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Ultrastructural Study of Secretory Granules in the Juxtaglomerular Cells —Particularly on Formation and Extrusion—

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The secretory granules in the juxtaglomerular cells which are believed to be renin or renin-like substance are of experimental and clinical interest since they are related to hypertension and Na balance. Electron microscopic observations were made with rats on the mechanism of formation and extrusion of secretory granules upon giving stimulation to the juxtaglomerular cells by means of restricted salt diet and administration of diuretics. As to the formation of granules, rough-surfaced endoplasmic reticulum developed into Golgi apparatus, immature granules and finally mature granules. At least, one of the extrusion types was diacrine mechanism since there was observed "fading" of granule content while the limit membrane was maintained. In view of the fact that extrusion by diacrine mechanism is generally of the substance of low molecular weight, it is presumed that the content of JG cell might be prmitive renin of relatively low molecular weight or might possibly include some substance other than renin.

In 1939, GOORMAGHTIGH and GRIMSON suggested for the first time that renin is produced in the juxtaglomerular apparatus (JGA)[19]. Since then, many studies have been carried out concerning the relationship between the JGA and renin. One of the most demonstrative arguments in favor of GOORMAGHTIGH's thesis was furnished by EDELMAN and HARTROFT who found that fluorescent anti-renin antibodies injected into the rabbit and dog were localized selectively in the juxtaglomerular cells (JG cells).

At present, the juxtaglomerular cell granule (JG cell granule) is generally believed to be renin-like substance[16),21),28),43]. Since the JG cell granules are formed and discharged in the arteriolar wall[20], and can be identified by light microscopy by means of special staining,[9),46] and electron microscopy[7),36] their relation with hypertension[6),12),34) 35),45] and Na balance[12] is of morphological interest.

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This paper deals with electron microscopic observations on the mechanism of formation and extrusion of JG cell granules in rats upon restriction of salt intake and administration of diuretics as the formation and extrusion of JG cell granules are slow in normal untreated animals.

MATERIALS AND METHODS

A) Animals

A total of nineteen female Wistar rats, each weighing 170 to 280 gm., were used for the present experiment.

The rats were classified into the following three groups:

I) Control group

Six rats were allowed free access to food and tap water till the time of sacrifice.

II) Group treated with acetazolamide (Diamox)

Five rats were given daily stomach-tubings of 60 mg. of acetazolamide per 100 gm. of animal weight for seven days, and were maintained on relatively low salt rat pellets (Orientaru Kōbo Kōgyō K.K.) and distilled water. They were sacrificed the day after the last tubing.

III) Group treated with furosemide (Lasix)

Eight rats were given daily intraperitoneal or intravenous injection

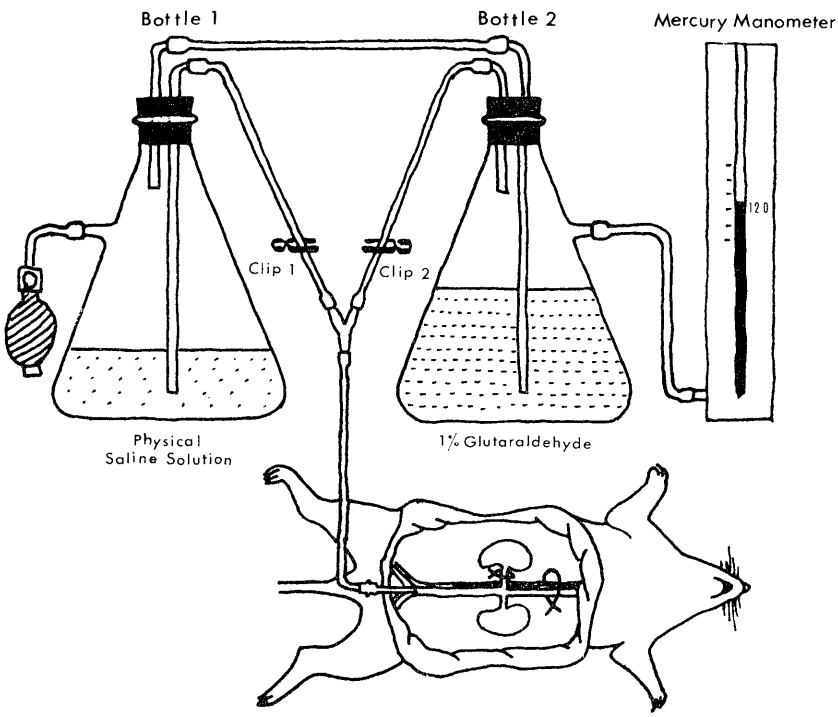

Fig. 1 Diagram of apparatus for pefusion fixation

of 0.1 mg. of furosemide per 100 gm. of rat weight for seven days, and were maintained on relatively low salt intake as group II. They were sacrificed the day after the last injection.

B) Perfusion fixation[37] (Fig. 1)

All animals were anesthetized with ether or nembutal. Blood sample for electrolyte evaluation was collected by a 5 ml cylinder from the inferior abdominal aorta. The cylinder was exchanged for fixative tube, and the main right renal vessels were clipped devoid of perfusion of physical saline solution and glutaraldehyde. The left renal vein was incised and the abdominal aorta was ligated at its superior portion while physical saline solution was perfused for approximately 30 seconds. Then, 1% buffered, isotonic glutaraldehyde fixative was perfused for about 20 minutes. During this period, the clipped right kidney was removed. In the perfusion of both physical saline solution and glutaraldehyde, two bottles were maintained at the pressure of about 120 mm. of mercury using a rubber bulb.

C) Preparation of sections for light microscopy and electron microscopy

For light microscopy, the tissue of the right kidney was fixed in Helly's fluid, embedded in paraffin, and sections were stained with hematoxylin and eosin stain, and with Bowie's stain.

Material of the left kidney for electron microscopy was fixed in buffered 1.2% osmic acid, dehydrated in a graded series of acetones, and embedded in Epon 812.

Blocks were cut on a Porter-Blum ultramicrotome MT-2. Sections were stained on the grids with uranyl acetate and lead citrate. They were photographed in JEM 6C electron microscope.

D) Electrolyte evaluation

Serum sodium and potassium were evaluated with Hitachi's flame photometer FPF-2A.

