Studies on a Scorpaenous fish *Sebastiscus marmoratus* CUVIER et VALENCIENNES —VI

Electron-Microscopic Study of Spermatogenesis

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Abstract

The Spermatogenesis and the structure of spermium of marine ovoviviparous teleost fish, *Sebastiscus marmoratus*, was observed by electron microscope. The author had formerly studied this problem by optical microscope, but the division of spermatozoa was observed erroneously in that study. During the sexual resting period, there is no spermatozoon in the seminiferous tubules of this fish, but in June the spermiogonia appear from the basement membrane under the seminiferous epithelium. The spermatogenesis of this fish is classified into two periods, one is multiplying period and the other is developing periods. In maltiplying period, the spermiogonium which appears on the basement membrane is divided repeatedly ane grow into many spermatozoa in a cyst. A cyst originates from a spermiogonium, and the growing stage of the spermatozoa in a cyst is quite equal, but different from that of the spermatozoa in any other cyst within a seminiferous tubule.

During the developing period after the multiplying period, the nuclear contents of the spermatozoa in the cyst are granulated and all the granules are collected at one end of the nucleus and finally become the sperm head. There is no head cap in the sperm head of this species. And then the mitochondorias in the protoplasm gather at one end and when the nuclear contents begin to grow in granular condition, the tail flagella appears from the nucleus and is surrounded by the mitochondorias. The tail flagella grows longer along with the progress of nucleus (sperm head) . The number of mitochondorias around the tail flagella of this species is less than that of mammals. The mitochondorias are not in immediate contact with the tail flagella but packed by the mitochondorial sheath at the surroundings of the base of tail flagella. The tail flagella consists of a central pair of tail axial fibril and nine pairs of fibril surrounding it.

Introduction

Recently, there have been relatively many electron microscopic studies on spermatogenesis, spermiogenesis and particularly the structure of spermium for mammals, but unexpecteldy there are few such observation for other anfmals, especially few for fishes. only FUZIMURA et al[7] reported their electron microscopic study of the spermium of *Cyprinus carpio L.*

Generally, studies of fish spermatozoa are less than those of ova. This is due to the fact that the fish spermatozoa themselves are very small, and besides to the difficulty that the time of collection has to coincide with the sexual period of that fish. The author had reported [1),2)] a few studies on the spermatogenesis and the seasonal cycle of mature testis of marine ovoviviparous and viviparous teleost fishes. NISHIKAWA studied on the seasonal spermatogenetic cycle of Medaka [3)] - *Oryzias latipes*, Loach [4)] - *Misgurnus anguillicaudatus* and Conger eel [4)] - *Muraenesox cinereus*. Recently, MOSER [5),6)] reported his studies on seasonal histological changes of the gonads of California rockfishes. But these studies were observations of paraffin section of fish testis by optical microscope. It is difficult to observe the fine structure of spermatozoa and spermatogenesis by optical microscope, and there still remain many unknown matters. The author was attempted to carry out this study especially because his previous report on the division of spermatozoa which was observed erroneously [2)] had to be corrected, and as the fishspermatogenesis and the structure of spermatozoa seemed considerably different from those of other animals like mammals. The author wished to express his appreciation Mr. T. SUEMATSU, School of Medicine, Nagasaki University, who kindly guided the author in technical operation of the electron microscope.

Material and Method

The author used [1),2)] the testis of *Sebastiscus marmoratus* as the material in this study since paraffin sections of their testis were formerly observed by optical microscope. This fish is the most popular rock fish in the Japanese coastal sea and is the typical bottom fish which does not migrate seasonally throughout the year. Moreover, it is possible to collect samples from the same population every month and it is convenient to prefix and fix the material of this species. Accordingly, this species is the best material for electron microscopic study of the marine fish gonad.

Fig.1 shows the monthly change in weight (Gonad Index) and the degree of ripenes of the gonads of *Sebastiscus marmoratus* in this region. It is evident that the testis of this fish is most ripe in September and October. The fishes were collected six times by the bottom long lines at the Nomo Fishery Laboratory of Nagasaki University from September 1966 to October 1967. Table 1 shows the date of collection, fixation, preparation of sections and photographing. After the catch, the fishes were bred for a day in the tank at the laboratory, and then the testes were collected. The collected material was immediately prefixed by Glutaraldehyde for two hours and fixation was made by osmic acid. Millonig buffer solution was used at the first and second collections, and Phosphoric acid buffer was used thereafter. And then the material was dehydrated by acetone and imbedded by epon 812. The microtome was JUM - 5 for optical microscope and LKB- 4801 for electron microscope. In the samples collected in September, spermatogenesis was not quite advanced yet, but the samples collected in October were in the midst of spermatogenesis.

