THE CYTOLOGY, HISTOCHEMISTRY AND ELECTRON MICROSCOPY OF THE GRANULAR CELLS OF THE METRIAL GLAND OF THE GRAVID RAT*

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Characteristic changes take place in the subplacental wall of the rat's uterus during gestation. The area involved, which includes the endometrium, myometrium and mesometrium at the placental site, has been called the 'metrial gland' by Selye & McKeown (1935). The most typical element of the 'gland' according to them is a 'granulated cell of maternal origin containing glycogen, eosinophilic, and sometimes also basophilic granules' which is located principally in the myometrial portion of this 'organ system'. Similar granular cells are present in experimentally induced deciduomas (Selye & McKeown, 1935; Asplund, Borell & Holmgren, 1940; Velardo, Dawson, Olson & Hisaw, 1953). Baker (1948) investigated the metrial gland of the rat by histochemical methods during pregnancy and lactation. According to him, the gland as it first develops is characterized by basophilic cells containing cytoplasmic ribonucleoprotein. Starting on about the 8th day of gestation, these cells gradually begin to lose their basophilia and become transformed into cells distinguished by the presence of conspicuous, eosinophilic granules. These cells persist until late in gestation when they decline and are gradually replaced by newly formed cells which are laden with lipides. Similar granular cells occur in the uterus of other rodents (mouse, guinea-pig) and rabbits.

The present investigation is concerned solely with the granular eosinophilic cells of the metrial gland. The cytoplasmic structure of these cells has been investigated in the present study by various cytological and histochemical methods, as well as by means of the electron microscope. The granules of the metrial cells have also been compared by the same techniques with the granules of the rat's eosinophilic leucocytes. Although the granules of both of these two types of cells are strongly eosinophilic, they exhibit characteristic histochemical differences which establish that they are different in chemical composition. They also differ markedly with respect to their structure as visualized by the electron microscope. The results also show that the procedures adopted can be utilized as a method for distinguishing different kinds of basic proteins.

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MATERIAL AND METHODS

The material was obtained from twelve female albino rats of the Sprague-Hawley strain maintained on a Purina chow diet. The rats were bred, and subsequently killed on the 13th, 15th and 18th days of pregnancy during the period when the granulated cells of the metrial gland are most plentiful.

Representative pieces of the chorio-allantoic placenta and the subplacental uterine wall were removed and placed in appropriate fixatives. The fixatives were: Zenker's acetic acid fluid, Rossman's ethanol-formalin-picric acid mixture, Orth's fluid, a 4 % aqueous solution of basic lead acetate, a mixture of 95 % methyl alcohol and 5 % glacial acetic acid, and a 10 % solution of buffered formalin. Excepting the formalin-fixed material of which frozen sections were prepared, the other blocks of tissue were embedded in paraffin, sectioned at 5 μ, stained, cleared and mounted in clarite.

Sections of tissues fixed in Zenker's acetic acid fluid were stained by Gomori's chrome alum-haematoxylin and phloxine method, and by eosin and methylene blue. Two series of sections, fixed in the same way, were stained separately with methylene blue and with orange G at graded pH, from pH 8 to 3. Other series were stained similarly with eosin from pH 6 to 12, before and after extraction in pyridine, chloroform and ether-methanol, and after acetylation by pyridine in acetic anhydride (1 : 1) for 24 hr. at room temperature (Weiss, 1953). Other individual sections were stained for $\frac{1}{2}$ hr. in a 1 % aqueous solution of toluidine blue; control sections were placed in a solution of ribonuclease for 1 hr. at room temperature before staining them. Other sections were stained for protein-bound disulphide groups by the sulphydril and disulphide method of Barrnett & Seligman (1952), and for arginine by Baker's method (1947). NH_2 groups were demonstrated by the method of Weiss, Tsou & Seligman (1954), modified by the use of a more alkaline buffer (pH 9.1 against pH 8·5) and a staining time of 6 hr.

Sections of tissues fixed in either Rossman's or Orth's fluid were stained by McManus's periodic acid-Schiff procedure. Other sections were stained after immersing them first for 1 hr. in saliva to remove all glycogen.

Sections of the basic lead acetate-fixed material were stained for $\frac{1}{2}$ hr. in a 1 % aqueous solution of toluidine blue. Corresponding control sections were first exposed to a solution of ribonuclease for 1 hr. at room temperature before staining them.

Sections of the formalin-fixed material were cut on a freezing microtome at 5 μ, stained in a saturated solution of Sudan black B in 70 % ethanol, and mounted in glycerogel.

For electron microscopy, metrial tissue was excised and cut into appropriately small pieces with a razor blade. The pieces were immediately fixed for 2 hr. in buffered osmic acid (Palade, 1952), washed in distilled water for $\frac{1}{2}$ hr. and dehydrated in 60 and 80 % ethanol for 1 hr. each. They were left overnight in two changes of 95 % ethanol. Next morning, they were placed in each of the following for 1 hr.: absolute alcohol, equal parts of absolute alcohol and *n*-butyl methacrylate, and two changes of pure *n*-butyl methacrylate. The tissues were embedded in gelatin capsules containing *n*-butyl methacrylate to which the catalyst 2 : 4-dichlorobenzoyl peroxide was added, and polymerization was carried out overnight in an oven at 45° F.

Ultrathin sections were cut with a glass knife on a Porter-Blum microtome, floated on a 20% acetone solution, picked up on a collodion-covered grid and examined and photographed in an RCA electron microscope, EMU-2E, without removing the methacrylate.

