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A CYTOCHEMICAL ELECTRON MICROSCOPIC STUDY OF GLANULOGENESIS IN NEUTROPHILS

PEROXIDASE AND DOPA-OXIDASE REACTIONS

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ABSTRACTS

The present study was undertaken to demonstrate, electron microscopically, a detailed localization of peroxidase and dopa-oxidase within the neutrophils of various maturities and further to correlate them with the development of cellular organelles and with the granulogenesis.

The bone marrow of rats were used as materials. Perdxidase and dopa-oxidase reactions, as seen through an electron microscope, localize in the granules, and these two stainings show similar distributions throughout the various developing stages of the neutrophil.

However, dopa-oxidase reaction never appear in Golgi region while peroxidase reaction stains either vesicles or cisternae in neutrophilie promyelocyte and myelocyte.

These two reactions show a distinct difference in intensity and localization of stainings in the granules between the specific and nonspecific granules in neutrophil. Particularly, wtih the peroxidase reaction, a discrimination is possible between the Golgi vesicle maturing to specific grannule and that maturing to nonspecific one.

The specific granules which do not stain with these two reactions appear for the first time in metamyelocyte and increase in number in more mature neutrophils.

INTRODUCTION

The rejuvenation of descriptive cytology using electron microscope was followed soon by an attempt initially made by Shelden and Btandes[1)2)] namely, application of enzyme cytochemical methods to the electron microscopy. With the introduction of the improved fixation technics devised by Holt *et al.*[3)] and Sabatini *et al.*[4)] further advances have been made possible and havc led to a recent rise of the electron microscopic enzyme cytochemistry.

So far, various cell types including hematopoietic cells have been studied with the use of enzyme cytochemical technics in combination with the electron

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microscopy[5)-10)]. Only several studies, however, have dealt with myeloid elements, especially granulocytic series[11)-15)]. Recently, granules of neutrophils have drawn much attention in conjunction with lysosomes[16)-17)], although the identity of these two structures is yet contraversial. In view of above, it would be desirable to study an exact localization of each of various enzymes within the neutrophil, particularly in relation to its granulogenesis.

At the level of the ordinary microscope, the real nature of peroxidase reaction in the neutrophil[18)-19)] first demonstrated by Fisehel[20)], has been much debated since the study by Agner[21)]. Meanwhile, Dopa-oxidase reaction, originally shown in melanocytes by Bloch[22)], is also known to take place in neutrophils. Bloch himself, however, in studying leukocyte oxidase believed that the reaction in the latter is due to a nonspecific oxidase and that the peroxidase staining can be replaced by the dopa-oxidase staining. Furthermore, Duijn[23)] has shown the similar inactivation pattern as in the peroxidase reaction in the dopa-oxidase reaction. Recently, Takikawa[24)] have shown, by separating various cellular organelles of horse neutrophils, that the Agner's verdoperoxidase activity resides in the mitochondrial fraction while the dopa-oxidase activity's in the granular fraction.

The present study was undertaken to demonstrate, electron micrcoscopically, a more detailed localization of peroxidase and dopa-oxidase within the neurtophils of various maturities and further to correlate them with the development of cellular organelles and particularly with the granulogenesis.

MATERIALS AND METHODS

Five normal adult rats of the Donryu strain were used as a source of the bone marrow. The rats were anesthetized with chloroform and their femurs excised. The bone marrow of the femur was extruded *in toto* into chilled 3% glutaraldehyde solution buffered at pH 7.4 with cacodylate and cut into pieces smaller than 1 mm^3 and then prefixed in the solution at 0-4°C for 30 minutes. Prior to the prefixation, some of the bone marrow was first extruded into pure methanol and kept in it for 5 minutes to eliminate both the peroxidase and the dopa-oxidase activities. All of the prefixcd tissue pieces were washed in the 0-4°C chilled cacodylate buffer solution (pH 7.4) containing 9% sucrose, the medium renewed 2 to 5 times every 15 minutes and then subjected to the following and sham procedures.

Peroxidase staining was perfomed by incubating the tissue pieces for 3 minutes in the saturated benzidine solution to which 3 drops of 3% peroxide per 100 ml of the solution were added just before use. Following the incubation the tissue pieces were washed in the cacodylate buffer solution by changing the medium twice at 30 minute-intervals, and then post-fixed in Millonig's sol-

ution[25,26] for 90 minutes. A control staining was carried out without benzidine.

Dopa-oxidase staining was performed according to the Bloch's method[22] and was followed by the same washing and postfixation procedures as in the peroxidase staining. As for the control, a few tissue pieces underwent solely the washing and the post-fixation procedure.

Inactivation tests were carried out by observing each of the same staining procedures as described above on the tissue pieces that had been pretreated with pure methanol prior to the glutaraldehyde prefixation.

After dehydration in a series of graded alcohols, all the tissue pieces were embedded in Epon (epoxy resin) and cut with the JUM-5-Type ultramicrotome. Ultrathin sections were either stained with uranyl acetate alone or doubly stained with uranyl acetate and then lead acetate. For viewing observation and photography taking, a JEM-5C-Type electron microscope was used. The micrographs were taken on films or plates at the original magnification of 4,000 to 10,000 and printed using projection enlargement.

RESULTS

General cytology in routine sections

Myeloblast: The myeloblast that is identified as the most immature myeloid cell in the bone marrow, is round or slightly elongated and is relatively small, being smaller than 10 μ in the longer axis. This cell is characterized by an ovoid nucleus with a diffuse nucleoplasm of nearly uniform density distribution, prominent nucleoli and the sparse cytoplasm lacking granules but containing abundant free ribosomes and numerous mitochondria, both evenly distributed. A few, usually 2 to 3, rough surfaced endoplasmic reticulums and a small Golgi complex may be seen in the cytoplasm.

Newtrophilic promyelocyte: The neutrophilic promyelocyte, the earliest developing stage of the neutrophil from the myeloblast, is easily identified by the presence of the granular structures in the cytoplasm, which is not seen in the earlier stage. The cell measures 10 to 16 μ in diameter and has a round nucleus though tend to be more irregularly shaped with the coarser nucleoplasm and less prominent nucleoli. The state of the nucleoplasm will distinguish an early promyelocyte from a later one: in the early promyelocyte, the nucleoplasm still appears diffuse while in the later promyelocyte, the coarsening of the nucleoplasm becomes apparent and condensation of chromatin takes place around the periphery of the nucleus. The nucleolar structure appears to fade in the latter. Generally, an overall amount of the cytoplasm appears to increase as the rough surfaced endoplasmic reticulum becomes more numerous, the Golgi complex enlarges and the granular structures accumulate in the cytoplasm. The ribosomes, though still abundant in the cytoplasm, are more diffusely distributed and the

mitochondria becomes progressively less in number through these stages.