RESULTS

The values of serum sodium, serum potassium and juxtaglomerular index (JGI)[24] by the group were as shown in Table I.

Table I Serum Electrolyte and JGI[a]

		No. of Rats	Serem Na[d] mEq/l	Sarum K[d] mEq/l	JGI[d]
I	Control	6	139.0±2.02	4.35±0.13	31.0±1.3
II	RLSD[b]+DW[c] +Acetazolamide	5	141.5±0.93	4.04±0.11	40.0±7.1
III	RLSD+DW +Furosemide	8	138.6±0.76	3.29±0.33	32.7±1.7

a Juxtaglomerular Index
b Relatively low salt diet (Orientaru Kōbo Kōgyō K.K.)
c Distilled water
d Mean ± standard error

Serum Na and JGI showed no statistical difference in value among the three groups ($P>0.05$) while the value of serum K was lower in Group III than in the other two ($P<0.001$, $\alpha=0.05$).

The vascular lumen and renal tubular lumen of non-perfusion fixation samples were collapsed but those of perfusion fixation samples remained open though the interstitium was narrowed (Fig. 2, 3). Granular precipitates were observed in the area from the Bowman's capsule to the renal tubule in non-perfusion fixation samples of Group II but they were washed away and were not observed in perfusion fixation samples (Fig. 2, 3).

In Group II and III, particularly in the latter, cloudy granular swellwing was observed in the epithelial cells of the renal tubule (Fig. 4).

In electron microscopy, JG cell granules were located in the epithelioid cells of the renal arteriolar wall. The epithelioid cells showed the character of smooth muscle cells with the myofibril and dense attachment (Fig. 5, 6).

The JG cells were generally remarkable in the development of Golgi apparatus and demonstrated in the Golgi sac some materials of approximately identical electrcn density to JG cell granule (Fig. 7).

Mature granules being round or oval in shape were surrounded by a layer of limit membrane, and the content of granule manifested crystalline structure of "dot pattern" or "band pattern"[3] (Fig. 5 to 12). The crystalline structure showed periodicity in one direction (Fig. 8, 9) or in two directions (Fig. 10) according to the direction of cut surface of granule. Some mature granules lacked part of the limit membrane[12] (Fig. 6) or had double membrane[33] (Fig. 10).

In Group II, small granules (daughter granules) were seen on the surface of mature granule. Electron density was low inside the daughter granules but contrarily high at the periphery (Fig. 9, 11). It is unknown whether the daughter granules have any relation to the formation and extrusion of granules.

Some masses of the size of mature granule or smaller lacking the limit membrane and of the same electron density as the JG cell granule were observed in the vicinity of mature granules (Fig. 6, 8, 9, 11, 12 Ms). Some of those masses demonstrated cystalline structure (Fig. 9 Ms↓).

In Group II and III as well as in normal rats (Fig. 5), several mature granules were fused into a giant granule (Fig. 9, 11)[12][34]. The electron density of granule content decreased at the periphery of granule while the limit membrane was maintained (peripheral fading) (Fig. 10) and the decrease of electron density extended diffusely to the entier granule (diffuse fading) (Fig. 11, 12).

DISCUSSION

There are several methods for the grading of JGA granulation such as juxtaglomerular index (JGA) by HARTROFT[24], index of hyperplasia and hypertrophy by RAPP[39], juxtaglomerular cell count (JGCC) by TURGEON and SOMMERS[8)45], a modification of JGCC by CELORIA and PUCHE[11], and OKAMOTO's index[38]. The JGI was adopted in this paper. Howeve, some investigators insist that JGI does not reflect increase in number of cells and the presence of those evidentally bright and hypertrophied cells without large granules which are frequently observed in abnormal kidneys[45]. In the JGA of sodium-depleted animals, JGI generally increases[11),12),24),43]. In this experiment, relatively low salt diet, distilled water and diuretics were used but sufficient hyponatremia could not be effected in rats. Accordingly, increase of JGI was not noted. However, since the JGA was stimulated by low salt diet and diuretics, the stimulation to the JGA might have been indicated by other grading methods though it failed to appear as an increase of the JGI.

Recently, CELORIA and PUCHE stated that no correlation exists between the JGI and plasma Na concentration but that a significant negative correlation is found between the JGI and logarithm of the urinary Na excretion, and paid attention to the urinary Na excretion rather than plasma Na[11].

Perfusion fixation by glutaraldehyde provides good specimens for electron microscopy[37]. However, it is inappropriate for observation of precipitates since precipitates in the urinary tubules are washed out by perfusion. In electron microscopy, the JG cell shows myofibril and dense attachment and transition from the smooth muscle cell[33),42]. JG cell granules are generally present in the afferent arteriole but occasionally in the efferent arterioles[1], lacis cells[33], or mesangial cells[15], all in the vicinity of the glomerular hilus. It is noteworthy that presence of JG cells in the arteriole away from the glomerular hilus was reported by SHIMAMURA[41].

BARAJAS considers that the crystalline pattern (or band pattern as named by BIAVA) exists as Golgi apparatus or protogranule which has immature character or is on the way of formation[2].

Immature granules are present near the Golgi apparatus developed in the JG cell. Like other protein secreta, the JG cell granules may be formed from rough-surfaced endoplasmic reticulum through the Golgi apparatus[10),30),32),34]. Thus, it may well be said that granule formation is made in the space surrounded by the membrane.

CHANDRA et al stated that JG cell granules are formed directly from the reticulum according to their understanding that the endoplasmic reticulum and JG cell granules are fused[12]. However, the granules which are said to be fused are mature and not in the course of maturation.

The electron dense mass indicated by Ms in the figure is not a

granule but a mass of secretory substance since the limit membrane is not present[31]. For the same reason, the mass is not being formed nor can be considered as mature granule but properly considered to be in the course of extrusion. This is equivalent to KUROSUMI's Excrusion type V (diacrine mechanism) subtype 1[32]. Discharge of such masses from the cytoplasm of JG cells into the intercellular space has been observed also by LEE et al[34]. Some of the electron dense masses presumably in the course of extrusion indicate crystalline pattern. If the crystalline pattern is of immature nature as stated by BARAJAS, it would signify that immature masses at the stage of formation are also extruded.