OBSERVATIONS

The development, topography and histology of the metrial gland of the rat have been described so extensively by previous investigators (cf. Selye & McKeown, 1935; Baker, 1948) that they need not be recapitulated here. Instead, for the orientation of the reader, we shall merely present two photographs to illustrate the location and general nature of the gland. Pl. 1, fig. 1, is of a transverse section of a rat's pregnant uterus on the 13th day of gestation. The placenta (*pl*), endometrial decidua (*de*) and myometrium (*my*) are visible. A rectangle encloses a field embracing a small portion of the decidua and a somewhat larger area of the myometrium. Pl. 1, fig. 2, shows the area in the rectangle enlarged, revealing numerous granular cells with their acidophilic granules differentiated by phloxine. The decidua in the upper part of the field is separated from the myometrium in the lower portion of the picture by an oblique line of interstitial material. The granular cells are far more abundant in the myometrial than in the decidual (endometrial) zone of the 'metrial gland'. However, in fig. 2 at the junction of the two zones they are visible in both regions. These granular cells are most abundant between the 12th and 18th days of gestation.

The granular cells are relatively large, mononucleate or binucleate elements with a conspicuous Golgi region situated to one side of the nucleus or between the two nuclei (Pl. 2). The granules are usually clustered immediately around the Golgi region in the centre of the cells. The extensive peripheral portion of the cytoplasm is in most instances clear and vacuolated with a variably distinct cell membrane separating it from the surrounding intercellular matrix.

The cell granules have distinctive characteristics. They are acidophilic, as revealed by their intense staining with phloxine (Pl. 1, fig. 2), eosin (Pl. 2, fig. 6) and orange G (Pl. 2, fig. 8). Investigation of their dye-binding capacity for eosin reveals intense staining of the granules at pH 10·3 with an estimated value of 3+ and a sharp decline in staining at pH 11·5 to an estimated value of only 1+. Following acetylation by pyridine in acetic anhydride (1:1) for 24 hr. at room temperature, the granules fail to bind eosin or they bind it at most to a very slight degree. Following extraction by pyridine, chloroform, or ether methanol, the eosinophilic staining remains undiminished. The granules are highly reactive for protein-bound amino groups (Pl. 2, fig. 11) and protein-bound disulphide groups (Pl. 2, fig. 10), but do not react with Baker's method for arginine.

The chemical properties of the specific granules of the metrial cells differ from those of eosinophilic leucocytes within placental and uterine blood vessels. In contrast to the granules of the metrial cells, those of the eosinophilic leucocytes show intense acid dye-binding at pH 11·5, which is unimpaired by acetylation; they give a strongly positive reaction with Baker's method for arginine and remain unstained by the procedure for NH_2 groups. The significance of these reactions will be interpreted in the discussion with respect to the chemical nature of the granules in the metrial cells and eosinophilic leucocytes.

Stained with toluidine blue at approximately neutral pH, the granules of the metrial cells are metachromatic after fixation in either basic lead acetate or Zenker's acetic acid mixture (Pl. 2, figs. 12, 13). Following immersion of the sections in ribonuclease previous to staining them, this metachromasia is undiminished (Pl. 2, fig. 14). Stained with methylene blue at pH 8 and below, the granules are unstained (Pl. 2, fig. 9). The granules are intensely reactive with the periodic acid-Schiff stain (Pl. 2, fig. 7). With Sudan black B they are quite unstained, the only visible reaction in the cells consisting of a faint coloration of the Golgi region.

The cells reveal a small to moderate degree of cytoplasmic basophilia when sections are stained with methylene blue. The basophilic substance is visible in clumps and strands located variously between the granules and peripheral vacuoles of the cells (Pl. 2, figs. 6, 9 and 12). The basophilia is almost completely abolished by pre-treatment of the sections with ribonuclease, a result indicating that the substance consists of ribonucleoprotein (cf. Pl. 2, figs. 12, 14). The basophilic substance stains with methylene blue at relatively low pH, ceasing around pH 4·5, a result characteristic of nucleoproteins which are acidic.

The large vacuoles in the peripheral cytoplasm of the cells contain glycogen, as can be demonstrated by appropriate means. Pl. 1, figs. 3 and 4, illustrate the total degree of staining of the granular cells by the periodic acid-Schiff procedure, whereas Pl. 1, fig. 5 and Pl. 2, fig. 7, illustrate the residual staining of the cells following exposure of the sections to saliva. The difference in amount of staining before and after treatment with saliva represents glycogen present in the large peripheral vacuoles. The intense residual periodic acid-Schiff reaction is confined entirely to a carbohydrate present in the granules (Pl. 1, fig. 5; Pl. 2, fig. 7), which is resistant to digestion by saliva. In the preparation shown in Pl. 1, fig. 3, it will be noted that the glycogen has drifted to one side of the cells as a result of the penetration of the fixative from the opposite side. There appears to be relatively more glycogen at 18 days (Pl. 1, fig. 4) than at 15 days (Pl. 1, fig. 3).

Examination of the metrial gland in the electron microscope confirms and amplifies some of the cytological and histochemical observations. The findings are illustrated in Pls. 3 and 4. In the electron micrographs, four distinctive organelles are visible in the cytoplasm, namely specific granules, the Golgi apparatus, mitochondria and endoplasmic reticulum (Pl. 3, fig. 15; Pl. 4, figs. 17, 18).