Table 1 Specimens

Collection and Fixation			Making the Section and Photographing		
3.	Sept.	1966	8.	Sept.	1966
13.	Sept.	1967	21.	Sept.	1967
14.	Sept.	1967	21.	Sept.	1967
26.	Sept.	1967	3.	Oct.	1967
27.	Sept.	1967	3.	Oct.	1967
15.	Oct.	1967	22.	Oct.	1967

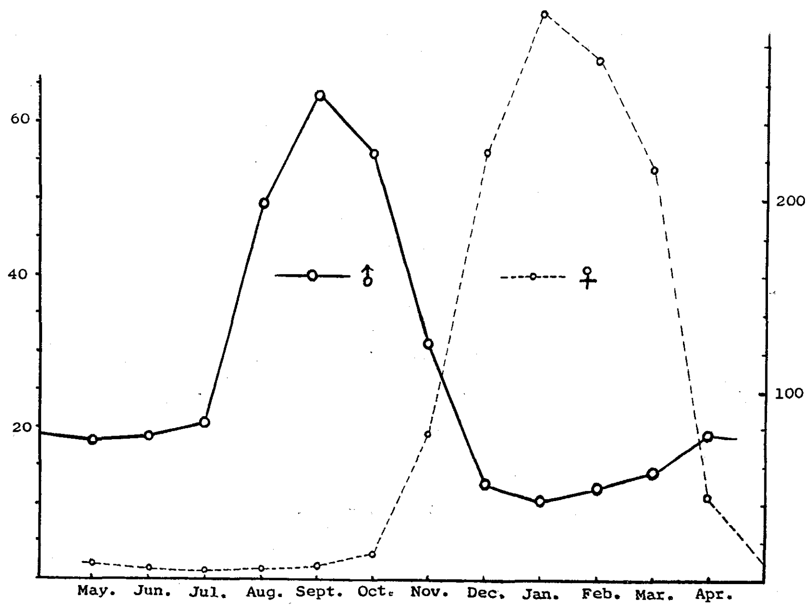

Fig. 1 Monthly Weight (Gonad Index) of Testis of *Sebastiscus Marmoratus*

From one of the testes collected in October the paraffin sections were made. And from other piece of testes the epon sections were made, stained by Toluidin blue or Plumbum Aceticum and Uranyl Acetate, observed by electron microscope and photographed. The electron microscope used for this study was JEM - 6C .

Observation and Discussion

Spermatogonium and its appearance

During the sexual resting period (November~May) , there is no spermatozoon in the seminiferous tubules of this fish, and there is a layer of seminiferous epithelium on the inner surface of the seminiferous tubule on the basement membrane, and the center of the seminiferous tubule is empty. These findings are the same as results of the optical microscopic observations which had been formerly reported by the author.

In June the gonad of the male fish begins to move actively, and the spermiogoniums appear from the basement membrane under the seminiferous epithelium in the seminiferous tubule, and accordingly the seminiferous epithelium is pushed up from the basement membrane to the lumen of the seminiferous tubule. These spermiogoniums can be observed without difficulty even in the testis which is collected in September. Pl.II-3 and Pl. II-4 show the spermiogoniums. the nucleus of spermiogonium is very large and plotoplasms of that cell are rather few. Many mitochondorias are contained in a plotoplasm. The mitochondorias are circular in shape. The cristaes in mitochondoria are not complete both in style and arrangement. the style of mitochondolia and arrangement of its cristaes remain the same at all stages of spermatozoa. The mitochondorias of spermiogonium are arranged around the mitochondorial rosettes. The findings on the spermatozoa which appeared between the basement membrane and the seminiferous epithelium but not divided yet are generally the same as the results of the optical microscopic observations previously made with paraffin sections [1),2)] These spermiogoniums begin to divide themselves from the first of September.

Division of spermatozoa

Pl.III-5 shows the division of spermatozoa. Three spermatozoas can be seen in this section. Pl.III-6 shows a more advanced stage of division. From these figures it is manifest that a spermiogonium is divided into many spermatozoas in a cyst, that all the spermatozoas in a cyst undergo exactly the same course of advancement and that one cyst originates from a spermiogonium. Pl.I-1, 2, optical microscopic photographs, shows that many cysts which are in different stages of advancement from each other are present in a seminiferous tubule. The author was erroneouos in his previons optical microscopic observation of the division of spermatozoa through use of paraffin sections. It was reported in his former papers [2)] that the nucleus of the spermatozoa is divided. The error is due to inadequate observations of the cell membrane between cells as the spermatozoas are very small and the cyst is densely filled with many spermatozoas.

During the maltiplying period, the mitochondorias in the protoplasm are very few in number and very small in size, althouge there are many large mitochondorias in the spermiogonium which appears from the basement membrane. However, at the last stage of maltiplying period, the mitochondorias begin to be clearly visible. In the protoplasm of Pl.IV-8 many mitochondoria are seen together with the golgi field. The spermatozoa in this photograph seems to be at the last stage of multiplying period.