The granular structures appearing in the neutrophilic promyelocyte are mostly round but some are elongated, varying in size from 90 to 550 mμ in diameter. They are composed of a granular structures of varying electron densities and a limiting membrane enclosing them. According to their electron density and the presence or absence of an electron lucent rim between the grannular content and the limiting membrane, two types of granules are identified. Those with the rim appear to have a less dense content and predominate in the early promyelocyte stage, while the other, without the rim, appear the denser and to increase in the later promyelocyte stage while the former by described in number diminishes. Generally the former is larger measuring 400 to 500 mμ in diameter while the later measuring 90 to 500 mμ. Both types of granules seem to be formed in the Golgi region (Fig. 1, 2). However, no clear evidence is available as to many possible interrelations between the site of the granule formation and a structural difference of the granules. Although it is never clearly demonstrated whether one type of the granule transforms to the other, it is often observed that the material being discharged from the granule having an electron lucent rim is forming a structure quite similar to the granule without the rim (Fig. 3, 4) Another interesting observation is that the granule somtimes has a protuberance which looks like another granule budding. (Fig. 13).

Neutrophilic myelocyte: The neutrophile myelocyte stage is characterized by the predominance of the rim-lacking-granule and notable nuclear indentation. The cell measures 9 to 12 μ in diameter. The nucleoplasm shows progressive condensation and clumping of chromatin. Although the ribosomes are still abundant in the cytoplasm, the endoplasmic reticulums and the mitochondria are less numerous as compared with the promyelocyte stage. The Golgi comlex, though appears still active, becomes smaller in this stage. Most of the granules seen in this stage are those lacking the rim though some having the rim are still present. Both types of granules appear to arise in and from the vesicles and cisternae of the Golgi complex (Fig. 5).

Neutrophilic metayelocyte and band form: With increase of the nuclear indentation, resulting in sausage-shape or band form, the neutrophilic metamyelocyte and band form are identified. The cells in these stages measure 7 to 8 μ in diameter. Except for the nuclear shape, both types of cells have common features as to the nuclear as well as the cytoplasmic structure. The nuclear outline is sharp, the chromatin coarse, and the nucleoli are no longer visible. The ribosomes and endoplasmic reticulums are apparently reduced in numbers together with the concomitant abundant cytoplasimic granulation and also few mitochondria are seen at these stages. The Golgi compex, though still visible, new appears inactive. The abundant cytoplasmic granulation is almost entiely

made up of the granules which do not have the electron lucent rim between the granular content and the limiting membrane. They vary in size from 90 to 500 mμ; and those smaller than 100 mμ in diameter appear to be more numerous in the periphery of the cytoplasm.

Segmented neutrophil: This fully developed cell is characterized by the segmented nucleus with the heavy peripheral condensation of the chromatin in the nuclear lobes. The diameter of the cell measures 7 to 8 μ. The Golgi region is barely identifiable only by the presence of a smaller complex ring of vesicles, at the same time and the mitochondria are rarely seen in this stage. The rough surfaced endoplasmic reticulums now appearing smaller than in earlier developmental stages are less numerous. All but one or two granules are those lacking the electron lucent rim and a close relation of the granules to the Golgi complex is hardly observable.

Cytoplasmic granulation on ordinary and electron microscopy

In a routine smear preparation of rat bone marrow, stained with MayGrünwald-Giemsa stain, immature neutrophils contain two types of granules which differ from each other in their stainability: the one is azurophilic, and another neutrophilic, as is so in the human neutrophils. The azurophilic granules are called nonspecific granules since they are also gresent in the other series of granulocytes, while the neutrophilic granules that define the neutrophil are called specific granules.

In order to identify the two types of granules observed in electron micrographs of the rat bone marrow neutrophils, parallel observations were made on the cytoplasmic granulation in the cells of various maturities by the ordinary and the electron microscopy.

Immature neutrophils of the rat bone marrow, when viewed by electron microscopy, also contain two different types of granules with respect to the size, the shape, the electron density, and the fine structure as described above. The behavier of two types of granules are also different with respect to the change in their numbers through the maturation stages. Those relatively larger (400 to 550 mμ in diameter) and less dense, granules with an electron lucent rim between the matrix and the limiting membrane, apear first and predominate on the early promyelocyte stage and there after decrease in number through the later promyelocyte and myelocyte stages, nearly disappearing in the metamyelocyte stage. Thus, the wax and wane of this type of granules exactly coincides with that of azurophilic (nonspecific) granules seen in a routine smear preparation.

On the other hand, those granules devoiding of the rim, relatively small (90 to 500 mμ in diameter) and denser, appear in the early promyelocyte stage and steadily increase in number until the cell matures fully. Thus, this type

of granules apparently behave as the neutrophilic (specific) granules.

From these parallelisms evidenced on observations using the ordinery and electron microscope, it can be said that the granule with the electron lucent rim corresponds to the nonspecific (azurophilic) granule and those without the rim, the specific (neutrophilic) granule.

These observations are essentially identical to those on the human neutrophils.

Electron microscopy of neutrophils stained for peroxidase

The final reaction product of peroxidase activity, revealed by electron microscopy, is identified as spot-like deposits of varying electron densities at a lower magnifiction. When visualized at a higher magnification, however, the spot-like deposit is identified as an aggregate of numerous microparticles of high electron density. Density of a deposit that apparently depends on a degree of aggregation of the electron dense microparticles is considered to indicate the presence of an activity of peroxidase there.

As will be detailed in the followings, the reaction product of peroxidase activity is mainly localized in most or a portion of granules in maturing as well as segmented neutrophils and also to a part of Golgi region of the earlier developing cells. There are, in general, two types observed with respect to the mode of the appearance of the reaction product in granules: in the granules with an electron lucent rim between matrix and limiting membrane (nonspecific granules), the reaction product diffusely covers the content; while, in the granules without the rim (specific granules), the reaction product heavily marks the perpihery of the granules leaving a core unmarked or slightly marked. As juged by an electron density of the deposit, the specific granule, in general, appears to have more afiinity to peroxidase staining than the nonspecific grannle does. (Fig. 6). In control preparations, either sham stained or inactivated for peroxidase, no reaction product is observed.

Myeloblast: None of the cell organelles of the myeloblast is reactive at all.

Neutrophilic promyelocyte: In the early promyelocyte, the majority of the granules is marked by the reaction product, but some of the nonspecific granules are unreactive. The vesicles of the Golgi complex are occasionally unreactive or weakly reactive (Fig. 7).

In the later promyelocyte, almost all of the granules of both types are reactive though the reaction of the each granule varies in its mode and intensity. as described above. The vesicles of the Golgi complex exhibit peroxidase activity in most of the later promyelocytes examined, whereas only the inner layer of the cisternae is occasionally reactive. The reaction product in the vesicles of the Golgi comlex appears in various fashions: it appears only along the outer surface of some vesicles or only inside of other vesicles, or else it

covers the entire vesicle (Fig. 8).