The specific granules (Pl. 4, fig. 17, *Gr*) which correspond in distribution and number to the acidic material are surrounded by a loosely fitting membrane (Pl. 3, fig. 15; Pl. 4, figs. 17, 18). The smaller the granule, the larger is the lacunar space between it and the surrounding envelope. The granules vary in density and some contain several clear vacuolar spaces within them. The surfaces of the granules may be smooth or irregular. The irregularities are due to minute vesicles which, in places, appear to be coalescing with the substance of the granules (Pl. 4, fig. 17, *V*). In some instances, these small vesicles are regularly dispersed in the lacunar space between the granule and its enveloping membrane (Pl. 4, fig. 18, *V*).

The endoplasmic reticulum in the metrial cells appears as double membranes or as flattened vesicles connected with lacunar spaces (Pl. 4, fig. 18, *R*). Palade's granules are present on the outer aspects of these membranes and some are randomly scattered throughout the cytoplasm.

The metrial cells also contain numerous vesicles of different sizes and shapes which are enclosed by double membranes. These structures are concentrated for the most part near the nucleus in the Golgi region (Pl. 4, fig. 17). The Golgi complex, clearly defined by an aggregation of flattened vesicles, is typically located between the granules and the nucleus (Pl. 3, fig. 15; Pl. 4, fig. 17, *G*) in the same position as it is seen with the light microscope.

A few short oval mitochondria (Pl. 4, figs. 17, 18, *M*) appear randomly distributed between the granules. They have a clear or vacuolated matrix and sharply defined cristae.

In addition to these organelles, the cytoplasm contains diffusely scattered, greyish flocculent material believed to represent the glycogen identified in preparations stained by the periodic acid-Schiff reagent.

The granules of the rat's eosinophilic leucocytes are quite different from those of the metrial cells as seen with the electron microscope. The leucocytic granules are distinguished by a sharply demarcated, dense, meridional band (Pl. 3, fig. 16). Moreover, there is no space and loose membranous envelope associated with the granules, and consequently there are no small vesicles associated with their surfaces. The marked differences between the two types of granules may be compared in Pl. 3, fig. 16, and Pl. 4, figs. 17, 18.

DISCUSSION

The most interesting question raised by the present findings concerns the nature of the protein composing the acidophilic granules of the metrial cells. The substance of the granules is quite basic since it binds an acid dye, such as eosin, up to relatively high pH. The observation that eosin is intensely bound up to pH 10·3, but that at pH 11·5 binding has practically ceased, suggests that the basic substance is composed of ϵ-NH_2 groups of lysine. These groups, while quite basic, are not as basic as the guanidonium groups of arginine or the tertiary and quaternary amines which bind acid dyes as high as pH 12 (Cohn & Edsall, 1943). The failure to bind eosin following acetylation indicates that NH_2 groups or hydrogenated amines are responsible for the observed dye-binding, because the guanidonium groups of arginine and the tertiary and quaternary amines, having no replaceable hydrogen, cannot be acetylated under the prevailing conditions (Weiss, 1953). Additional support for this conclusion is seen in the persistence of staining after extraction by fat solvents, because, unlike the protein-bound ϵ-NH_2 of lysine, tertiary and quaternary amines which are typically associated with phospholipid would be dissolved. The negative reaction with Baker's method for arginine also provides evidence that the eosinophilia is not due to the guanidonium groups of arginine. Finally, the strong reaction with the method for NH_2 groups also supports the thesis that those groups are responsible for the eosinophilia. From these data, it is concluded that the eosinophilia of the granules of the metrial cells is due mainly to the ϵ-NH_2 groups of lysine.

The histochemical methods adopted here provide a means of differentiating and distinguishing different basic proteins, as is illustrated in the following table which compares, in the rat, some of the histochemical reactions of the granules of the metrial cells with those of eosinophilic leucocytes (Table 1).

From these chemical differences, it is concluded that the granules of the eosinophilic leucocytes are composed of a basic protein which is rich in the amino-acid arginine, instead of lysine which characterizes the metrial cells. These comparisons illustrate the possibility of distinguishing basic proteins from one another by histochemical means. The validity of the conclusion that the metrial cell granules differ in composition from eosinophilic leucocyte granules, is also borne out by their markedly different morphology in the electron microscope (cf. Pl. 3, fig. 16; Pl. 4, figs. 17, 18).

Table 1. *Comparisons of some histochemical reactions of the granules of the metrial cell and of eosinophilic leucocytes*

Method	Metrial cell	Eosinophilic leucocyte
Acid dye-binding (with eosin)	Intense at pH 10·3, slight at pH 11·5	Intense at pH 11·5
Acid dye-binding after acetylation	Unstained	Stained
Reaction for arginine	Negative	Strong positive
Reaction for NH_2 groups	Strong positive	Negative

Besides the evidence advanced that the granules of the metrial cells contain lysine rather than arginine, the intense staining for disulphide groups by Barrnett & Seligman's sulphydril-disulphide method suggests that the substance of the granules is also rich in cystine.

Another point in need of brief clarification is the assertion by Selye & McKeown (1935) that the granulated cells contain eosinophilic as well as basophilic granules in one and the same cell. The former, they say, stain with fuchsin or eosin, while the latter stain with aniline blue. This is a misconception based on the false assumption that aniline blue is a basic dye and hence stains acidic or basophilic granules. Actually, aniline blue is an acid dye which has an affinity for basic or alkaline substances. The staining of some granules by aniline blue in contrast to the majority which was eosinophilic as reported by Selye & McKeown, signifies merely that the substance of individual granules varies somewhat with respect to its isoelectric point and consequently its affinity for individual acid dyes.

