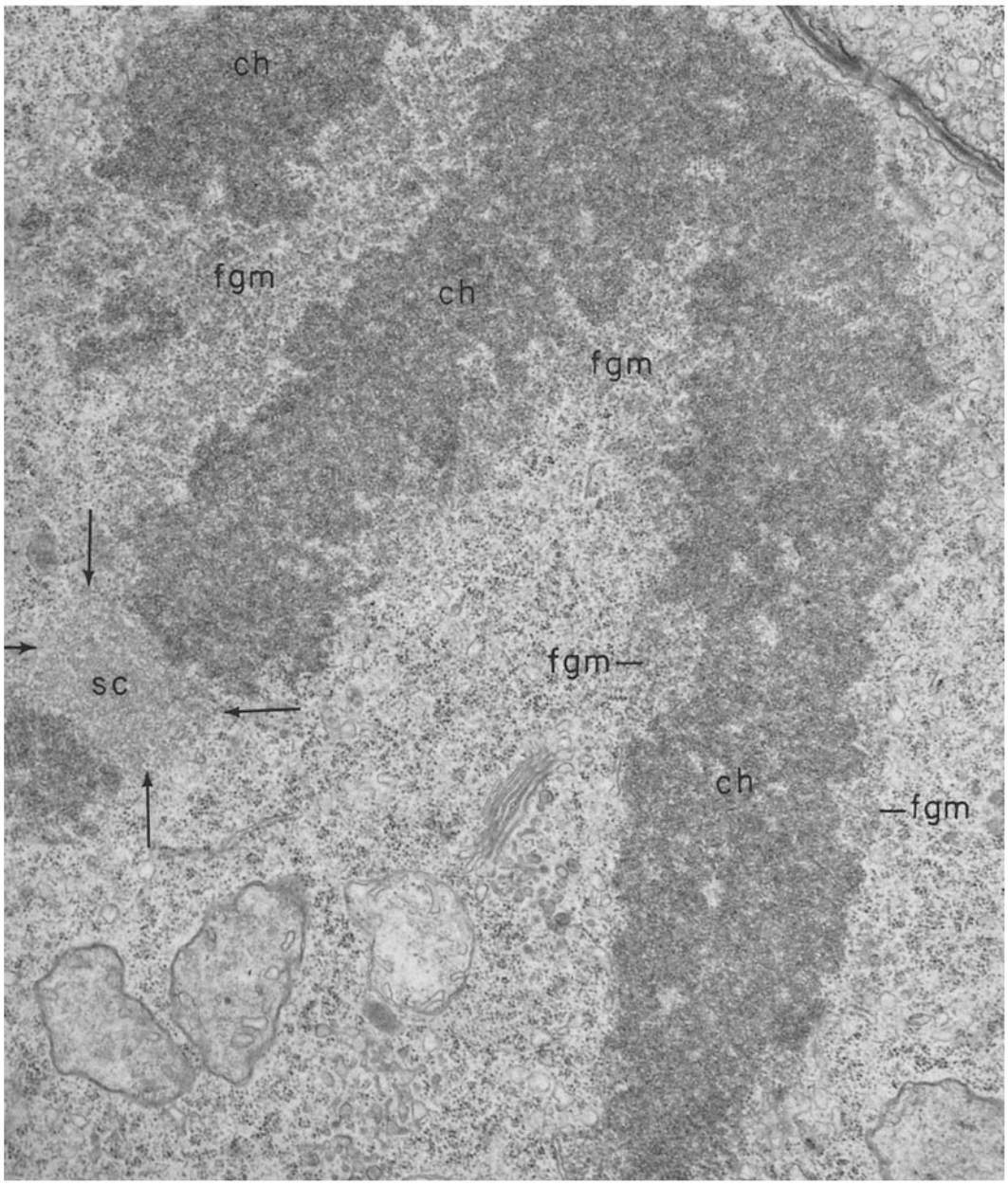


FIGURE 33

This micrograph shows segments of three early telophase chromosomes (*ch*) at the cell pole. The chromosomes are still quite dense except for the presence of a number of tiny light areas within their mass. One chromosome shows a nucleolar secondary constriction (*sc*) filled with fibrillar material of low density. A fibrillogranular material (*fgm*), the density of which is about intermediate between that of the chromosomes and that of the spindle, is seen to form in many places a coating of variable thickness over the wavy surface of the chromosomes. In the upper left-hand corner of the micrograph, the respective fibrillogranular coatings of two neighbouring chromosomes have apparently merged and, in this area, appear to be arranged into convoluted, coarse ($\sim$0.1 μ), thread-like structures. The granular elements of the chromosomal coating are indistinguishable, both with respect to density and size, from the neighbouring spindle ribosomes. A few dictyosomes, mitochondria and plastid-like organelles are present in the V shaped spindle area between two of the chromosomes. $\times$ 22,000.