Another possible type of extrusion is that the granule extrudes its content after having undergone peripheral fading and then diffuse fading while maintaining the limit membrane. This is equivalent to KUROZUMI's Extrusion type V subtype 2. A schemic diagram of formation and extrusion of JG cell granules in consideration of the theories of the above-mentioned investigators is shown in Fig. 13.

In view of the fact that the mechanism of extrusion type V is of the materials of relatively low molecular weight[32] and that the molecular weight of renin is currently said to be 48,000, the content of JG cell granule might be primitive renin of relatively low molecular weight or might possibly include some substance other than renin.

SUMMARY

1) The rats treated with relatively low salt diet and diuretics showed no difference from the control group in the value of serum Na and JGI but a decrease in the value of serum K.
2) The 1% glutaraldehyde perfusion fixation employed in this study proved to be outstanding for electron microscopy but unsuitable for observation of materials in the renal tubule as it washes out precipitates therein.
3) The JG cells showed the character of smooth muscle cells with the myofibril and dense attachment, and were remarkable in the development of Golgi apparatus.
4) Mature granules were surrounded by the limit membrane and the content showed dot pattern or band pattern. Some granules lacked part of the limit membrane or had double membrane.
5) In the group treated with acetazolamide, daughter granules were observed.
6) Secretory masses were observed in the cytoplasm, which are considered to be in the course of extrusion. Some of the masses showed crystalline structure.
7) One of the types of extrusion of granule content in the cytoplasm was diacrine mechanism in which the electron density decreased at

the periphery of granule while the limit membrane was maintained (peripheral fading) and further decreased diffusely in the entire granule (diffuse fading).

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REFERENCES

1) BARAJAS, L. and LATTA, H.: *Lab Invest* 12: 257, 1963.
2) BARAJAS, L.: *J Ultrastruct Res* 15: 400, 1966.
3) BIAVA, C. and WEST, M.: *Amer J Path* 47: 287, 1965.
4) BIAVA, C. and WEST, M.: *Amer J Path* 49: 679, 1966.
5) BIAVA, C. and WEST, M.: *Amer J Path* 49: 955, 8966.
6) BIAVA, C. and WEST, M.: *Lab Invest* 15: 1902, 1966.
7) BOHLE, A.: *Verk Deutsch Ges Path* 43: 219, 1959.
8) BOUGHTON, R.M. and SOMMERS, S.C.: *J Urol* 89: 133, 1963.
9) BOWIE, D. J.: *Quart J Exp Physiol* 25: 247, 1935.
10) CAJAL, S.R.: *Trabajos Lab Invest Biol* (Madrid) 12: 17, 1914.
11) CELORIA, G.C. and PUCHE, R.: *Acta Physiol Lat Amer* 17: 6, 1967.
12) CHANDRA, S., HUBBARD, J.C., SKELTON, F.R., BERNADIS, L.L., and KAMURA, S.: *Lab Invest* 14: 1834, 1965.
13) DESPREZ, J.: *Amer J Clin Path* 18: 953, 1948.
14) DUNIHUE, F.W.: *Amer J Path* 23: 906, 1947.
15) DUNIHUE, F.W. and BOLDOSSER, W.G.: *Lab Invest* 12: 1228, 1963.
16) EDELMAN, R. and HARTROFT, P.M.: *Circ Res* 9: 1609, 1961.
17) FISHER, E.R. and KLEIN, H.Z.: *Proc Soc Exp Biol Med* 113: 37, 1963.
18) FISHER, E.R., PEREZ-STABL, E. and PARDO, V.: *Lab Invest* 15: 1409, 1966.
19) GOORMAGHTIGH, N. and GRIMSON, K.S.: *Proc Soc Exp Biol Med* 42: 227, 1936.
20) GOORMAGHTIGH, N.: *Proc Soc Exp Biol Med* 42: 688, 1939.
21) GOORMAGHTIGH, N.: *Rev Belge Sci Med* 16: 65, 1945.
22) GRIFFITH, L.D., BULGER, R.E. and TRUMP, B.F.: *Lab. Invest* 16: 220, 1967.
23) GUILLAN, R.A., ZELMAN, S. and MURPHY, F.J.: *Arch Path* 84: 598, 1967.
24) HARTROFT, P.M., HARTROFT, W.S.: *J Exp Med* 97: 415, 1953.
25) HARTROFT, P.M.: *J Exp Med* 105 (Suppl. 6): 501, 1957.
26) HARTROFT, W.S. and HARTROFT, P.M.: *Fed Proc* 20: 845, 1961.
27) HARTROFT, P.M.: *Circ Res* 12: 525, 1963.
28) HARTROFT, P.M., SUTHERLAND, L.E. and HARTROFT, W.S.: *Canad Med Ass J* 90: 163, 1964.
29) KAUFMAN, W.: *Amer J Path* 17: 620, 1941.
30) KIRKMAN, H. and SEVERINGHAUS, A.E.: *Anat Rec* 70: 413, 1938.
31) KUROSUMI, K., YAMAGISHI, M. and SEKINE, M.: *Z Zellforsch* 55: 297, 1961
32) KUROSUMI, K.: *J Electron Micr* 14: 12, 1965 (Japanese).
33) LATTA, H. and MAUNSBACH, A.B.: *J Ultrastruct Res* 6: 547, 1962.
34) LEE, J.C., HURLEY, S. and HOPPER, J.: *Lab Invest* 15: 1459, 1966.
35) NISHIMORI, I.: *Jap J Nephrol* 7: 2, 1965.
36) NISHIMORI, I., TSUDA, N. and TAKESHITA, K.: *Saisin Igaku* 23: 1812, 1968 (Japanese).
37) MAUNSBACH, A.B.: *J Ultrastruct Res* 15: 242, 1966.

38) OKAMOTO, K.: *Folia Endocr Jap* 38: 782, 1962 (Japanese).
39) RAPP, J.P.: *Amer J Physiol* 206: 93, 1964.
40) SHIMAMURA, T. and SHAW, E.H.: *S Dakota J Med* 21: 33, 1968.
41) SHIMAMURA, T.: *S Dakota J Med* 21: 49, 1968.
42) TAKESHITA, K.: *Arch Histol Jap* 29: 237, 1968.
43) TOBIAN, L., THOMPSON, J., TWEDT, R. and JANECEK, J.: *J Clin Invest* 37: 660, 1958.
44) TOBIAN, L., JANECEK, J. and TOMBOULIAN, A.: *Proc Soc Exp Biol Med* 100: 94, 1959.
45) TURGEON, C. and SOMMERS, S.C.: *Amer J Path* 38: 227, 1961.
46) WILSON, W.: *Anat Rec* 112: 497, 1952.