Although it has been known for a long time that the granulated metrial cells of the pregnant rat's uterus contain glycogen (cf. Selye & McKeown, 1935; Bridgman, 1948), ours is the first detailed description of the staining of these cells by the periodic acid-Schiff method. By this technique it is apparent that, in addition to a relatively large amount of glycogen which is diffusely distributed in the cytoplasm and can be removed by treatment with saliva, there is a pronounced saliva-resistant, residual staining of the specific granules (Pl. 1, fig. 5; Pl. 2, fig. 7) indicating the presence in them of a glycoprotein or mucopolysaccharide.

The possibility of a carbohydrate being conjugated with the protein of the cell granules leads us to a consideration of the significance of the metachromasia of the granules. Asplund *et al.* (1940) first called attention to the fact that the specific eosinophilic granules of the metrial cells of the rat, mouse and rabbit stain metachromatically in an aqueous solution of toluidine blue, particularly after fixation in a 4% aqueous solution of basic lead acetate. Metachromatic staining after this particular procedure they accepted as proof of the presence of a sulphated muco-

polysaccharide. Furthermore, from observations of the influence of acid, alkaline and physiological salt solutions upon the staining of the granules of the metrial cells they concluded that the granules contain a sulphated mucopolysaccharide of a low degree of esterification.

In recent years the thesis of Lison (1936) that all metachromatic staining by thiazine dyes should be attributed to the presence of sulphated acid mucopolysaccharides has been questioned (cf. Gomori, 1952; Pearse, 1953). According to Pearse, relatively alcohol-resistant metachromasia in paraffin sections is most likely to be due to sulphate esters. However, metachromasia of lesser intensities can be caused by polymerized carbohydrates by virtue of their carboxyl groups (Michaelis, 1947) or by phosphate-containing compounds. Ribose nucleic acid, for example, sometimes shows metachromasia in paraffin sections (Wislocki, Bunting & Dempsey, 1947), which can be prevented by removing the nucleic acid with ribonuclease. However, our failure to prevent the staining of the granules of the metrial cells by ribonuclease indicates that their metachromasia is not associated with ribose nucleic acid. That the substance producing the metachromasia must be relatively feebly acidic is indicated by its failure to bind methylene blue even at pH 8.

It may be presumptuous to offer any explanation of the intense acidophilia of the granules combined with their observed metachromasia. Nevertheless, the speculation is tentatively advanced that one is dealing here with a strongly alkaline protein conjugated with mildly acidic prosthetic groups. If that assumption is correct, the strongly alkaline protein would account for the intense affinity of the granules for acid dyes and their weak reaction towards methylene blue, whereas the acidic prosthetic groups would have to be held specifically responsible for the metachromatic staining with toluidine blue. The intense reaction of the granules with the periodic acid-Schiff reagent following removal of all glycogen should probably be attributed to a mucopolysaccharide representing possibly the same prosthetic groups responsible for the metachromatic staining.

The electron micrographs of the metrial cells reveal a Golgi complex close to the nucleus, specific cell granules, mitochondria and some endoplasmic reticulum.

The specific granules are peculiar in many respects, including the presence of numerous minute vesicles in the space between the granule and the capsule which loosely surrounds it. The association of these vesicles with the smaller granules and their apparent incorporation into the granules suggest that the vesicles may be related to the formation and growth of the granules of the metrial cells.

In some places the membrane which encloses the granule and the perigranular space appears to be related to endoplasmic reticulum. In Pl. 4, fig. 18, the membrane surrounding the granule and irregular space marked '*V*' appears to be continuous at its lower border with a strand of endoplasmic reticulum. Other similar appearances have been noted. If this speculation is correct, then the outer membrane and the lacunar space of each granule would be equivalent to a dilated cisterna of endoplasmic reticulum. The cytological observation made by Baker (1948) that cytoplasmic basophilia disappears inversely with the development of the acidophilic granules would seem to support such an assumption. These considerations suggest studying the origin of the metrial cells and the mechanism of formation of their granules by observing earlier stages than those examined here.

Brief comment and speculation may be welcome regarding the possible function of the granular cells of the 'metrial gland' of rodents and rabbits. In recent years the formation of relaxin, steroid hormones and histamine has been variously ascribed in some of these species to the activity of the decidually transformed uterine wall in pseudopregnancy or pregnancy. Thus, relaxin has been found in the uterus and placenta of the guinea-pig and rabbit (Hisaw & Zarrow, 1950), as well as in the uterus and maternal placenta of the rat in pseudopregnancy and pregnancy respectively (Zarrow, 1956). Frieden & Hisaw (1953) characterize relaxin as a simple protein which according to Kraintz (1951) contains lysine and cysteic acid (representing cystine and cysteine) among several amino-acids, whereas arginine is absent. The resemblance of relaxin in these respects to the protein of the granules of the metrial cells of the rat, as observed in the present investigation, is apparent.

The granular cells in question would not seem to be implicated in any possible steroid synthesis since they do not contain lipid material, aside from a slight staining of their mitochondria and elements of the Golgi complex with Sudan black B. With respect, however, to the possible synthesis of steroid compounds by the metrial gland, observations by Baker (1948) are of interest. He describes lipid-laden cells in the rat's metrial gland which gradually replace the granular cells and of which he remarks that, judged by their histochemical reactions, they may contribute to steroid metabolism late in gestation. These cells are, however, totally different from the granular cells described in the present study.

With respect to histamine, Shelesnyak (1952, 1954) observed that antihistaminic drugs suppress the development of the decidual reaction in pseudo-pregnant rats, from which he postulated and discussed the possibility 'that histamine release may be the operative mechanism in initiating natural placentation'. These observations offer the slight connexion with the present study that histamine is regarded as being produced by mast cells (Riley & West, 1953; Fawcett, 1954) which are characterized by the presence of strongly basophilic cytoplasmic granules which are intensely metachromatic. This recalls to mind the metachromatic attribute of the eosinophilic granules in the metrial cells discussed above.