Growth of spermatozoa

Upon completion of division, the spermatozoas in a cyst begin to grow into sperms. It is the developing period. In the beginning of this period, the mitochondorias which become clearly visible again in the protoplasm gather at one part of the protoplasm. And the tail flagella begins to appear from the nucleus and is surrounded by the mitochondorias as shown in Pl.V-9. And then the nucleus begins to become granular and the tail flagella is gradually elongated. then, the granules in the nucleus become clear and they begin to gather at one border of the nucleus. The tail flagella grows much longer. These developments are shown in Pl.V-10. The part of the nucleus where granules gather densely becomes the head of sperm of this fish, and the wider part of the nucleus where granules are not present shrinks eventually (Pl.VII-13). Pl.VII-14 and Pl.VIII-15 show the sperm cell which is almost completed, granules are no longer seen in that complete head of the sperm. There is no head cap at the tip of the sperm head of this species which can be usually seen at the head of mammals sperm. In the mammals sperm, there are many mitochondorias around the tail flagella being directly attached thereto, but in the fish sperm the number of mitochondorias is much smaller than that of the mammals and they are not in direct contact with the tail flagella but are interrupted by the membrane of the sperm cell. In other words, they are packed by a mitochondorial sheath and there is a cavity between the mitochondorial sheath and the tail flagella. Those are shown in Pl.VI.-11, 12 and Pl. VII-13, 14. The tail flagella of this fish is very long in comparison with the sperm head. The cross sections of the tail flagella at the base, middle and tip are shown in Pl.IX-17, 18, 19. The tail flagella consists of a pair of central tail axial fibril and nine pairs of fibril surrounding it.

Summary

1. The testes of *Sebastiscus marmoratus*, a marine ovoviviparous teleost fish, in the reproductive season were collected, and the spermatogenesis and the structure of spermatozoa were observed by electron microscope.
2. During the sexual resting period, there is no spermatozoa in the seminiferous tubule and a layer of seminiferous epithelium is present on the basement membrane in the seminiferous tubule.
3. In June, the spermiogoniums appear from the basement membrane under the seminiferous epithelium of the seminiferous tubule.
4. The nucleus of spermiogonium is large and its protoplasms are rather few. Many of mitochondorias in the protoplasm are arranged around the mitochondorial rosettes.
5. The mitochondorias of this spermatozoa are circular in shape, and the cristaes of mitochondoria are irregular.

6. The spermiogonium begins to be divided at the first of September, indicating the multiplying period of spermatozoa.
7. The spermatozoas continue to be divided in a cyst, and one cyst originates from one spermiogonium.
8. During the multiplying period, mitochondorias in the protoplasm of the spermatozoa are very few in number and very small in size.
9. After the multiplying period, the spermatozoas stop their division and grow into sperms, indicating the developing period of spermatozoa.
10. At the beginning of the developing period, mitochondorias become clearly visible again in the protoplasm of spermatozoa and they gather densely in one corner. The nucleus grows into many granules and the tail flagella begins to appear from the nucleus and then is surrounded by mitochondorias.
11. And then the granules in the nucleus become more clearly visible and gathered in one border of the nucleus. The tail flagella grows longer.
12. The granules densely gathered in one border then become nongranular and finally become the sperm head. The wider part of the nucleus where granules are not present shrinks eventually.
13. The tail flagella of this sperm is very long in comparison with the sperm head, and consists of a pair of central tail axial fibril and nine pairs of fibril surrounding it.
14. There is no head cap in the sperm head of this species.
15. The number cf mitochondorias of this sperm is very few than that of the mammals, and they are not in direct contact with the tail flagella but are interrupted by the membrane of sperm cell. The base of the tail flagella is surrounded by a mitochondorial sheath.

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Explanation of Plates

c - Cyst, bm - Basement membrane, st - Seminiferous tubule,

sp - Spermiogonium, sz - Spermatozoa, n - Nucleus, nu - Nucleolus,
nm - Nuclear membrane, m - Mitochondoria, mr - Mitochondorial rosette,
ms - Mitochondorial sheath, p - Protoplasm, cm - Cell membrane,
g - Golgi complex, t - Tail flagella, cf - Central fibril,
sf - Surrounding fibril, ca - Cavity, cr - Cristae, h - Sperm head,

Plate I

1. ×300, The sections were prepared from the specimen imbeded in epon 812 by JUM-5 (microtome) , stained by toluidinblue and observed by optical microscope. There are many seminiferous tubles each containing many cystes.
2. ×600, Enlarged photograph of Fig.1. In the seminiferous tubule there are many cysts containing spermatozoas developing stage of which are different from each other.