Neutrophilic myelocyte: In the myelocyte, as in the later promyelocyte, almost all the granules are reactive and most of them are the specific granules which exhibit peroxidase activity only in their peripheral portion (Fig. 9), although small number of nonspecific granules which are diffusely reactive except for the rim are stil present. It is noteworthy that in this stage are there a few granules which appear as transitional forms, from nonspecific to specific granules, in appearance of their peroxidase activity (Fig. 10). The majority of the cells in this stage reveal peroxidase activity diffusely over the Golgi vesicles, and the number of the cells exhibiting the activity inside the Golgi vesicles are apparently less (Fig. 11). In a few myelocytes the inner layer of the Golgi cisternae is partially reactive (Fig. 12).

Neutrophilic metamyelocyte and band form: Besides those peroxidase positive granules present in the cells of these two stages, numerous small granules newly appear in the cytoplasm, mostly in its periphery, as many as are seen in the earlier developing stages. They are all smaller than 100 mμ in diameter and are apparently negative for peroxicase activity. These peroxidase negative granules are considered to be belonging to the specific granules since they develop only after the metamyelocyte stage and increase in number till the cell fully matures. In these two stages, the Golgi region is seldom reactive except for a small portion of membrane of a few Golgi vesicles (Fig 14).

Segmened neutrphil: In the segmented neutrophil, the peroxidase positive specific granule apparently decreases and the negative specific granule increases in number. Consequently, the number of the negative granule becomes as many as or surpasses the number of the positive granule. These negative specific granules, however, now include a considerable number of large granules, besides those small ones which have been present since the metamyelocyte stage.

Any part of the Golgi region is no longer reactive in this stage (Fig. 15). However, on some electon micrographs there is a finding suggesting that peroxidase-negative granules originated from Golgi complex (Fig. 16).

Electron microscopy of neutrophils stained for dopa-oxidase

When observed with electron microscopy, the presence of the final reaction product of dopa-oxidase activity *i.e.*, melanin, is indicated by a notably augmentated electron density of varying degrees. As compared with the peroxidase staining in which the final reaction product is clearly recongnizable as an aggregate of microparticles with a high electron density, the dopa-oxidase staining has disadvantages: it is often difficult, when specimens are stained with uranyl acetate alone, to distngiuish some weakly reactive granules from absolutely unreactive ones. This disadvantage is, however, overcome by double-staining using uranyl acetate and lead acetate. Another disadvantage of the dopa-oxidase

staining is the disappearance of the elecrton lucent rim between the granular content and the limiting membrane; the presence or the absence of the rim has been a cardinal differance between two types of granules seen in sections stained for peroxidase activity as well as those not stained as described above. Therefore, in this staining, dopa-oxidase positive granules are typed according to the appearance of the reaction product within a granule; namely Type I granule is the one which exhibits dopa-oxidase activity only in a peripheral part of the granule; Type II granule showing the activity only in the central core; and Type III granule which exhibit the activity evenly distributed over an entire granule. (Fig. 17). With this dopa-oxdidase staining, no other part but granules are marked by the reaction product. This is an apperant distinction between the peroxidase and dopa-oxidase stainings: the former may stain a part of Golgi region but the latter will never do so.

Judged by an augmentation of electron density, in general, more of the dopa-oxidase activity, as appears when the activity localizes in either the periphery (as in Type I) or in the central core (as in Type II) of a granule and much less when the activity spreads evenly over the entire granule (as in Type III).

As will be described later, the majority of the dopa-oxidase positive granules consist of either Type I, Type II, or of both granules, depending on a maturation stage; which Type III granules are rather component throughout maturation stages.

There is no evidence indicating of transfiguration between three types of granules.

Myeloblast: The myeloblast is totally negative for dopa-oxidase activity since there are absolutely no granular structures in this cell.

Neutrophilic promyelocyte: In the early promyelocyte, the Type II granule predominates and only a few of the Type I granules are present (Fig. 18). In the later promyelocyte, all granules are positive for dopa-oxidase activity. About 3/4 to 4/5 of the granules seen in this stage are of Type II, the rest consisting of Type I and III granules (Fig. 19).

Neutrophilic myelocyte: As in the later promyelocyte stage, all the granules present in this stage exhibit the dopa-oxidase activity. A notable distinction between the two stages, however, is that the majority of the granules in the myelocyte are shared fifty-fifty to eight to one ratio with Type I and II granules while the granules in the later promyelocyte mainly consist of Type II granules. This transition is due to a change in proportion of the number of Type I and II granules. (Fig. 20).

Neutrophilic metamyelocyte and band form: In these two stages, the cells contain smaller dopa-oxidase negative granules, intermingled with the positive granules, notably in periphery of the cytoplasm. Although the total number of the positive granules remains unaltered through cut these, a further increase

in the number of Type I granules takes place together witn a decrease in Type II granule number. (Fig. 21).

Segmented neutrophil: as to the gegmented neutrophil, about a half of the granules are negative for dopa-toxidase activity. These neutrophils at this stage contain a certain number of larger granules besides those small ones which have been increasing in number since the previous stage. Dopa oxidase positive granules now consist of a large number of Type I granules and a small number of Type III granules. Type II granules are rarely seen in this stage.

Identificatiou of dopa-oxidase positive granules and negative granules

As stated in the preceding sections, presence or absence of an electron lucent rim between the granular content and the limiting membrane of a granule is the point of distinction between the nonspecific and the specific granules in the neutrophils stained for peroxidase as well as those not stained. In neutrophils stained for dopa-oxidase, however, this distinction has been lost. In order to identify nonspecific and specific granules in the neutrophils stained for dopa-oxidase, dopa-oxidase positive granules were tentatively classified into three types as previously described and their rise and fall through maturation stages were separately followed and compared with those of nonspecific and specific granules observed in routine sections.

The Type I granule which measures 100 to 500 mμ in diameter appears in a small number through the early and later promyelooyte stages, notably increasing in the myelocyte stage and becoming the predominat granule throughout the subsequent stages.

The Type II granule which measures 400 to 550 mμ in diameter develops in the early promyelocyte stage, reaching to the maximum number in the later promyelocyte and begins to decrease in the myelocyte stage, almost disappearing thereafter.

The Type III granule measuring around 100 mμ in diameter is present as a minority in every maturation stage except the myeloblast stage with out notably change in number throughout the stages.

The dopa-oxidase ngative granules appear only after the metayelocyte stage and increase gradually until the cell fully matures.

The above observations make it evident that the Type II granule is nonspecific one and the Type I, Type III, and the negative granules being specific.

DISCUSSION

The present observation on positive peroxidase reaction in the Golgi region of the rat neutrophils is well in agreement with the observation made by Yamada and Yamauchi[15] on the rat eosinophils, but disagrees with that of Enomoto

and Kitani[14] on the human neutrophils. Whether this discrepancy is due to a species difference remains to be elucidated.

Of most significance is, in the present study, the observation that the localization of peroxidase activity is different from that of dopa-oxidase activity. They differ frem each other with respect to their mode of appearance within a granule as well as to their presence or absence in the Golgi region of certain immature neutrophils. Obtained observation clearly points to a basic difference between peroxidase and dopa-oxidase reactions, both of which have been considered to be of the same meaning and thus to be interchangeable in studying leukocyte granules[22)23)].