SUMMARY

An account is given of the cytology, histochemistry and electron microscopy of the granular cells of the metrial gland of the gravid rat. The granules of these cells bind an acid dye (eosin) intensely at pH 10·3 and only slightly at pH 11·5, do not stain after acetylation, do not give a reaction for arginine and give a strongly positive reaction for NH_2 groups. From these data it is concluded that the eosinophilia of the granules is due mainly to the ϵ-NH_2 groups of lysine.

The granules also exhibit moderate metachromasia after staining with toluidine blue and stain intensely with the periodic acid-Schiff reaction. These staining properties are discussed, and it is concluded that they pertain to a mucopolysaccharide conjugated with the alkaline protein.

In electron micrographs the cytoplasm of these cells is observed to contain numerous specific granules, mitochondria, endoplasmic reticulum and glycogen. The specific granules are electron-dense, spherical objects surrounded by an

encapsulating membrane and a variously extensive, intervening space which contains minute vacuoles.

The granules of the metrial cells were compared with the granules of the eosinophilic leucocytes of the rat. In sharp contrast to the former, the eosinophilic granules of the leucocytes bind acid dyes intensely at pH 11·5, bind acid dyes after acetylation, react strongly for arginine and do not give a reaction for NH_2 groups. From these chemical differences it is concluded that the granules of the eosinophilic leucocytes consist of a basic protein rich in the amino-acid arginine, instead of lysine which characterizes the granules of the metrial cells. The histochemical procedures adopted in comparing these two types of granules provide a means of distinguishing different basic proteins. That the two kinds of granules differ is shown also in electron micrographs, where the granules of the eosinophilic leucocytes are observed to possess a wide, meridional, electron-dense band and not to have a perigranular space or capsule.

With respect to the possible function of the rat's metrial cells, the suggestion is offered that their basic protein granules which contain lysine but little or no arginine might be related to the presence of relaxin. Recent chemical evidence is cited which indicates that relaxin is a simple protein having lysine but not arginine amongst its amino-acid constituents.

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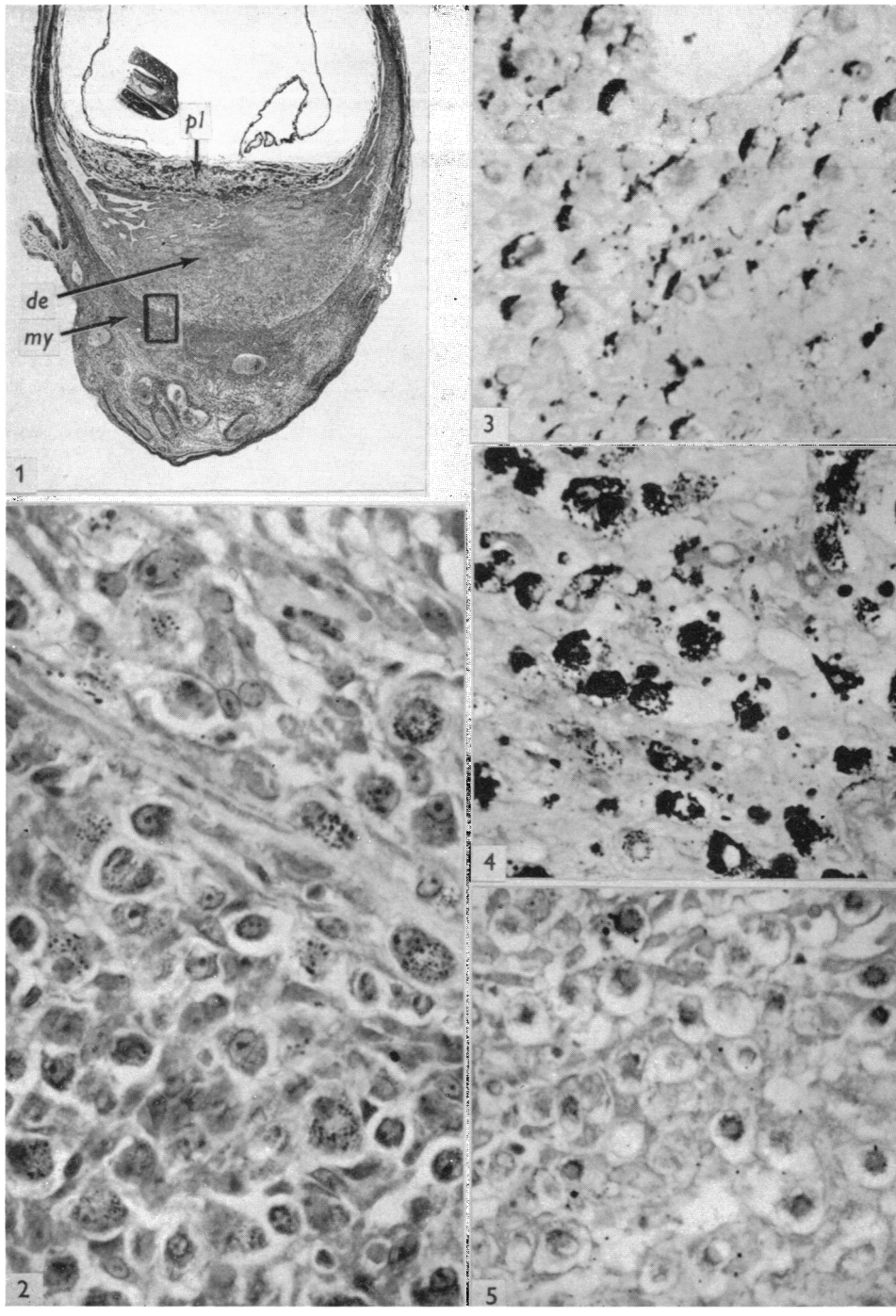