Plate II

3. ×8600, Spermiogonium which appeared from the basement membrane. The nucleus is large and there are many large mitochondorias in the protoplasm. Large mitochondorial rosettes are characteristic.
4. ×9400, Spermiogonium.

Plate III

5. ×12000, Three spermatozoas can be seen in a cyst.
6. × 8400, Spermatozoas in a cyst. It is difficult to observe the cell membrane between spermatozoas.

Plate IV

7. ×11200, The cell membrane between spermatozoas can be seen clearly. These spermatozoas are in the multiplying period and mitochondorias in the protoplasm are very few and very small at this stage.
8. ×7200, The mitochondorias in the protoplasm begin to show clearly together with golgi complex. These spermatozoas are at the last stage of the multiplying period.

Plate V

9. ×11000, These spermatozoas are at the beginning of the developing period. The mitochondorias gather at one corner of the protoplasm and surround the tail flagella which has appeared from the nucleus.
10. ×17600, The many nuclear granules begin to gather at the border of the nucleus, and the tail flagella grows longer.

Plate VI

11. ×12800, More advanced stage thad Fig 10. The tail flagella is surrounded by a mitochondorial sheath at the base.
12. ×64200, Enlarged photograph of Fig. 11.

Plate VII

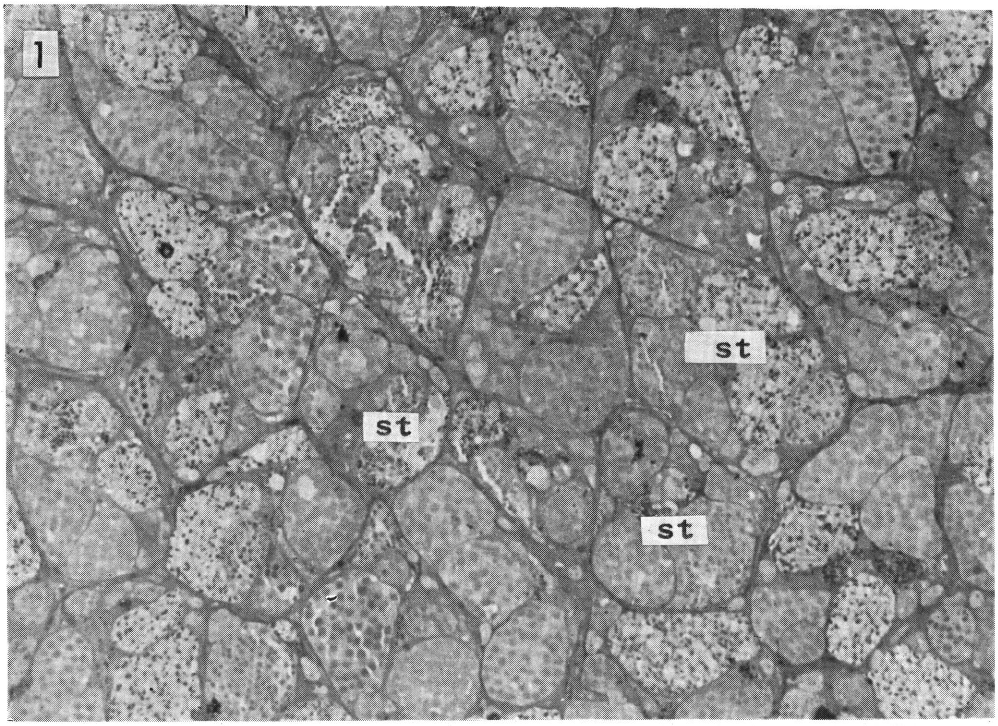
13. ×14400, The wider part of the nucleus where granules are not present shrinks.
14. ×18600, Spermatozoa which has mostly grown to be a sperm.

Plate VIII

15. ×3000, All spermatozoas in a cyst have practically grown to be sperms.
16. ×13600, vertical section of tail flagella.

Plate IX

17. ×13600, Cross section of tail flagella at the base.
18. ×16000, Cross section of tail flagella at the middle. It is clear from this and next figures that the tail flagella consists of a pair of central tail axial fibril and nine pairs of fibril surrounding it.
19. ×21000, Cross section of tail flagella at the tip.