Many studies have so far suggested from a purely morphological basis that the Golgi complex plays a definite role in the granule formation[27)-30)]. In any of those studies, however, a solid evidence is lacking and a more precise site where granule formation initiates remains nearly conjectural. Only with the use of peroxidase reaction in combination with electron microscopy is it possible to study the granulogenesis in the neutrophil by tracing back to the very initial site of granule formation. In the present study it is clearly demonstrated that an embryonic form of both nonspecific and specific gronules first appears in a part of Golgi cisterna, subsequently emerging as a minute vesicle-like structure as is called a nascent by Hirsh[31)] and then is transformed into a fully developed granule. Furthermore, the observation that the presence of predominant small vesicle like structures with peroxidase negative rim coincided with an active formation of nonspecific granules in the promyelocyte stage while the presence of those without peroxidase gegative rim corresponded with a nearly selective production of specific granules in the myelocyte stage strongly supported the general concept proposed by Bainton and Farquher[30)] of functional specialization of Golgi complex in granulogenesis.

As to the fate of the nonspecific granules, there have been two views: one that the nonspecific granule will degenerate by extruding its content[33)]; another that the nonspecific granule will be transformed into the specific granule[33)23)34)]. In the present study, however, both views were equally but only partially supported, yet neither of them could be fully evidenced.

Recently, on the human neutrophil Kondo *et al.*[35)] have shown that there are two types of specific granules and that they are structurally different from each other. In the present study is it also clearly shown that there are tow types of specific granules in the rat netrophil, *i.e.*, peroxidase or dopa-oxidase positive granules and negative granules, although whether the identical granules are either positive or negative for both enzymes is not clear. However, no evidence is available for any possible transformation between these two granules.

The neutrophilic granules are heterogeneous from the morphological as well as from the cytochemical (peroxidase and dopa-oxidase) points of view. This

concept seems to be acceptable to the other cytochemical components. Weitzel *et al.*[13] reported by an electron microscopical study of rabbit neutrophils (heterophils) stained with acid phosphatase reaction, that non-specific granules correspond to the lysosomes. our study on human and horse neutrophils showed that the specific as well as in the non-specific granules are the seat of acidphosphatase reaction. The present findings on the neutrophilic granulogenesis and the transition of these granules indicate again complexity of chemical composition of the granules in neutrophil.

CONCIUSION

1. In rat neutrophil, peroxidase and dopa-oxidase reactions, as seen through an electronmicroscope, localize in the granules, and these two stainings show similar distributions throughout the various developing stages of the neutrophil. However, dopa-oxidase reaction never appear in Golgi region while peroxidase reaction stains either vesicles or cisternae in neutrophilic promyelocyte and myelocyte.

2. These two reactions show a distinct difference in intensity and localization of the stainings in the granules between the specific and non-specific granules in neutrophil. With the peroxidase reaction, a few transitional figures from the non-specific to the specific granules are found, and with this staining a discrimination between the Golgi vesicle maturing to specific granule and that maturing to nonspecific one is possible

3. The specific granules which do not stain with these two reactions appear for the first time in metamyelocyte and increase in number in more mature neutrophils.

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LEGENDS FOR FIGURES

FIGURES 1- 5. Electron micrographs of rat bone mrrrow neutrophils stained with uranyl acetate alone.

FIGURES 6-16. Electron micrographs of rat bone marrow neutrophils incubated for demonstration of peroxidase activity and stained with uranyl acetate.

FIGURES 17-21. Electron micrographs of rat bone marrow neutrophils incubated for demonstration of dopa-oxidase activity and double-stained with uranyl acetate and lead acetate.

FIG. 1. A promyelocyte showing many nonspecific granules and a few specific granues. Note the presence of abundant mitochondria, ribosomes, and well developed endoplasmic retculums. × 9000

FIG. 2. A higher magnification photograph of well developed Golgi region of the promyelocyte shown in Fig. 1 indicating a close interrelationship between granuls and Golgi vesicles. Note nonspecific granules (A) and specific granules (S). × 23400

FIG. 3 and 4. A part of an early promyelocyte showing a granule-like structure budding from the surface of a nonspecific granules. Fig. 3: × 39900. Fig. 4: × 44000.

FIG. 5. A myelocytes containing many specific granules (S) and a several nonspecific granules (A). Note a smaller Golgi region, a fewer mitochondria, ribosomes, and less developed endoplasmic reticulums, as compared with the early promyelocyte shown in Fig. 1. × 14600

FIG. 6. Neutrophil granules showing peroxidase positive granules: nonspecific granules (A) and specific grauules (S). Note the difference in localization and intensity

of peroxidase activity between two types of granules. × 29500

FIG. 7. An early promyelocytes showing peroxidase granules: nonspecific granules (A) and specific granules (S). × 9800

FIG. 8. A part of Golgi region of a later promyelocyte showing peroxidase positive nonspecific granules together with peroxidase positive Golgi vesicle. × 26400

FIG. 9. A myelocyte in a relatively earlier stage showing predominant specific grauules positive for peroxidase reaction. Endoplasmic reticulums are still well recognizable. × 13400

FIG. 10. A myelocyte in a relatively earlier stage containing a few peroxidase positive granules (M) which appear as transitional form from nonspecific to specific granules. × 37000

FIG. 11. A part of Golgi region of a typical myelocyte showing peroxidase positive vesicles. × 26400

FIG. 12. A part of Golgi region of a myelocyte showing cisterna partly positive for peroxidase reaction. × 30000

FIG. 13. A peroxidase positive specific granule extruding its content. × 48000

FIG. 14. A band form neutrophil containing peroxidase negative granules of around 100 mμ diamenter, intermingled with peroxidase positive granules. × 12500

FIG. 15. A segmented neutrophil showing a surpassing number of peroxidase negative specific granules (N) against the positive specific granules (S). The negative granules in this stage are mostly larger than those in the preceeding stage. Golgi region is entirely negative for peroxidase reaction. × 12800

FIG. 16. A higher magnification photograph of Golgi region of the segmented neutrophil shown in Fig. 15 indicating a close interrelationship between peroxidase negative and Golgi complex. × 50000