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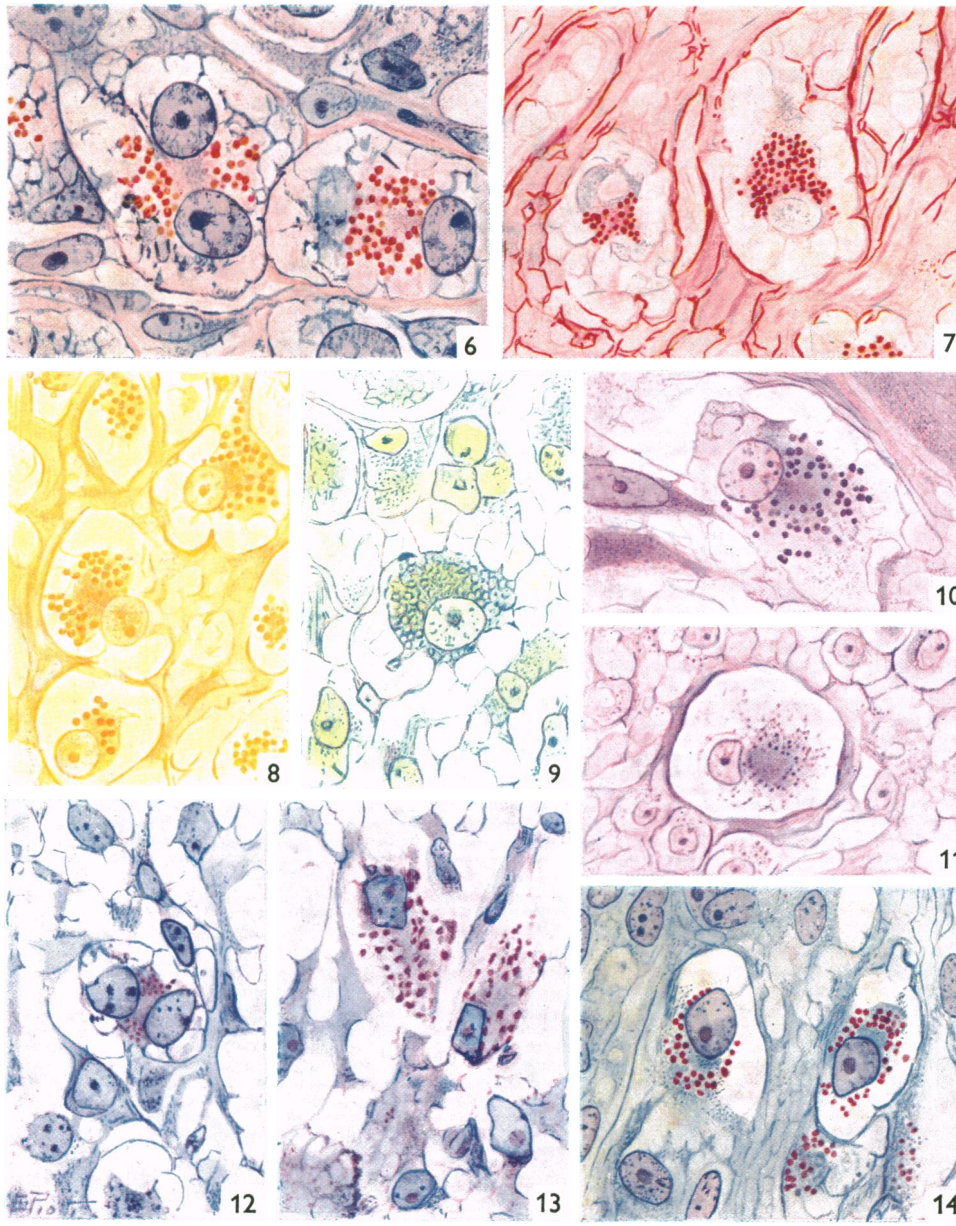
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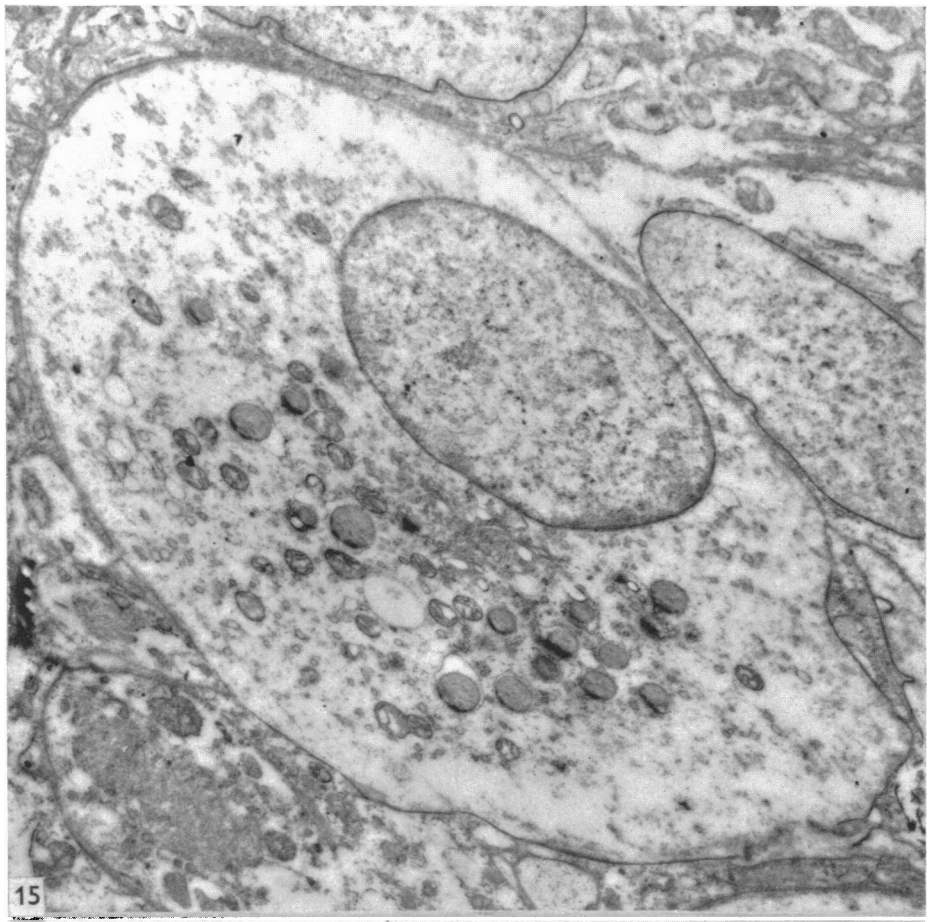