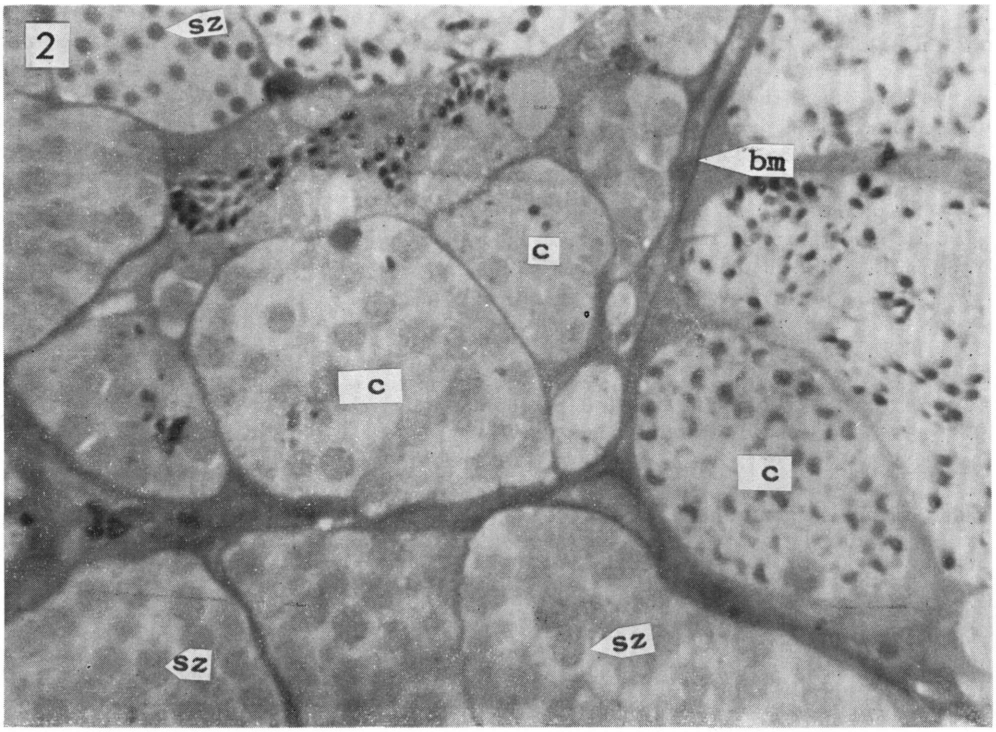
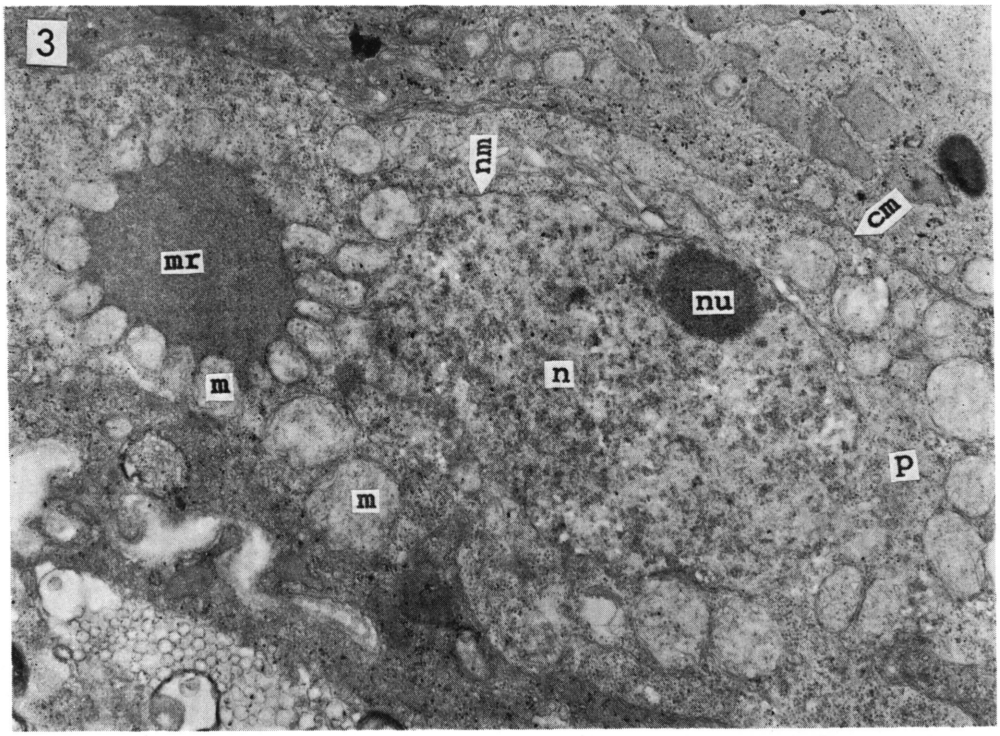