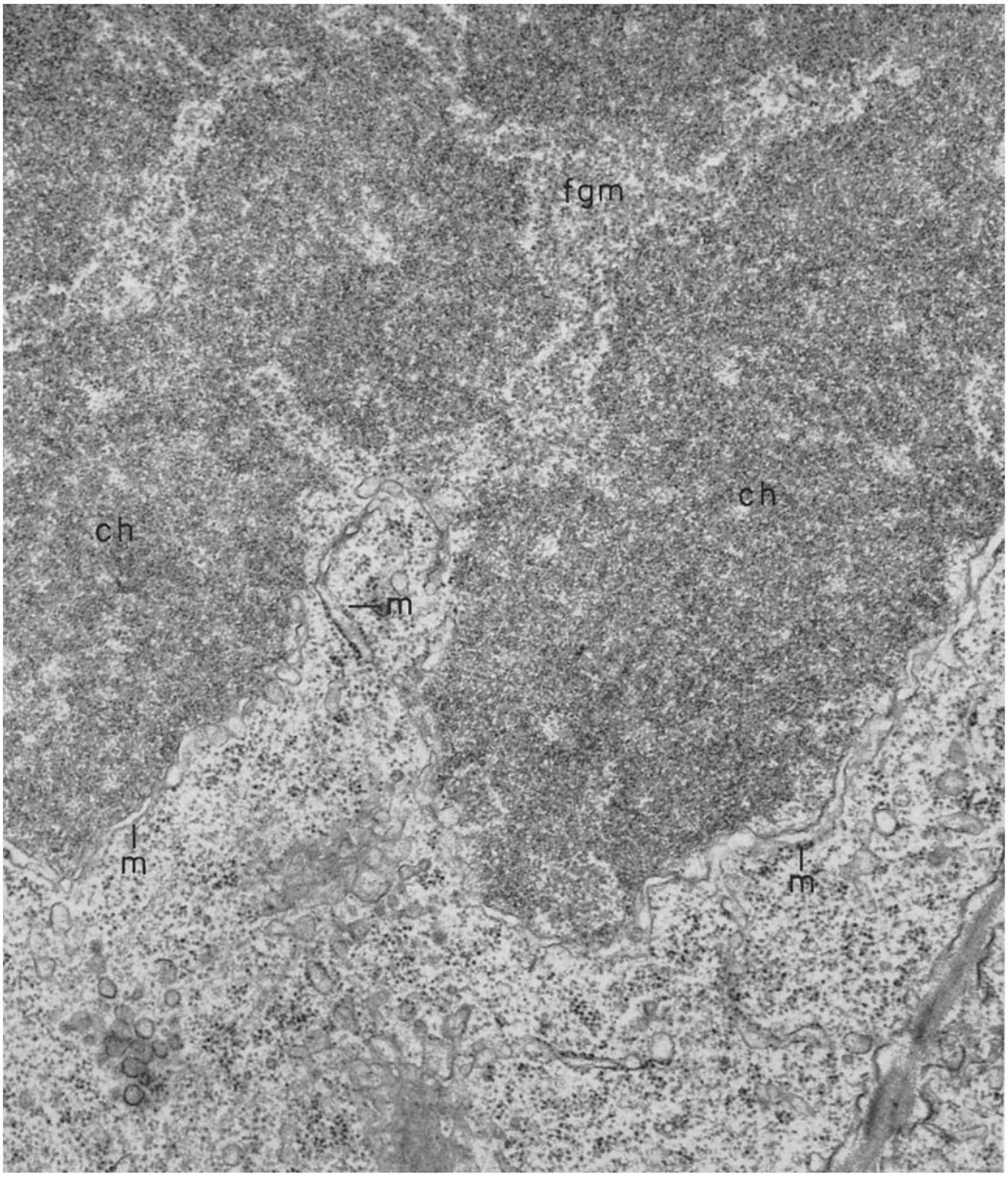


FIGURE 34

Lower portion of an early telophase nucleus at the time when the elements of the forming nuclear envelope have already circumscribed most of the telophasic figure. These membranous elements (*m*) lie in rather close contact with the chromosomal surface even in places where spindle material is found between neighbouring tips of chromosomes (*ch*). The fibrillogranular material (*fgm*), in relatively small amounts, is squeezed between the chromosomes. × 32,000.