EXPLANATION OF PHOTOGRAPHS

Fig. 2 Rat treated with acetazolamide

A granule is noted on the afferent arteriolar wall (AA) at the glomerular hilus. Distal convoluted tubules approach the afferent arteriole and become macula densa (MD). The renal tuft contains erythrocytes, and the Bowman's capsule and renal tubule contain precipitates.

Helly fixation, Paraffin embedding, Bowie stain

Fig. 3 Rat treated with acetazolamide

In specimen processed by glutaraldehyde perfusion fixation, the renal tuft, Bowman's capsule and renal tubule are open, and the interstitium is narrowed. No erythrocyte is present in the renal tuft and no precipitate in the renal tubule.

Glutaraldehyde perfusion fixation, Paraffin embedding, H & E stain

Fig. 4 Rat treated with furosemide

The epithelial cells of collecting tube show cloudy swelling.

Helly fixation, H & E stain

Fig. 5 Normal rat

JG cells of the afferent arteriole. Developed Golgi apparatus, and myofibril and dense attachment are seen. Secretory granules are round or oval in shape. Fading granules are seen in normal rat as well (JGC_1).

AL: arteriolar lumen MD: macula densa ×6,000

Fig. 6 Rat treated with acetazolamide

Golgi apparatus (Ga) is noted in the JG cell. In the dilated Golgi sac, some substance with the same electron density as the content of JG cell granule is present.

E: endothelial cell ×5,200

Fig. 7 Rat treated with acetazolamide

JG cell has myofibril (Fs) and dense attachment (Da). The content of mature granules shows dot pattern. Some granules (Gp) lack part of the limit membrane. Secretory masses (Ms) totally lacking the limit membrane are also observed. ×29,000

Fig. 8 Rat treated with acetazolamide

JG cell granule shows crystalline pattern (arrow). A secretory mass (Ms) with lower electron density than JG cell granule without the limit membrane is seen. ×50,000

Fig. 9 Rat treated with acetazolamide

Two granules are fused into one large granule (G_1). Indicated by large arrow at the bottom is a small granule or daughter granule protruding on the surface of a granule. Several secretory masses (Ms) are present among mature granules and some of them have crystalline pattern (Ms small arrow). Dilatation of the endoplasmic reticulum is remarkable. ×32,000

Fig. 10 Rat treated with acetazolamide

G_1 shows crystalline pattern in two directions. G_2 is low in electron density at the periphery showing peripheral fading. The limit membrane of G_2 is partially double layered. ×58,000

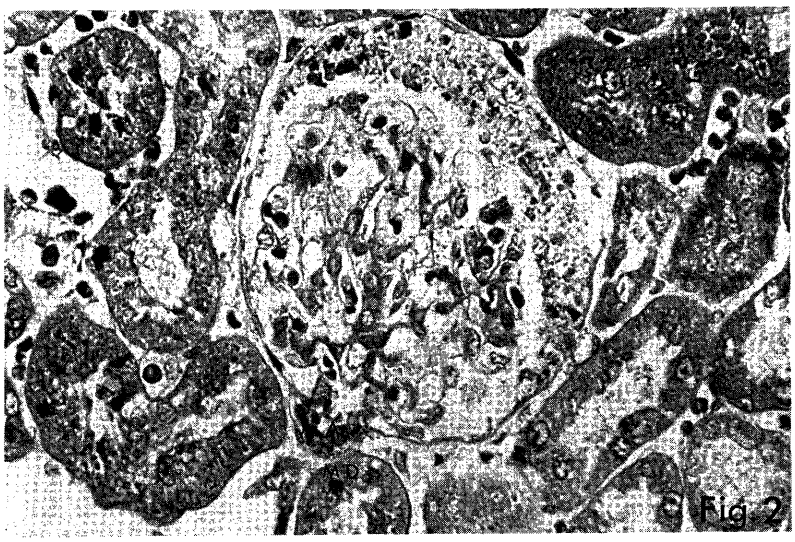
Fig. 11 Rat treated with acetazolamide

G_1 is a large fused granule. In G_2, the electron density of the content is decreased while the limit membrane is maintained (diffuse fading). The arrow indicates a daughter granule.

Ms: secretory mass E: endothelial cell ×32,000

Fig. 12 Rat trated with furosemide

Present at the central part of the photograph are granules at the stage of diffuse fading showing only the limit membrne. Secretory masses (Ms) are also noted. ×27,000



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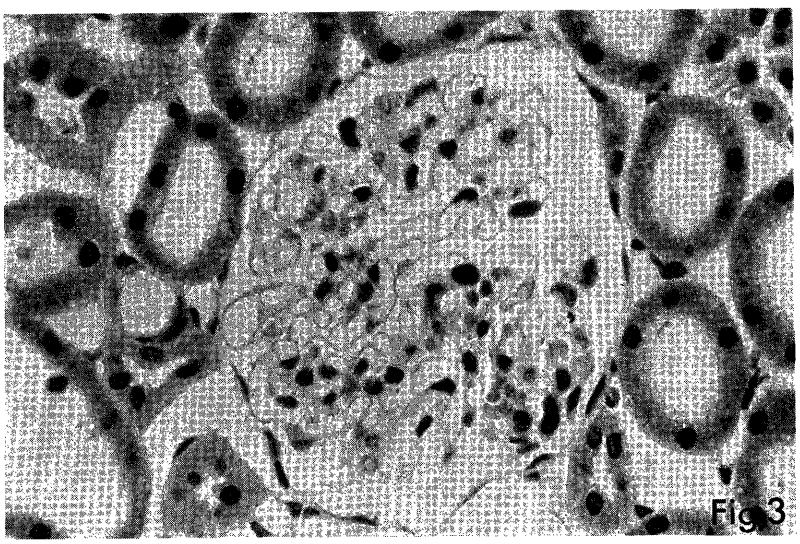