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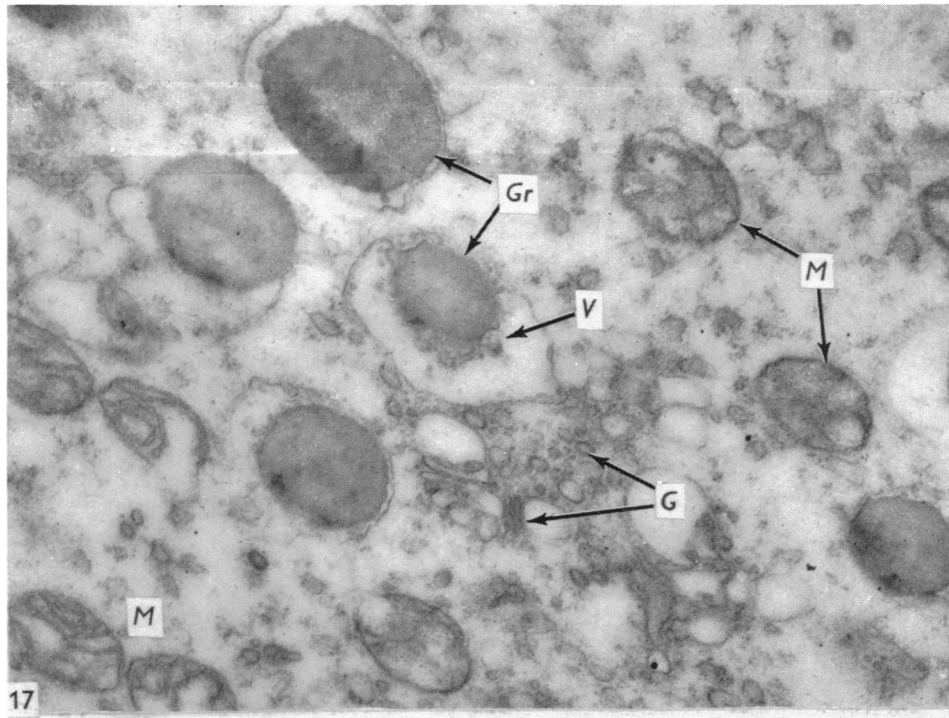
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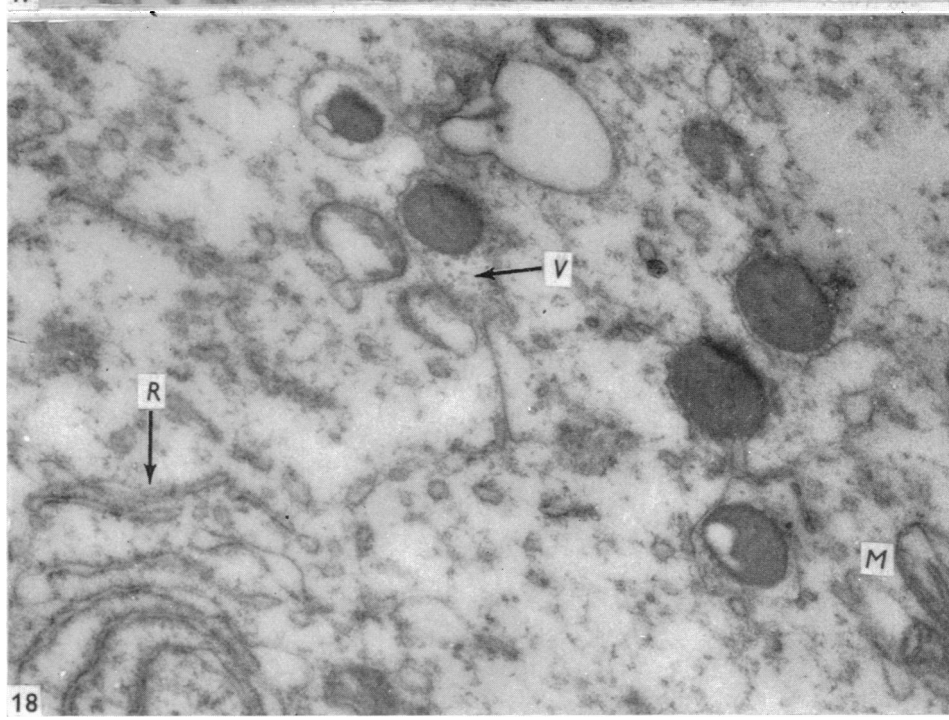
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Histological section of a pregnant rat's uterus at 13 days of gestation. *pl*, the chorio-allantoic placenta; *de*, the endometrial decidua; *my*, the myometrium. The area contained in the rectangle is shown at higher magnification in fig. 2. Chrome alum-haematoxylin and phloxine stain. × 13.