Plate I



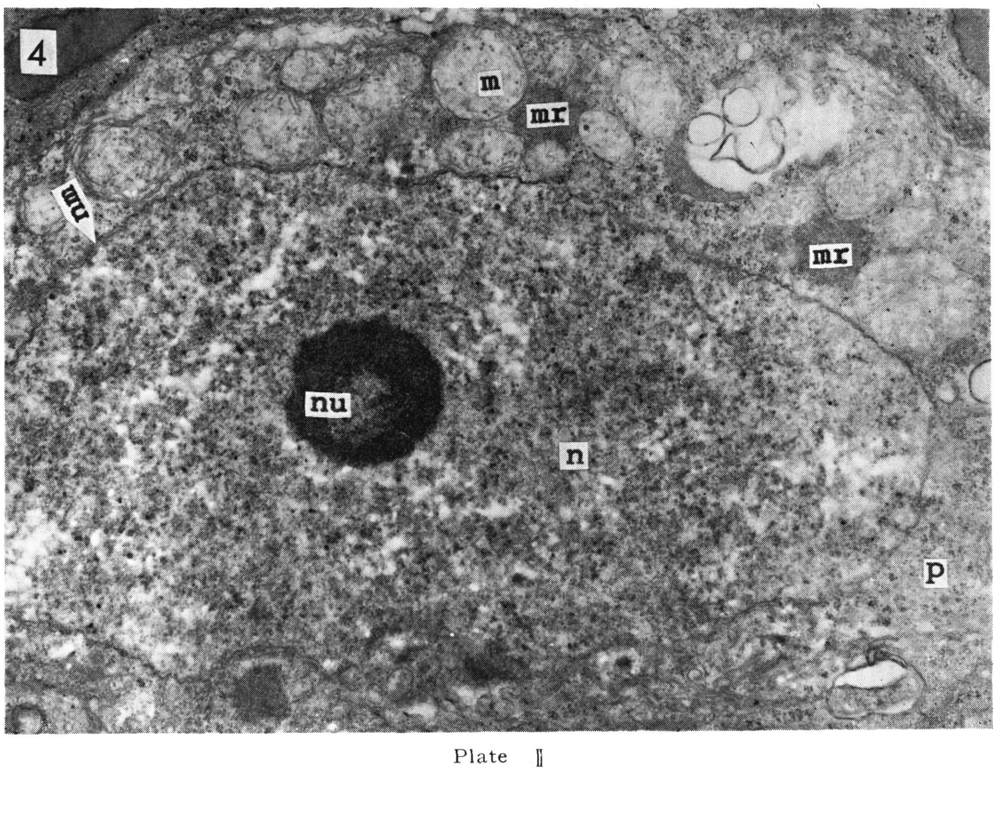
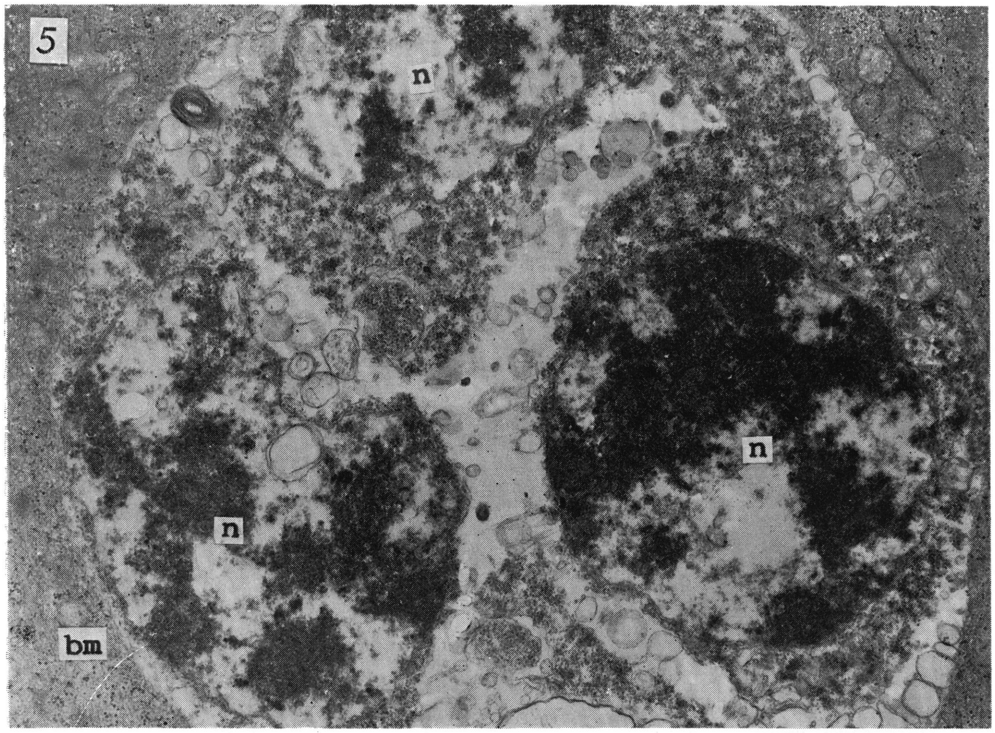