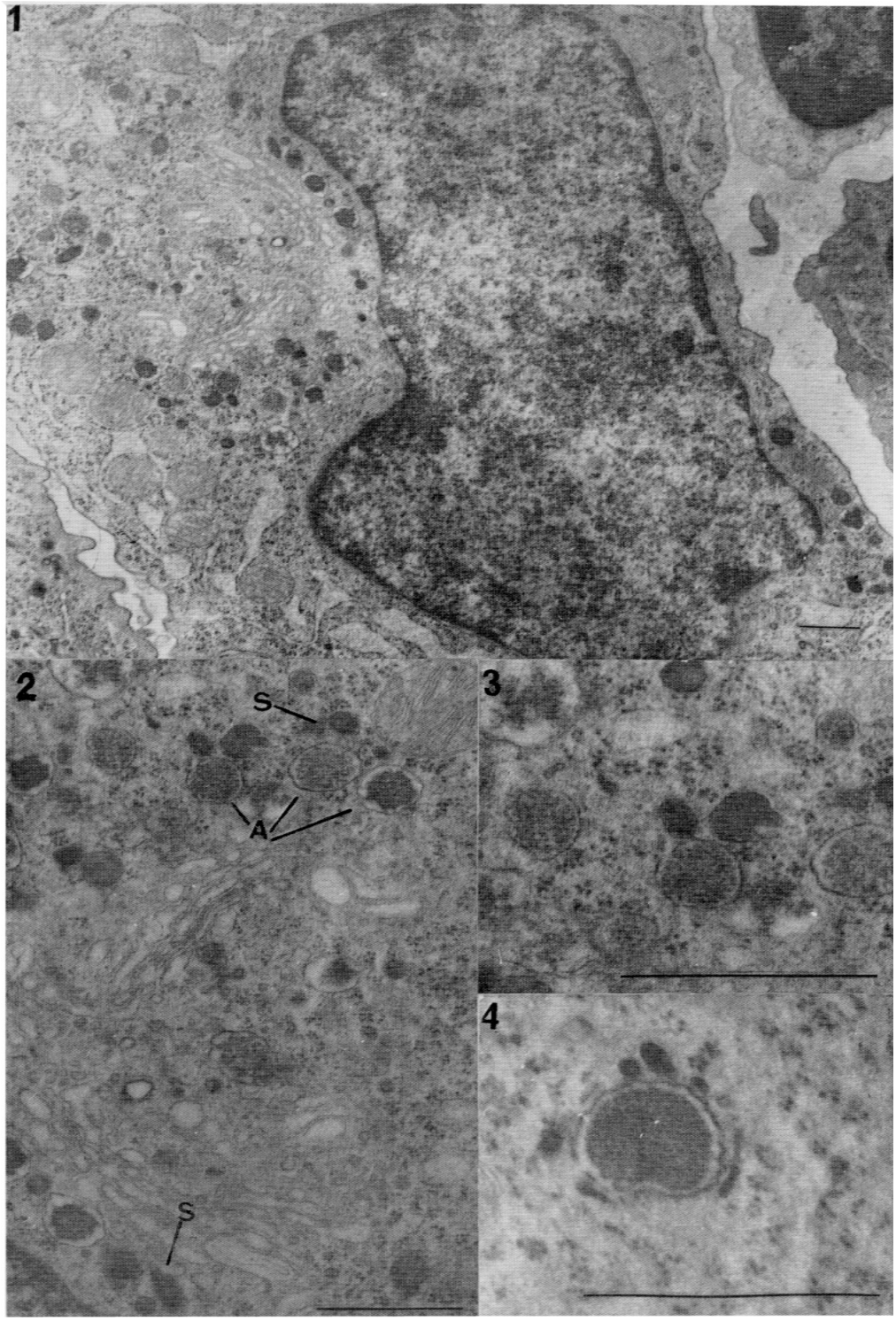
FIG. 17. A higher magnification photograph of an early promyelocyte showing three types of granules as revealed by dopa-oxidase reaction: Type I, Type II and Type III granules (see text). × 42600

FIG. 18. An eearly promyelocyte containing many Type II (nonspecific granules. × 14900

FIG. 19. A later promyelocyte showing many Type II (nonspecific) granules and a few Type I and II (specific) granules. Note unreactive Golgi region. × 12100

FIG. 20. A myelocyte containing a surpassing number of Type I (specific) granules. No dopa-oxidase activity in Golgi region. × 12100