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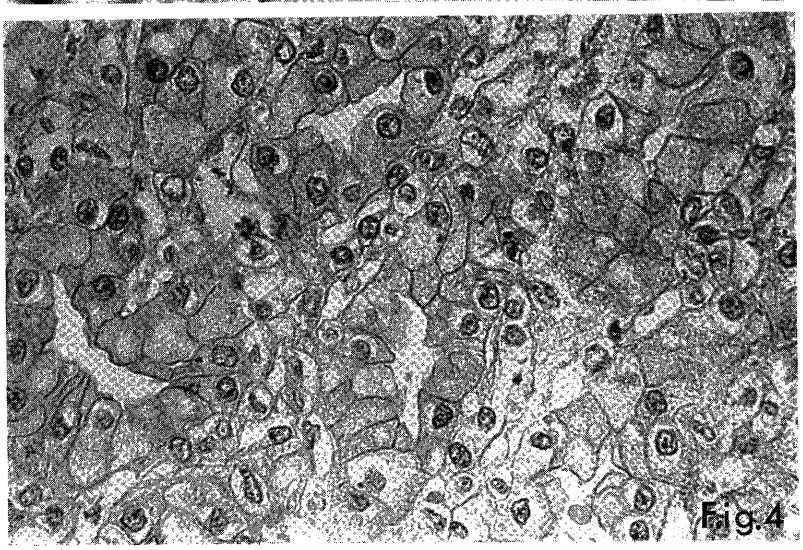

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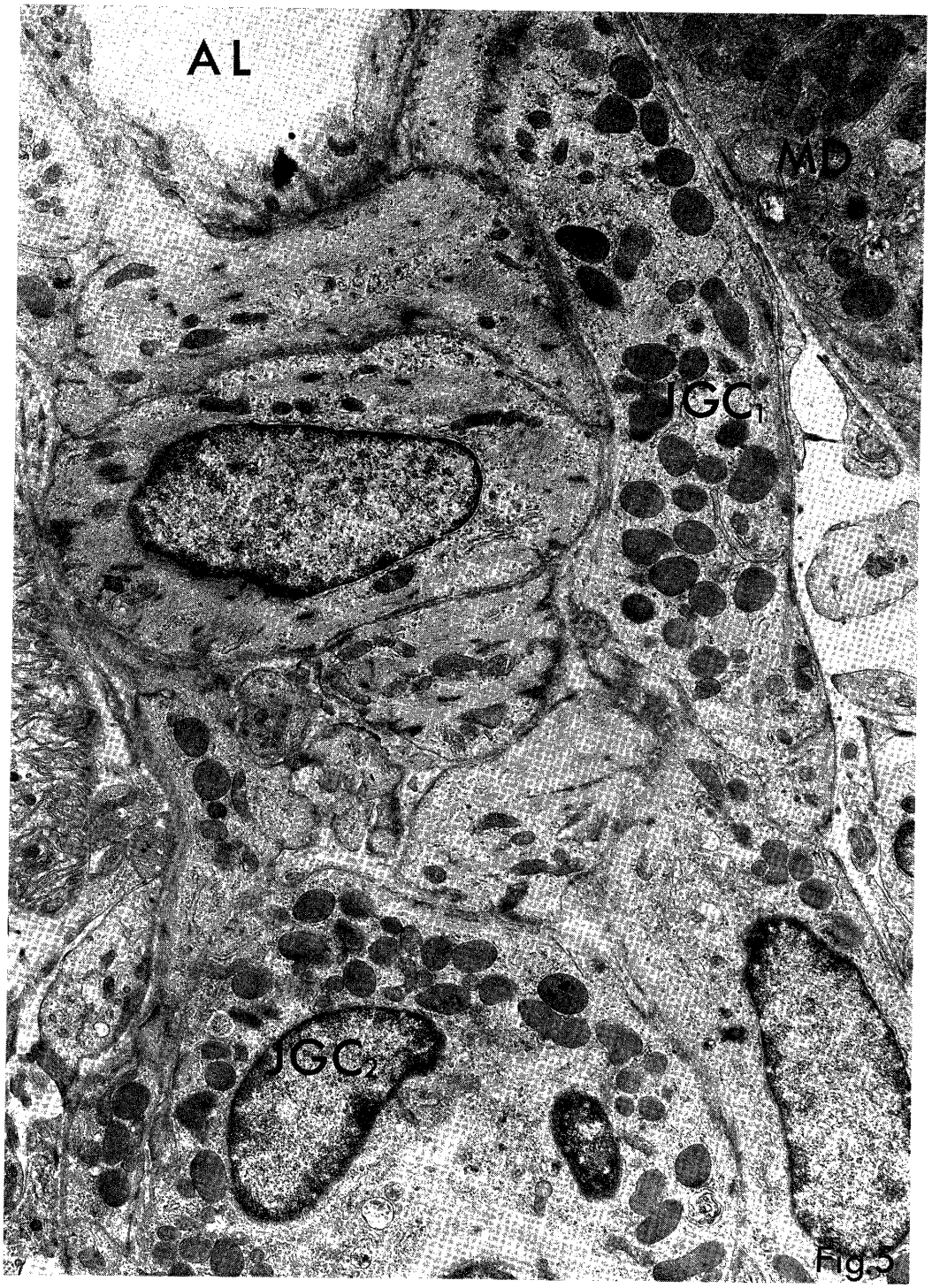

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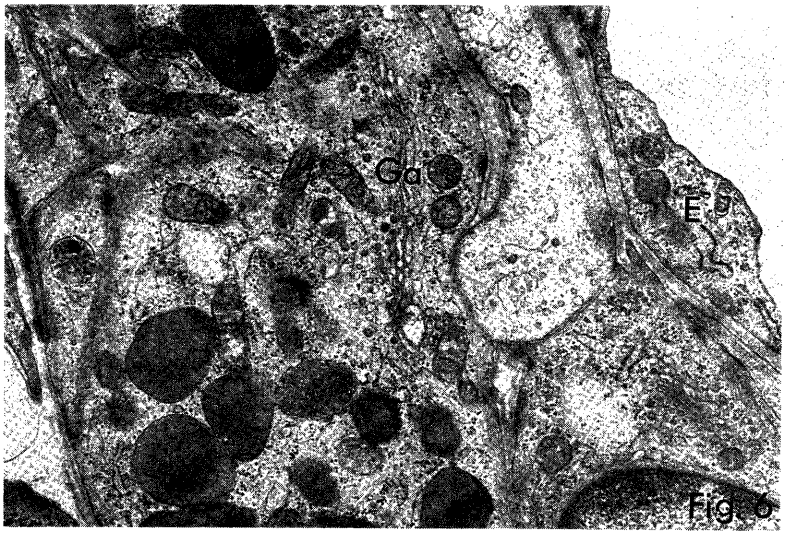