Plate II



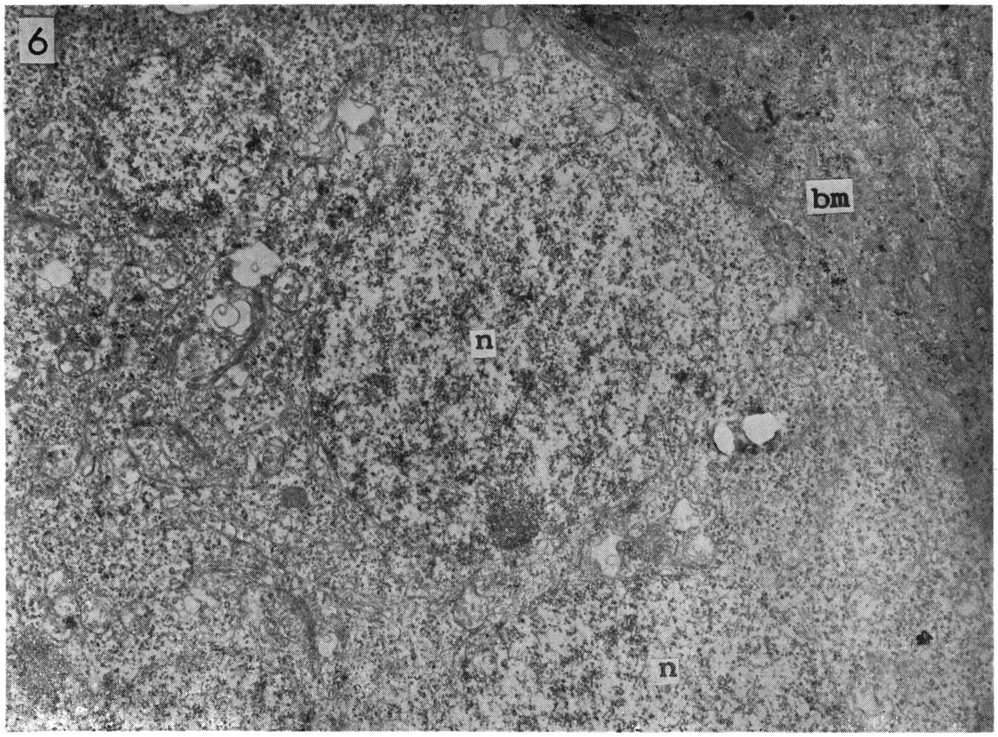
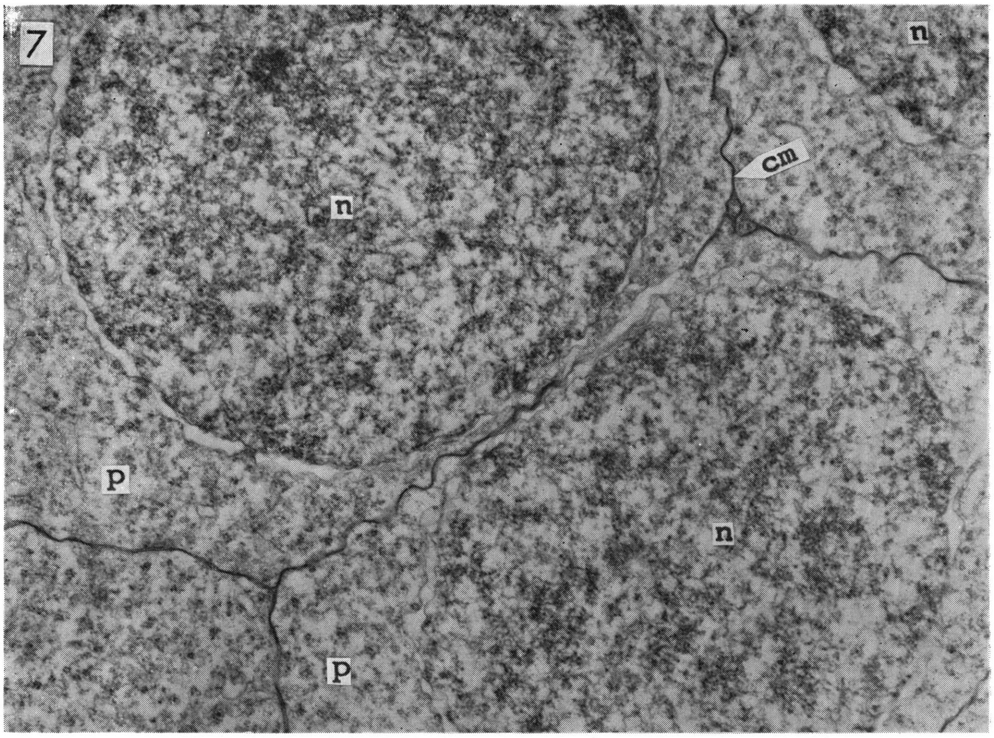