FIG. 21. A metamyelocyte showing the presence of dopa-oxidase negative small granules intermingled with the positive Type I (specific) granules. × 12500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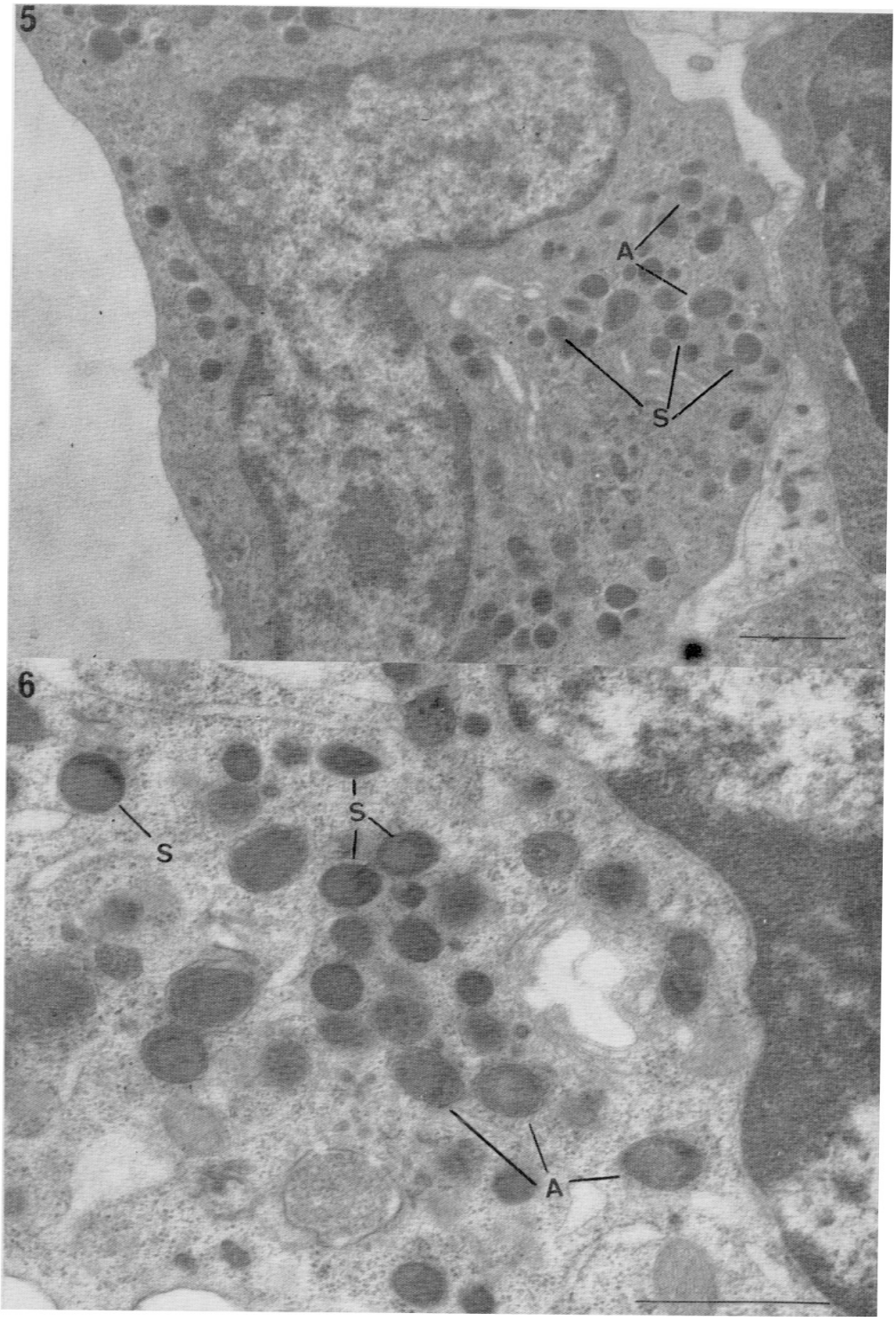