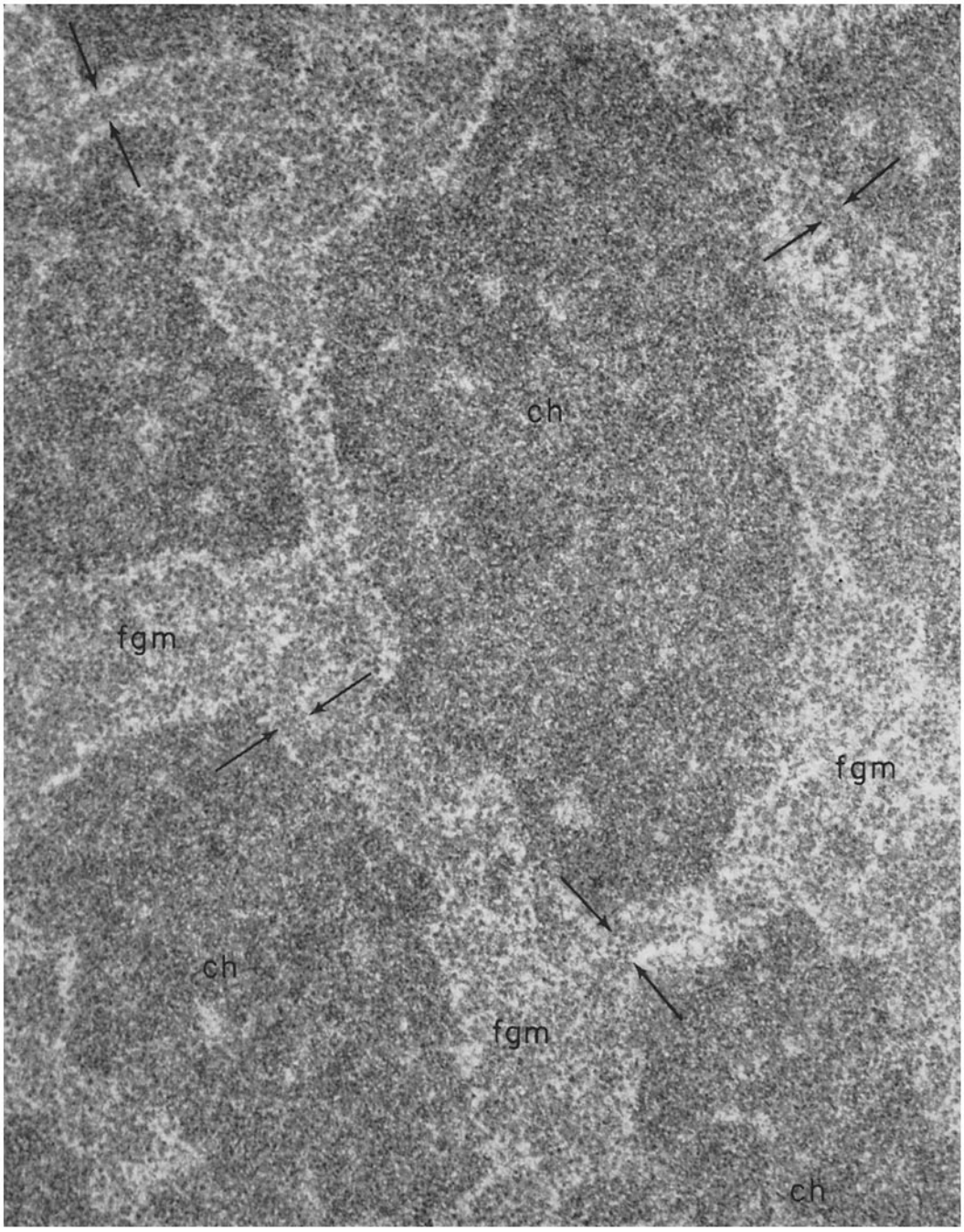


FIGURE 35

Micrograph of part of a midtelophase nucleus showing the enlarged interchromosomal spaces filled with fibrillogranular material (*fgm*) which in many places (arrows) is seen to be arranged into thread-like structures some 0.1 μ in diameter. Note that the chromosomes (*ch*) are quite dense except for a few tiny lighter areas within their mass. × 50,000.