Fig. 2. A portion of the endometrial decidua and myometrium (area contained in the rectangle in fig. 1), illustrating the granular cells of the metrial gland which are characteristic of the second half of gestation. Chrome alum-haematoxylin and phloxine. × 600.

Fig. 3. The metrial gland of a rat on the 15th day of pregnancy. The granular cells which contain glycogen have been stained by the periodic acid-Schiff reagent. The deeply stained glycogen has drifted to the side of the cells opposite to the direction of penetration of the fixative. Rossman's alcohol-formalin-picric acid fixative. × 480.

Fig. 4. The metrial gland on the 18th day of pregnancy, stained with the periodic acid-Schiff reagent. Same fixative as the preceding. × 480.

Fig. 5. The metrial gland on the 13th day of pregnancy. The section was exposed to saliva to remove all glycogen before staining with the periodic acid-Schiff reagent. An intense residual reaction due to a saliva-resistant substance regarded as a mucopolysaccharide or glyco-protein, is visible in the granules in the vicinity of the nucleus. The detailed appearance of two such cells is illustrated in Pl. 2, fig. 7. × 480.

PLATE 2

The figures on this plate all represent granular cells of the rat's metrial gland, drawn with a × 7 ocular and × 90 objective, excepting fig. 6 which was drawn with a × 10 ocular and figure 11 with a × 60 objective.

Fig. 6. Two binucleate granular cells from the 15th day of gestation, stained with eosin and methylene blue. Zenker's acetic acid fixative. Intensely eosinophilic granules surround the Golgi region, whereas the peripheral cytoplasm contains basophilic material representing ribonucleoprotein, and vacuoles.

Fig. 7. Two granular cells from the 18th day of gestation, illustrating the strong affinity of the granules for the periodic acid-Schiff stain. The section was exposed to saliva to remove glycogen prior to staining it. Rossman's fixative.

Fig. 8. Granular cells stained with orange G at pH 5·13, on the 15th day of gestation. Zenker's acetic acid fixative.

Fig. 9. A section similar to the preceding one, stained with methylene blue at pH 5·13. Note that the acidophilic granules have no affinity at this pH for methylene blue, a basic dye, whereas the dye has an affinity for cytoplasmic basophilic substance surrounding the granules. The latter substance, which is also visible in the cells in fig. 6, can be abolished by ribonuclease.

Fig. 10. A granular cell stained by the method of Weiss, Tsou and Seligman for the demonstration of protein-bound amino groups, showing the intense reaction of the cell granules.

Fig. 11. A similar cell, at lower magnification, illustrating the presence of protein-bound disulphide groups in the cell granules.

Fig. 12. A binucleate metrial cell, showing the metachromasia of its granules following staining with toluidine blue. Observe that the granules surround the Golgi region. Zenker's acetic acid fixative.

Fig. 13. Two granular cells stained with toluidine blue, following fixation in a 4 % aqueous solution of basic lead acetate. This is a technique recommended by Holmgren & Wilander (1937), following which they observed metachromatic staining of the granules of the metrial cells in a variety of rodents. The metachromatic staining depicted in the drawing confirms their observation.

Fig. 14. A section similar to the one in fig. 13, revealing the metachromasia of the granules following exposure of the section to ribonuclease.

PLATE 3

Fig. 15. An electron micrograph of a granulated cell of the rat's metrial gland, illustrating the nucleus and the smooth plasma membrane of the cell. The cytoplasm contains a number of electron-dense granules and some mitochondria, as well as a dense Golgi complex situated near the nucleus. The greyish flocculence throughout the cytoplasm is regarded as representing glycogen. The granules in this cell correspond in number, size and distribution to the specific acidophilic granules encountered in the light microscope. × 5,818.

Fig. 16. An electron micrograph of a portion of a rat's eosinophilic leucocyte. Part of the cell nucleus is present in the upper part of the figure. The characteristic eosinophilic granules present in the cell cytoplasm possess a distinct meridional band. Compare with Pl. 4, figs. 17, 18. × 32,000.

PLATE 4

Fig. 17. A portion of the cytoplasm of a granular metrial gland cell at higher magnification. The Golgi apparatus (*G*) is visible surrounded by five electron-dense, encapsulated granules (*Gr*) and several mitochondria (*M*). The smaller granules are usually surrounded by a space which contains minute vacuoles (*V*). × 30,600.

Fig. 18. Another portion of the cytoplasm of a granular cell revealing a number of granules, one of which is surrounded by an irregular capsule and space which contains minute vacuoles (*V*). Sheets of endoplasmic reticulum (*R*) are visible in the lower left-hand corner of the electron micrograph. Two mitochondria (*M*) are shown. Amorphous background material is believed to represent glycogen. The organelles seen in the electron micrograph (Pls. 3 and 4) should be compared with the cytological structures revealed at lower magnification in the light microscope (Pl. 2). × 16,525.