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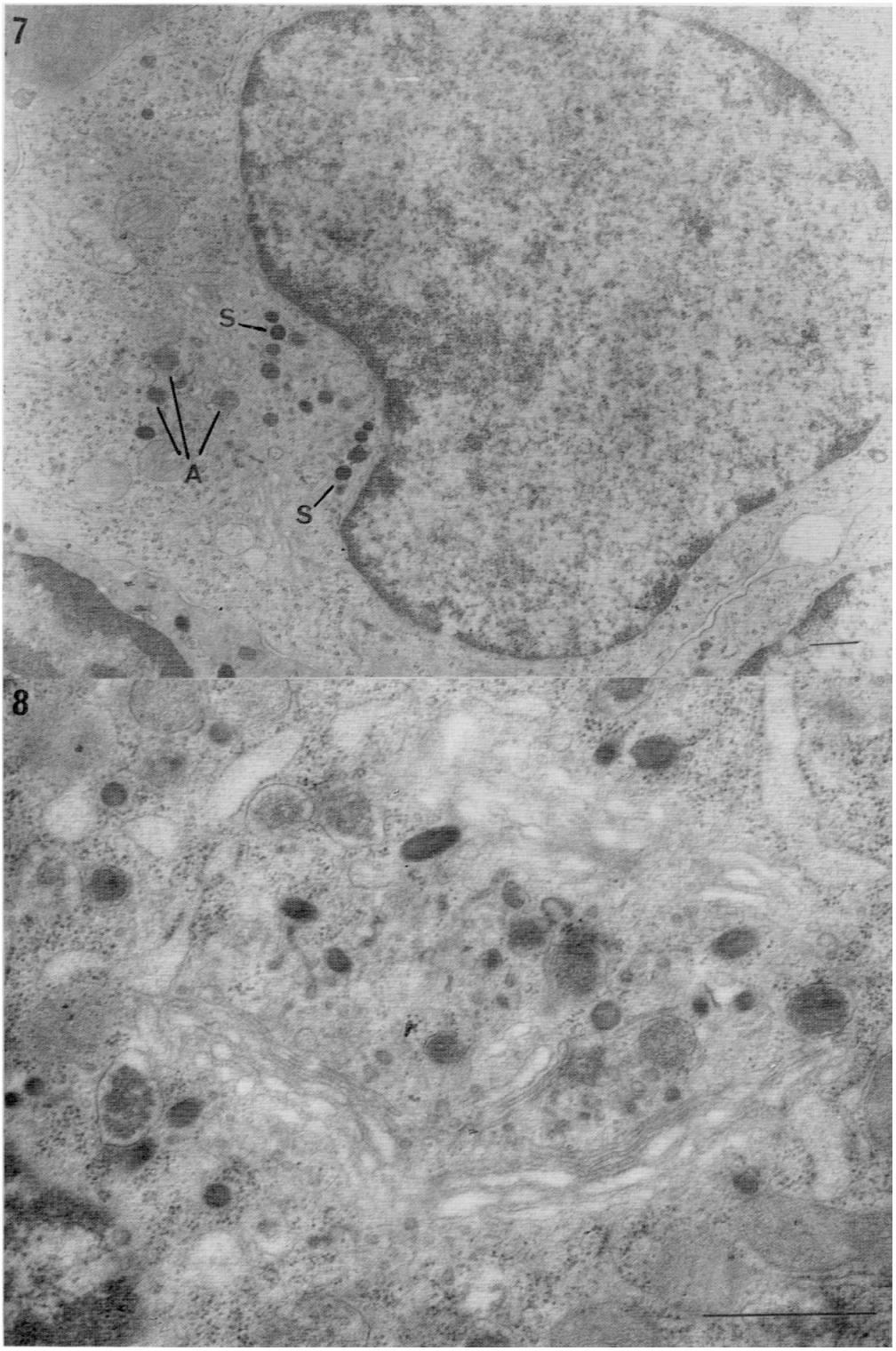
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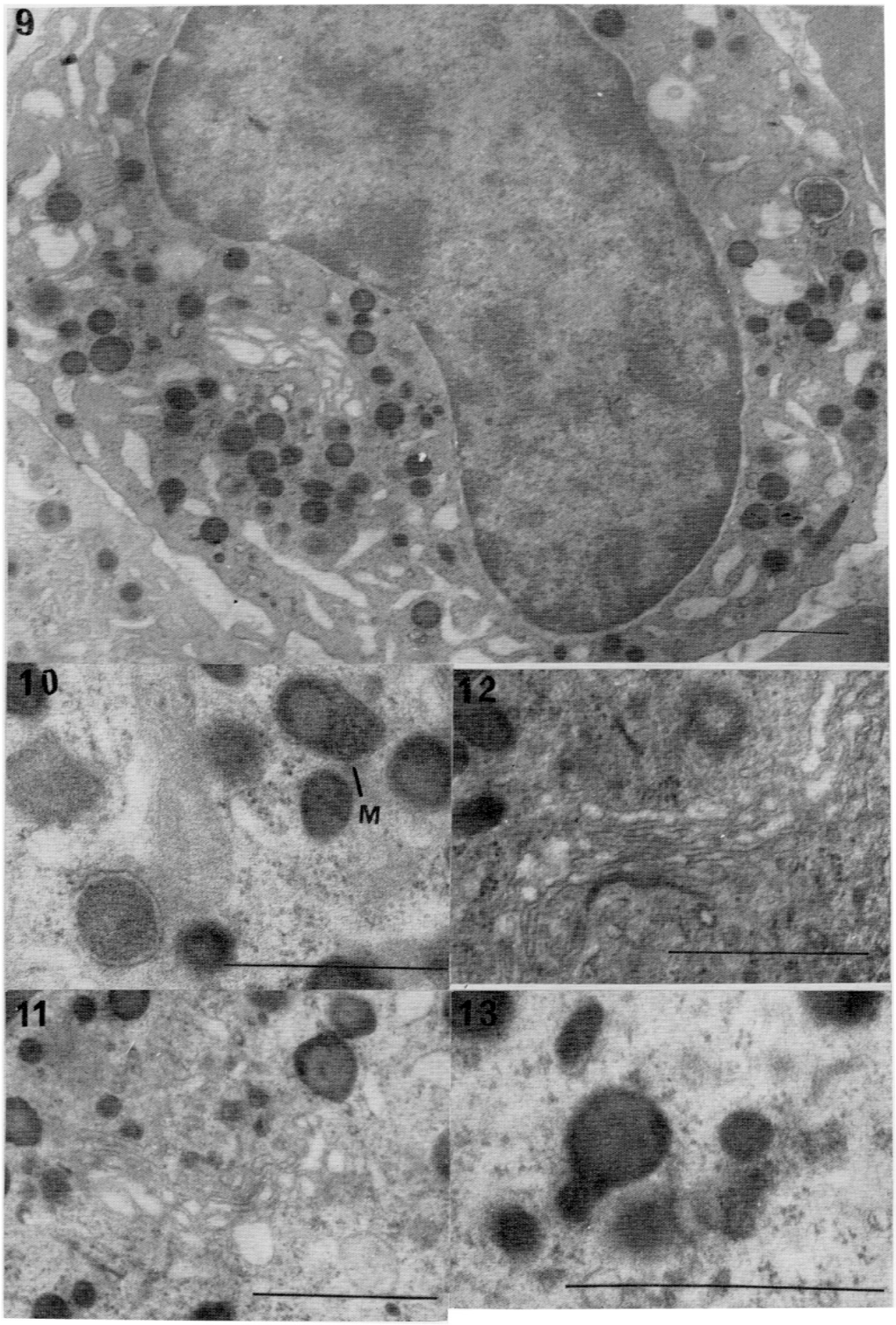
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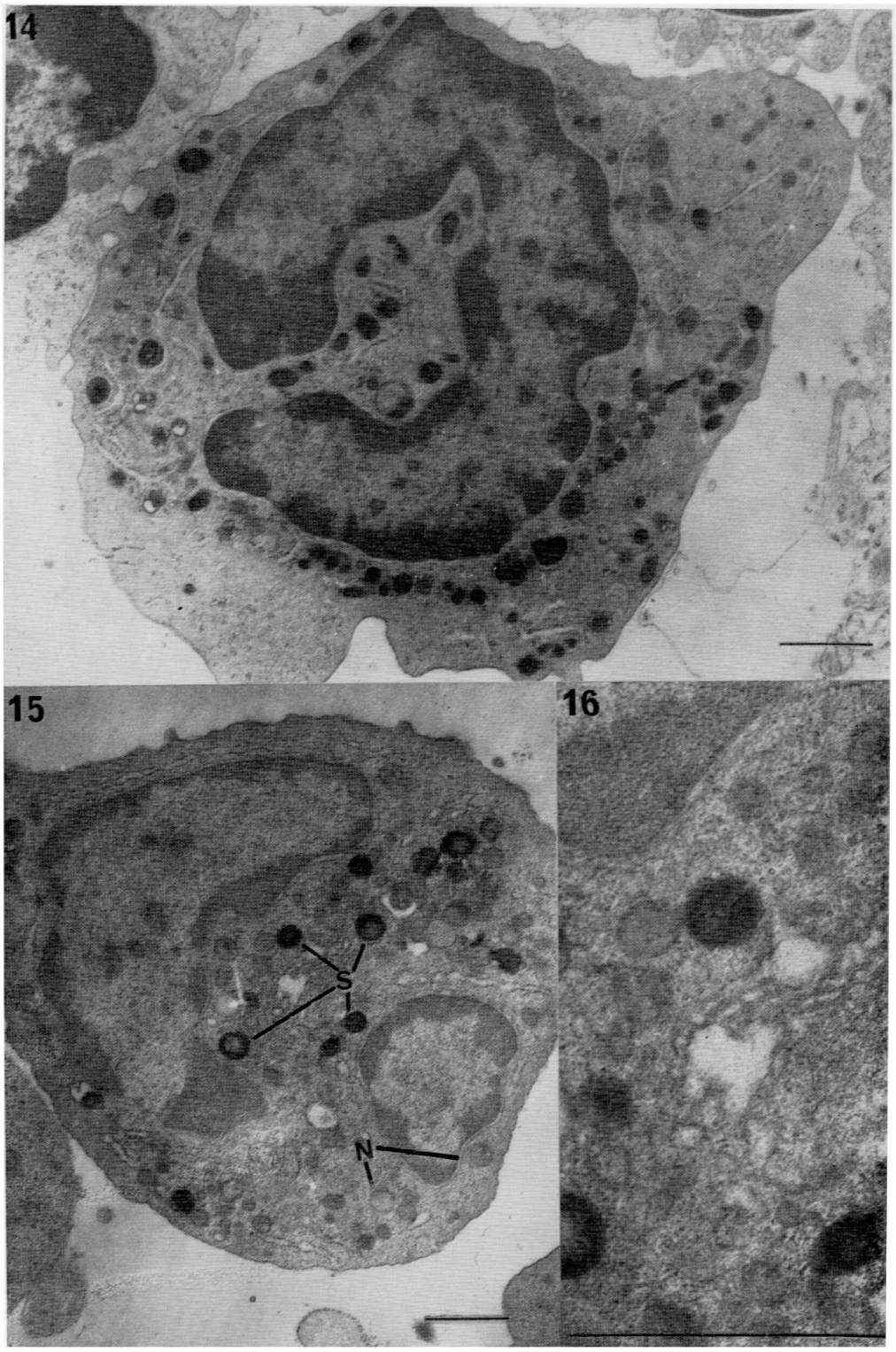
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