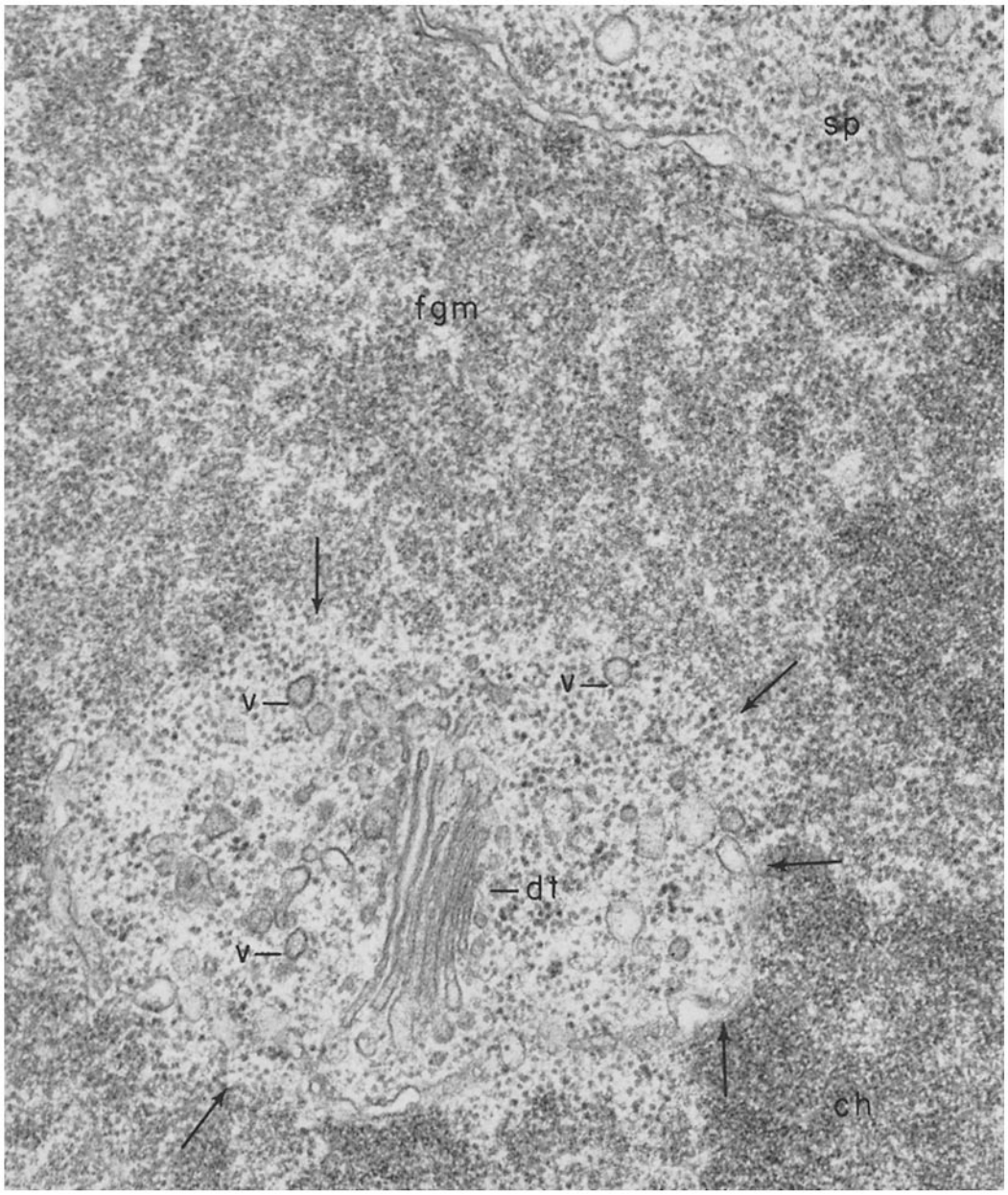


FIGURE 36

Portion of a midtelophase nucleus showing a circular area (arrows) partly circumscribed by a double-layered envelope and containing spindle remnant material. This area also shows, in addition to a dictyosome (*dt*), a number of roundish vesicles (*v*) and 150 A granules similar to those observed in the poleward region of the spindle (*sp*). × 54,000.

indistinguishable from that of the nucleoplasm at late prophase after nucleolar disintegration (Fig. 20). Slightly later on, the remnants of the nuclear envelope having dispersed, the central portion of the cell as well as the poleward region of the spindle are seen to contain, among other structural elements, granules and fibrils likewise indistinguishable from those found in the nucleus after nucleolar disintegration (Fig. 16). From these observations, the conclusion can hardly be avoided that at least part of both the granular and fibrillar components of the spindle are of nucleolar origin, the rest being evidently contributed by the cytoplasm.