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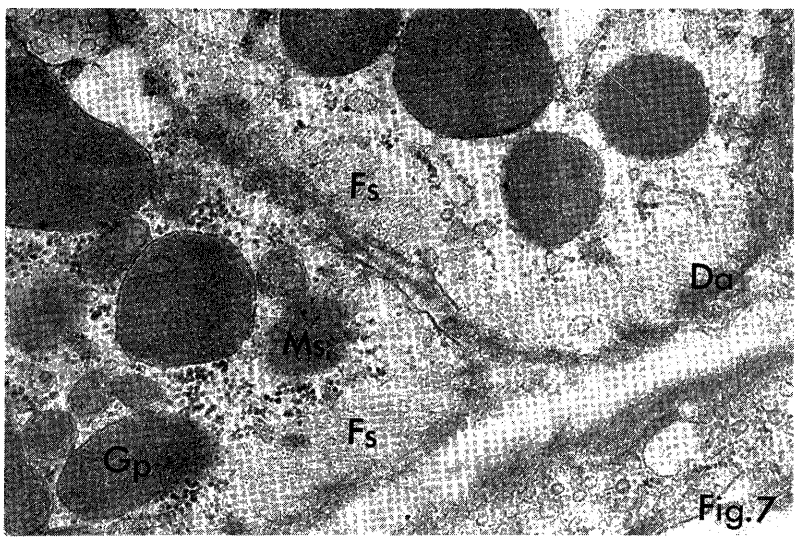

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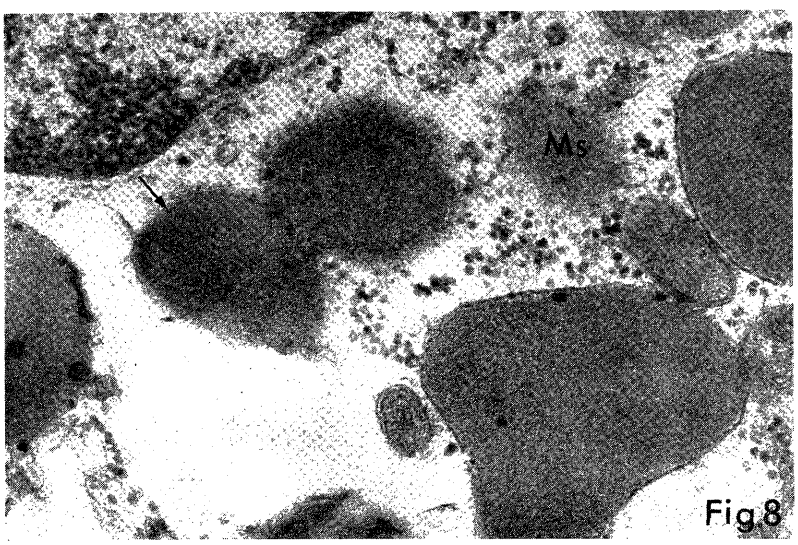

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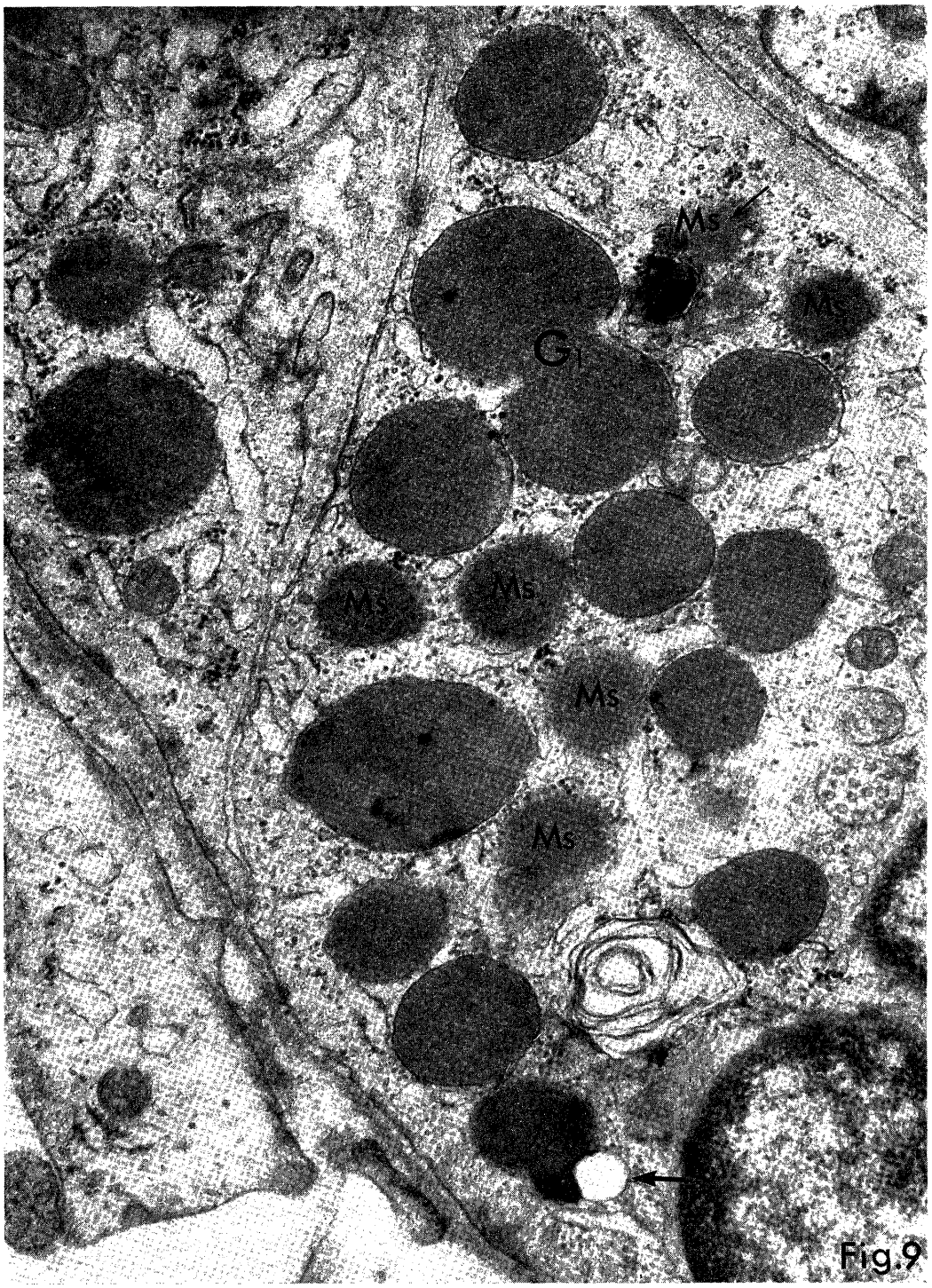