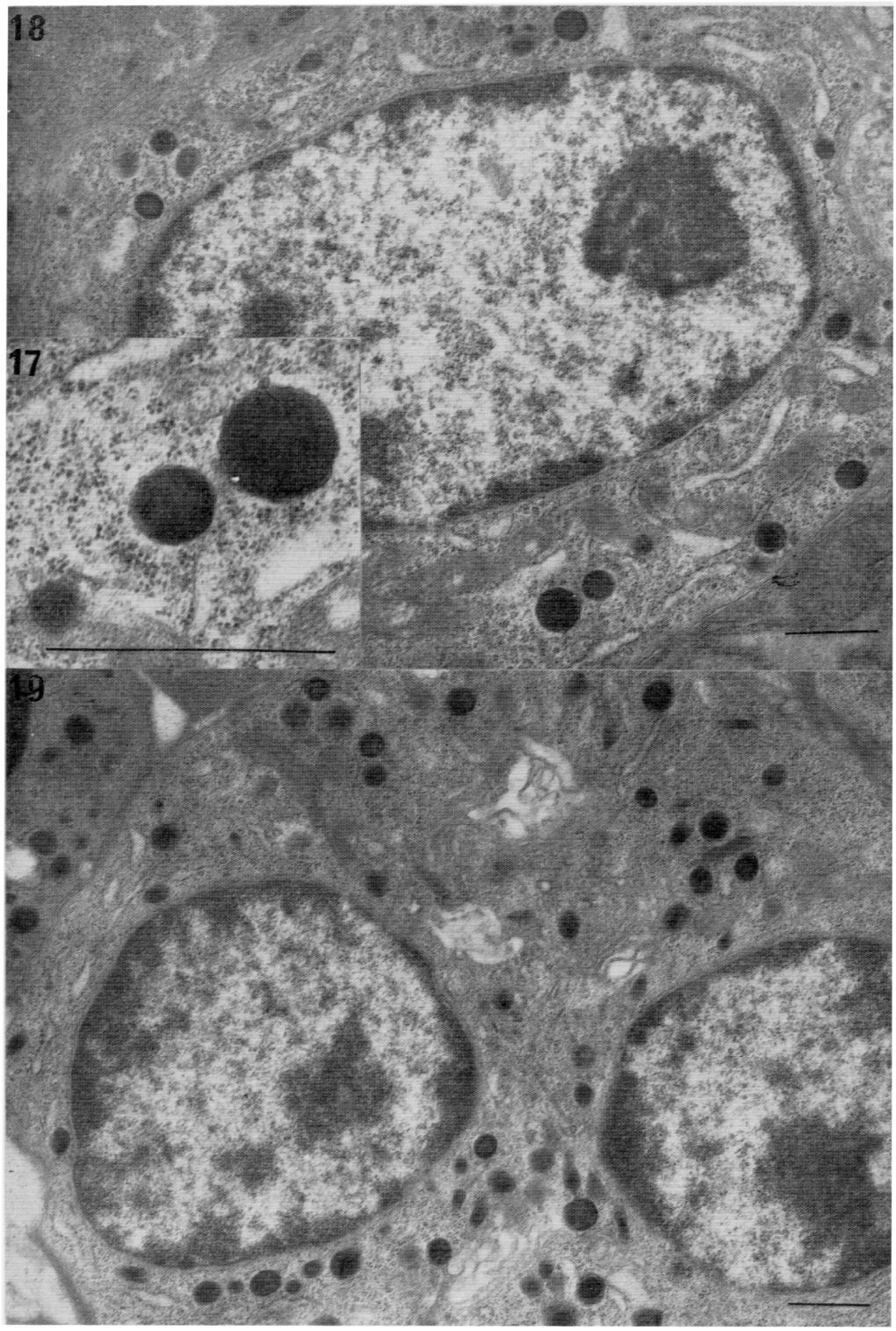
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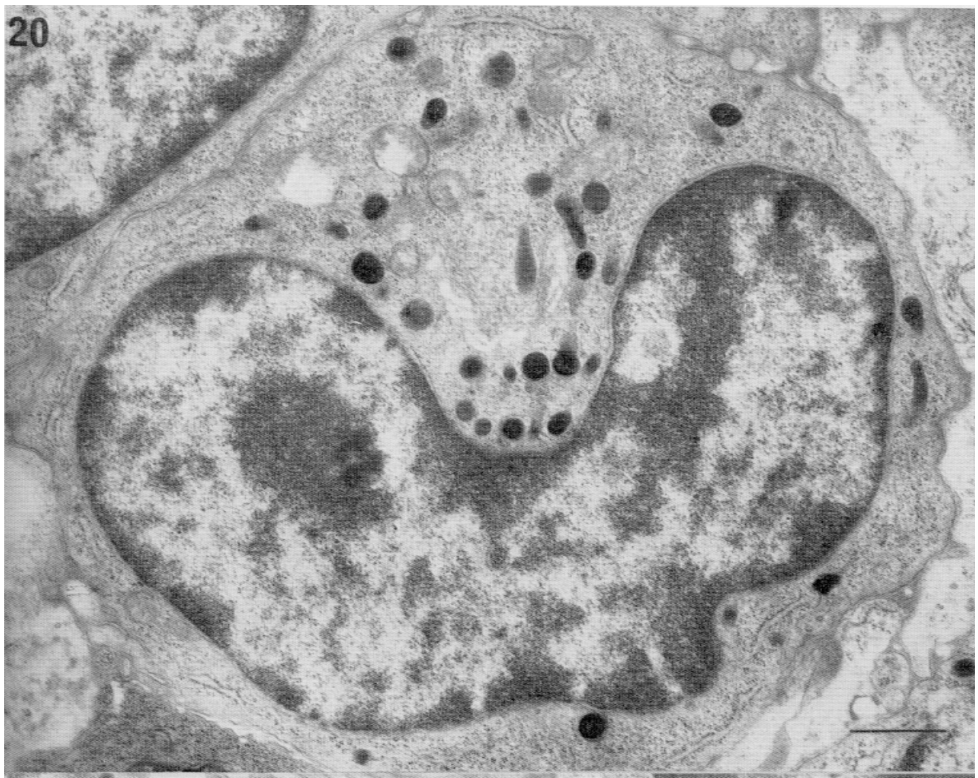
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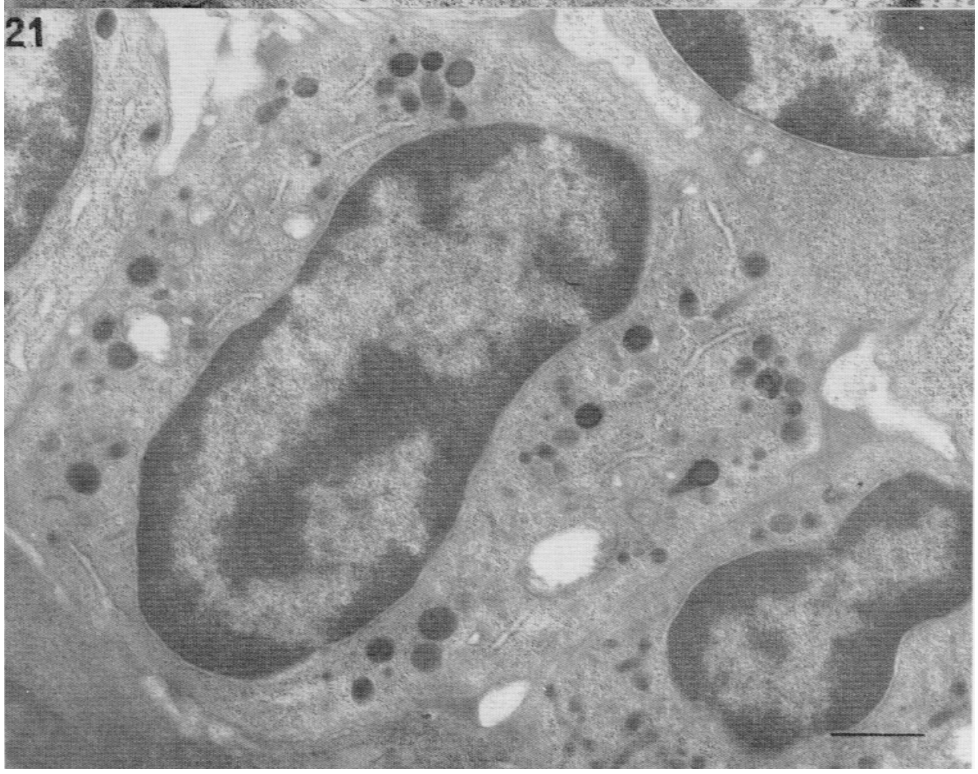
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