Evidence for the Existence of Matrix Material within the Chromosomes

Evidence for the existence of a matrix material within late prophase, metaphase, and anaphase chromosomes has been obtained with both light and electron microscopes. Grazing sections of metaphase and anaphase (Fig. 19) chromosomes, examined under light microscopy, indicate the presence of a lightly stained matrix substance between their chromonemal gyres. Under electron microscopy, metaphase chromosomes in longitudinal sections appear homogeneously dense and of uniform fibrillar fine structure in spite of the fact that their wavy contours strongly suggest the presence of coiled chromonemata within their mass. It has been observed, moreover, that thin (0.25 μ) transverse sections of metaphase chromosomes (Fig. 17 and insert) clearly show a Feulgen-negative chromatid core, the presence of which is not at all obvious under electron microscopy. Corresponding electron micrographs (Fig. 22) reveal, on the contrary, that the central portion of each chromatid is homogeneously dense and, as far as can be judged, of a fine structure similar to that of the remaining portion of the chromatid. Cross-sections of anaphase chromosomes (Fig. 23) exhibit a structure similar to that just described for the metaphase chromatids except that, occasionally, a small light core may be observed in the electron microscope. These observations, therefore, are in complete agreement with the view that an achromatic matrix fills the central core as well as the space between the coiled chromonemata of metaphase and anaphase chromosomes (see reference 13). This matrix, it would now appear, is as dense as the chromonemata and of a similar fine texture. The metaphase chromatid core, it must be noted, appears much lighter after potassium permanganate–fixation (8) than it does in the present study. The seeming disappearance of some of the material from the central core of certain anaphase chromosomes is, perhaps, related to the often observed release of chromosomal material into the spindle at that stage (26, 28–32) or at early telophase (33). As already suggested (13), such a release may indicate that the matrix filling the central portion of the condensed chromosomes is more loosely bound than that which is associated with the chromonemata themselves.

It is not unlikely that the matrix substance discussed above becomes associated with the chromosomes during prophase (25–27). In our material, indeed, the late prophase chromosomes already show a compactness, under both phase (Fig. 12) and electron microscopy (Figs. 15 and 16), approaching that of the metaphase and anaphase chromosomes. The present study does not permit us, however, to draw any definitive conclusion concerning the origin of this matrix substance. It is conceivable, for instance, that some of this matrix substance represents nuclear sap material and/or fibrillar material of nucleolar origin which has become trapped within the late prophase chromosomes during the coiling process of the chromonemata. Another possibility would be that the matrix substance results from the accumulation of a newly synthesized material.

Mode of Reconstitution of the Nucleolus at Telophase

Although it is well established that telophase nucleoli form in specific sites on the so called nucleolar chromosomes (14, 34), it is not yet known whether such sites merely serve to collect nucleolar material dispersed elsewhere in the nucleus or whether they actually synthesize the nucleolar material. A number of workers have presented data suggesting that the nucleolar material first appears in the form of a coating or droplets on the surface of the telophase chromosomes and is subsequently simply collected at the nucleolar sites or zones (1, 4, 5, 14, 22, 34–38).

In this study the very earliest stages of nucleolus development at the secondary constriction have either not been observed or not recognized. This may indicate, perhaps, that early growth of the nucleolus is quite a rapid process or that such small nucleoli exhibit a texture indistinguishable from the surrounding fibrillogranular material. At any

rate, our observations suggest that growth of the nucleolus, at least from the time it is first recognizable as a formed body, results mainly from an incorporation of a material that accumulates in the interchromosomal spaces from early to midtelophase. The relevant observational evidence can be summarized as follows: (*a*) the growing nucleolus exhibits a metachromatic staining characteristic identical with that of the interchromosomal material (Figs. 29, 30, and 31) and under electron microscopy both are seen to contain similar granular and, as far as can be judged, fibrillar components (Figs. 37 and 39); (*b*) throughout its growth period the surface of the nucleolus always appears continuous with patches of this interchromosomal material (Figs. 37 to 39); (*c*) as the nucleolar mass enlarges there occurs a corresponding gradual decrease in the amount of interchromosomal material and, by very late telophase, the remnants of such material are observed in the immediate vicinity of, or adjacent to, the nucleolar surface (Figs. 31 and 38). The above observations and conclusions are difficult to reconcile, therefore, with Swift's recent claim (6) that the so called prenucleolar material has no connection whatsoever with the formation of the nucleolus at telophase.

The present study also shows that growth of the telophase nucleolus is more than a mere accumulation of the interchromosomal fibrillogranular threads ($\sim$ 0.1 μ) since, even in the relatively young nucleolus, the granular and fibrillar components of these threads are apparently already segregated into different zones. It is conceivable, then, that in *Vicia faba* the secondary nucleolar constriction also plays an important role in determining the organizational pattern of the growing telophase nucleolus. In this regard it should be recalled that earlier investigations have shown that the RNA content (39), and mass and size (40) of the mature nucleolus, at least, are influenced by the chromosomal nucleolar organizing regions.

Since the very beginning of nucleolus formation has not been studied, no information was obtained concerning the possible role played at that time by the low density fibrillar material filling the nucleolar secondary constrictions. With regard to this problem it is of interest to note that in *Vicia faba* a material, likewise observed within the nucleolar secondary constrictions as early as metaphase (41), has previously been interpreted (4) as indicating a precocious nucleolar material formation.