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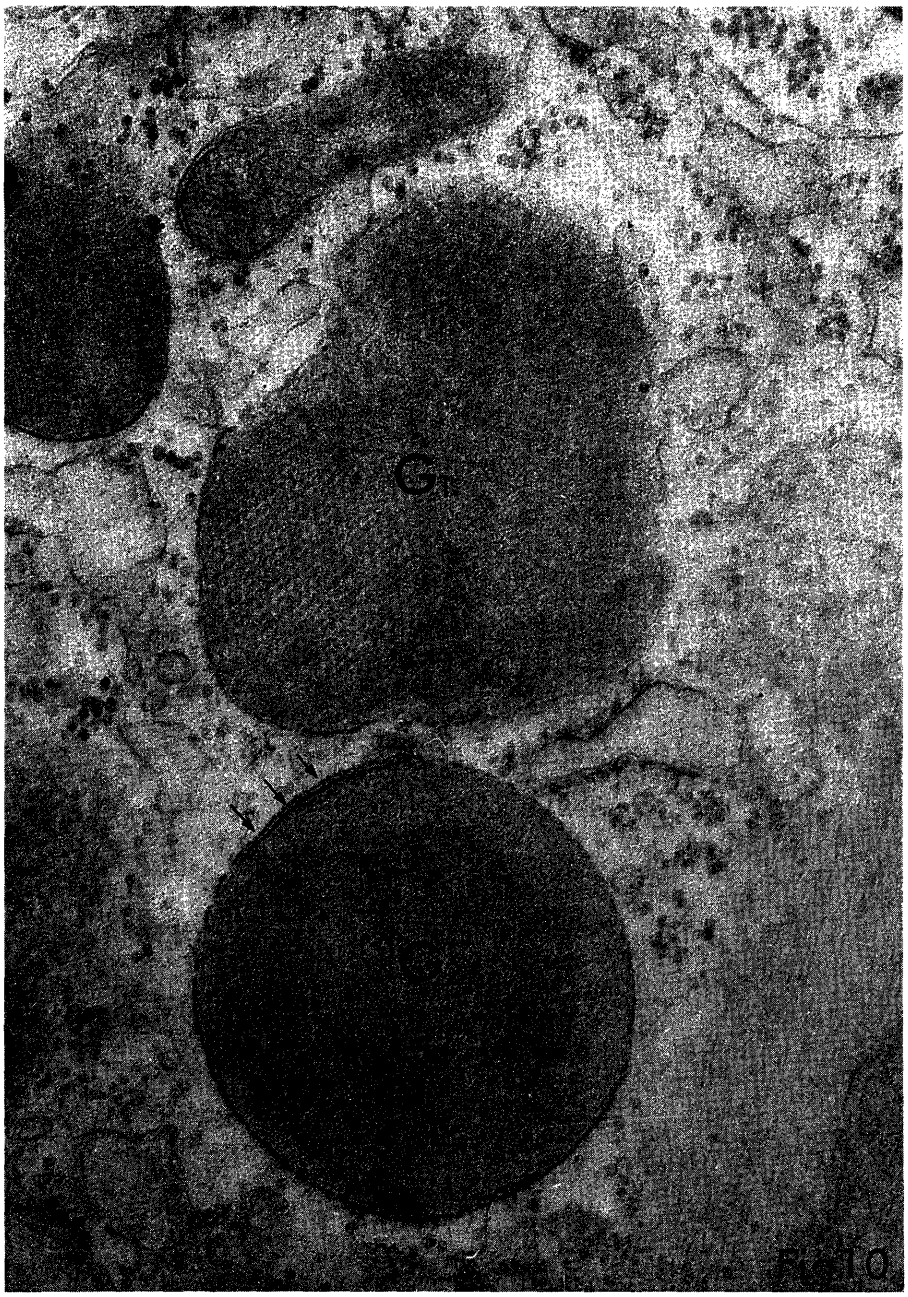
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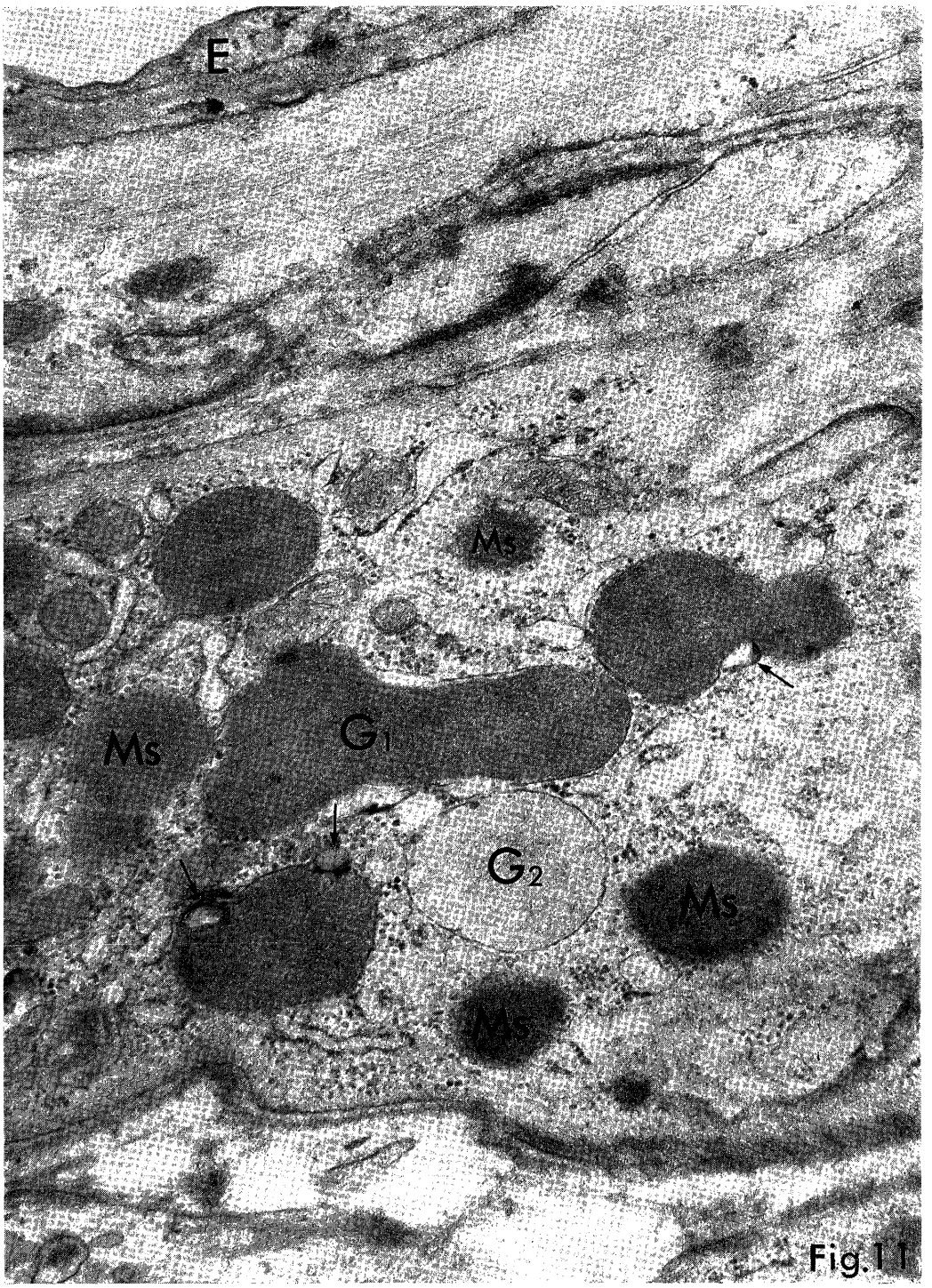