Origin of the Prenucleolar Material at Telophase

In the preceding section evidence has been presented suggesting that the bulk of the telophase nucleolus is derived from a fibrillogranular material which accumulates within the interchromosomal spaces during early and midtelophase. The question now arises as to the origin of this prenucleolar material. The present study, being essentially morphological in character, does not permit us to draw any definitive conclusions concerning this problem. It might be worthwhile, nevertheless, to reevaluate in the light of our own findings the hypotheses that have been formulated in relation with the origin of this prenucleolar material.

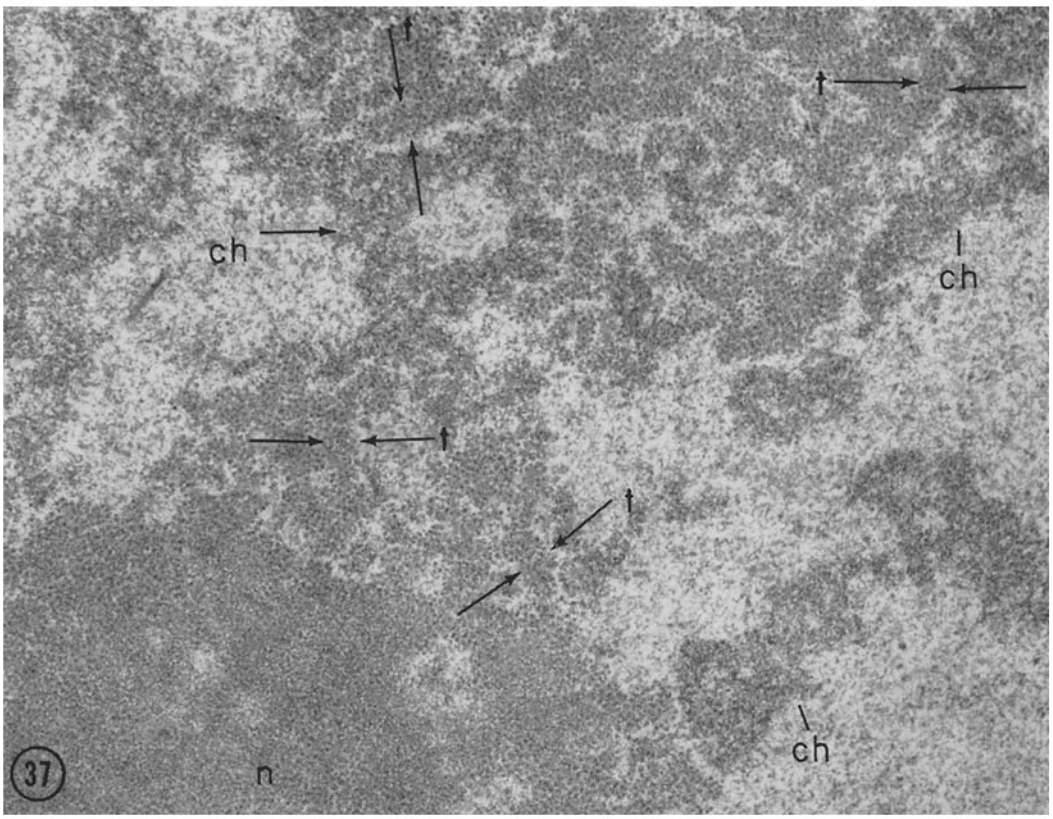
A first hypothesis, often sponsored in the past (1, 22), postulates that the bulk of the prenucleolar material is derived from the matrix of the