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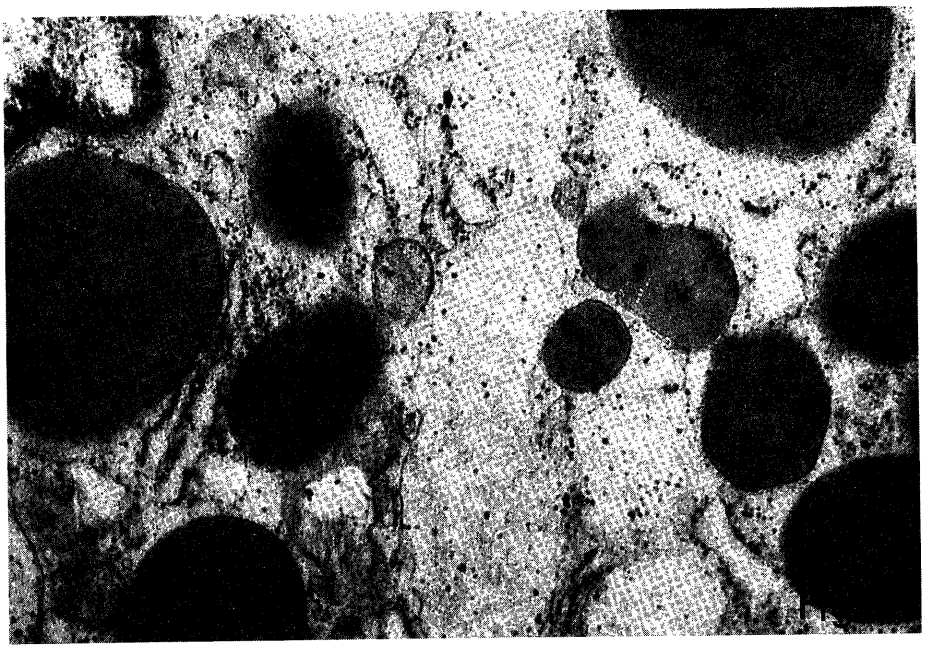

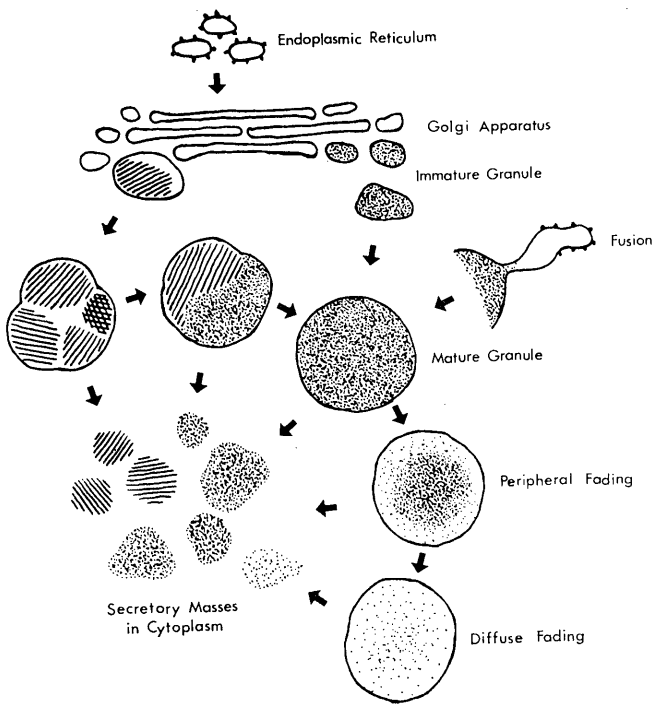

Fig. 13 Schematic Representation of Fomation and Extrusion of JG Cell Granules