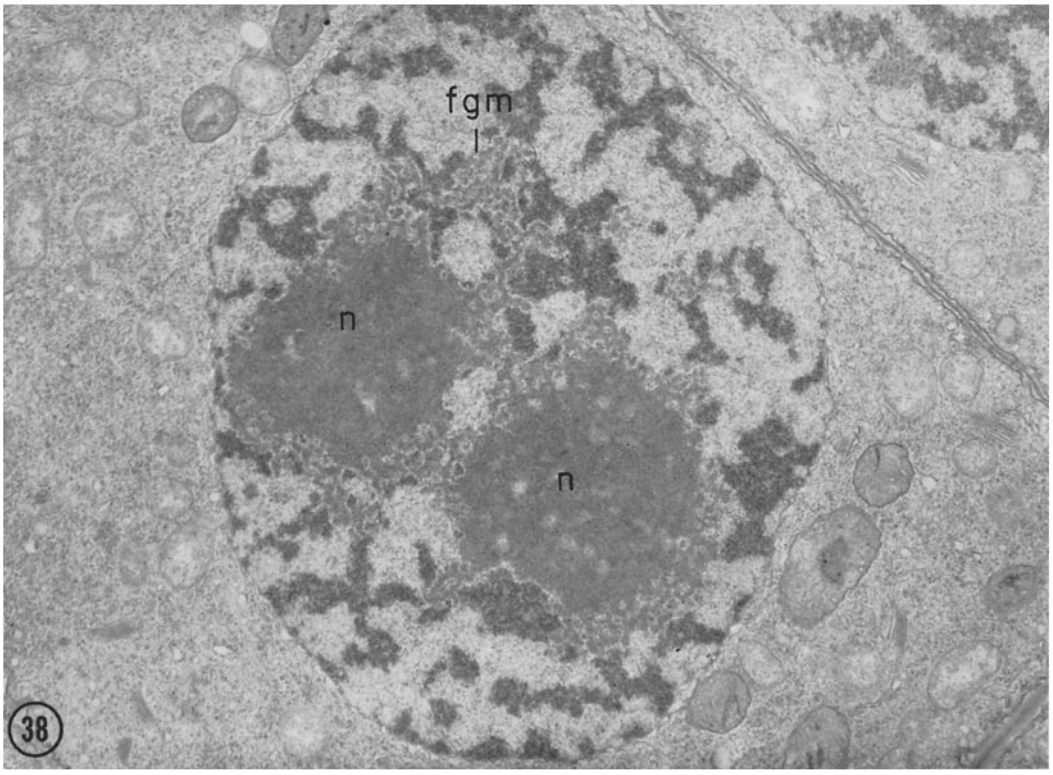
FIGURE 37

Electron micrograph of part of a midtelophase nucleus. The central portion of the small nucleolus (*n*) is quite dense and consists of granular and fibrillar elements, each grouped into irregularly shaped zones. The surface of the nucleolus, on the other hand, exhibits a fluffy appearance in places resulting from the presence of a network of fibrillogranular threads (*t*). A large patch of material, equally fluffy in appearance, and consisting of fibrillogranular threads as well as of segments of chromonemata (*ch*) can be seen in the vicinity of the forming nucleolus. × 30,000.

FIGURE 38

Electron micrograph of a post-telophase nucleus. The two nucleoli (*n*) are quite compact except for a number of small vacuole-like structures. The fibrillogranular material (*fgm*) is seen at that stage to be located adjacent to or in the vicinity of the nucleolar surface. × 9,500.


ch
ch
ch
n
37


fgm
n
n
38

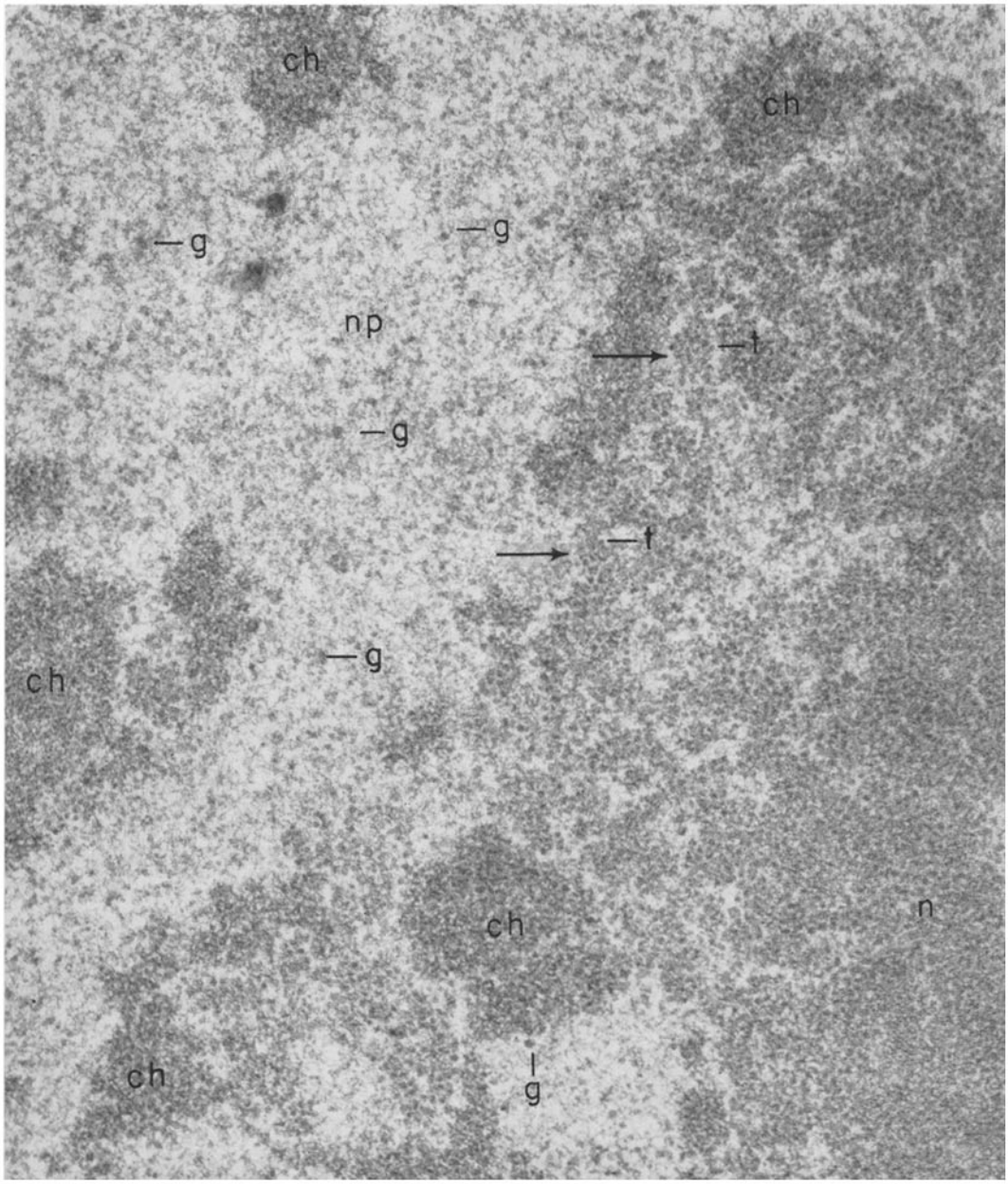


FIGURE 39

Higher magnification micrograph of portion of a post-telophase nucleus depicting the close relationship which exists between the nucleolus (*n*) and the surrounding fibrillogranular threads (*t*). The latter form the fluffy layer over the surface of the nucleolus and merge imperceptibly with the nucleolar mass proper. The nucleoplasm (*np*) is made up of loosely scattered fibrils as well as of a number of granules (*g*). The diameter and density of most of these granules correspond to those of the granules found within both the nucleolus and the thread-like structures. Chromosome, *ch*. × 50,000.

telophase chromosomes. Several of our observations are in disagreement with such an interpretation, at least as formulated. First, the amount of matrix material present within the early telophase chromosomes, in our opinion, would not be sufficient to account for the relatively large quantity of prenucleolar material that accumulates in the interchromosomal spaces from early to midtelophase. Moreover, the relatively small number of 150 A granules observed within the condensed early telophase chromosomes could not account for the large number of similar granules observed later in the prenucleolar material. Finally, during the period of accumulation of the prenucleolar material, the compactness of the chromosomes, as judged under both light and electron microscopy, does not change to the extent expected if most of this material was shed from the chromosomes.

If the bulk of the prenucleolar material does not originate from the chromosomal matrix as argued above, then the latter might be involved in the formation of the nucleoplasm. This possibility, at any rate, is suggested by the fact that the appearance of this nucleoplasm is more or less concomitant with the unraveling of the chromosomes during mid- and late telophase. That some of the granular components of the forming nucleoplasm are also derived from the prenucleolar material is not excluded.

Rattenbury and Serra's suggestion (4) that the perichromosomal plasm is responsible for the formation of most of the interchromosomal prenucleolar material is likewise difficult to reconcile with our observations on the reconstitution of the telophase nucleus. Indeed, at the time of the "polar clumping" of the early telophase chromosomes, only a relatively small amount of plasm of spindle or cytoplasmic origin is left within the narrow interchromosomal spaces where the prenucleolar material has already begun to accumulate. Furthermore, as the nuclear envelope completes its formation, the nuclear content, soon thereafter, becomes separated from the surrounding spindle and cytoplasmic elements. The possible significance, with respect to nucleolar formation, of the small amount of spindle material incorporated within the early telophase nucleus remains, however, to be determined.

Recent autoradiographic and microphotometric studies suggest a resumption of the synthesis of proteins and RNA within the telophase nucleus (32, 42, 43). Moreover, according to Prescott and Bender (43), this synthetic activity is already well under way before the nucleolus becomes visible within the telophase nucleus. In the light of these findings it might not be unreasonable to suppose that the prenucleolar material in *Vicia faba* originates mostly as a result of the synthetic activity of early and midtelophase chromosomes. The relevant observational evidence and considerations in favour of such a view could be summarized as follows: First, at the beginning of its formation, the prenucleolar material appears intimately associated with the surface of the chromosomes. Second, the prenucleolar material, which subsequently accumulates from early to midtelophase in the interchromosomal spaces, exhibits staining characteristics and a fine structure similar to that of the prenucleolar material first observed on the chromosomal surfaces. Third, a continued release of prenucleolar material synthesized within the chromosomes could, perhaps, account for both the relatively large amount of such material observed in the interchromosomal spaces and for the fact that the chromosomes remain quite compact during early and midtelophase. (Figs. 34 to 36).

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