Plate Ⅲ



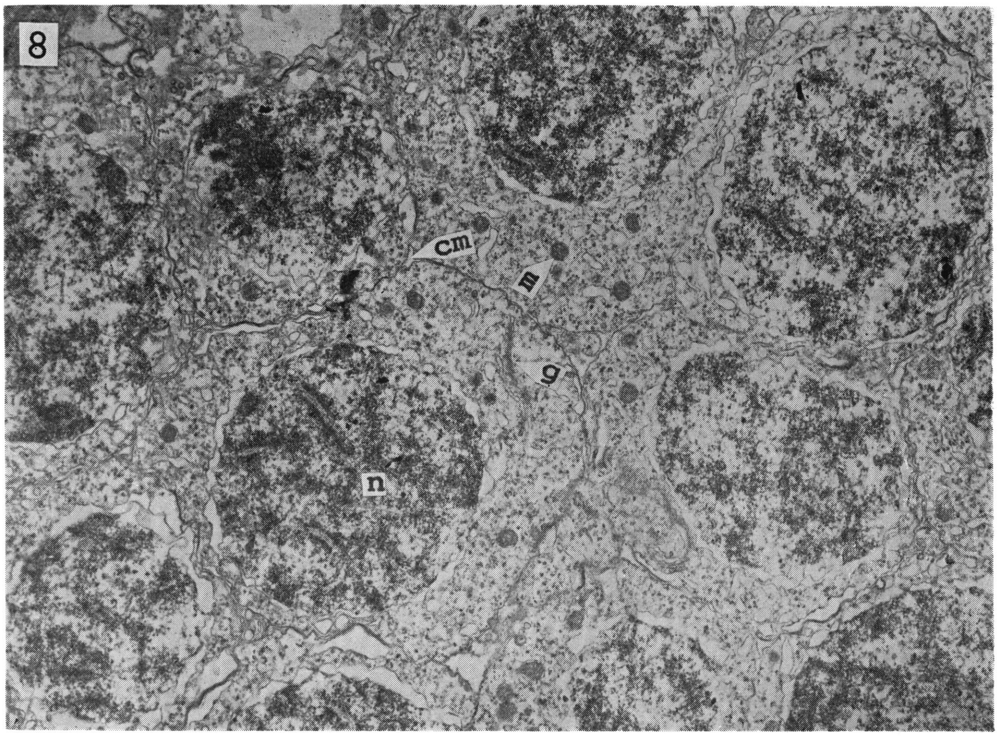
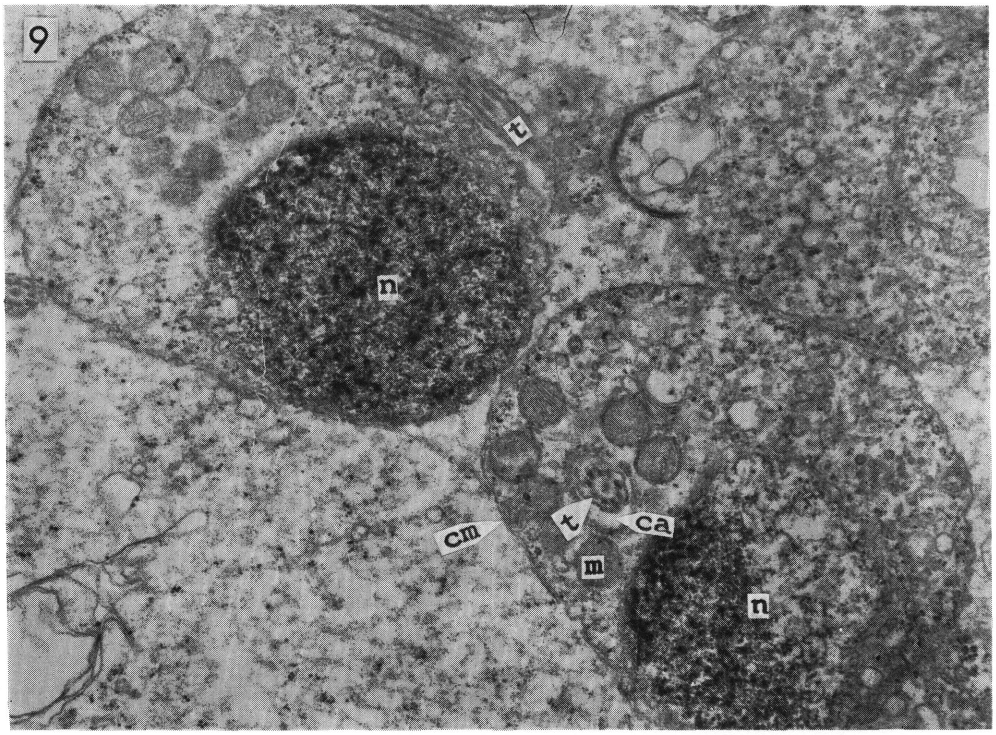


Plate IV



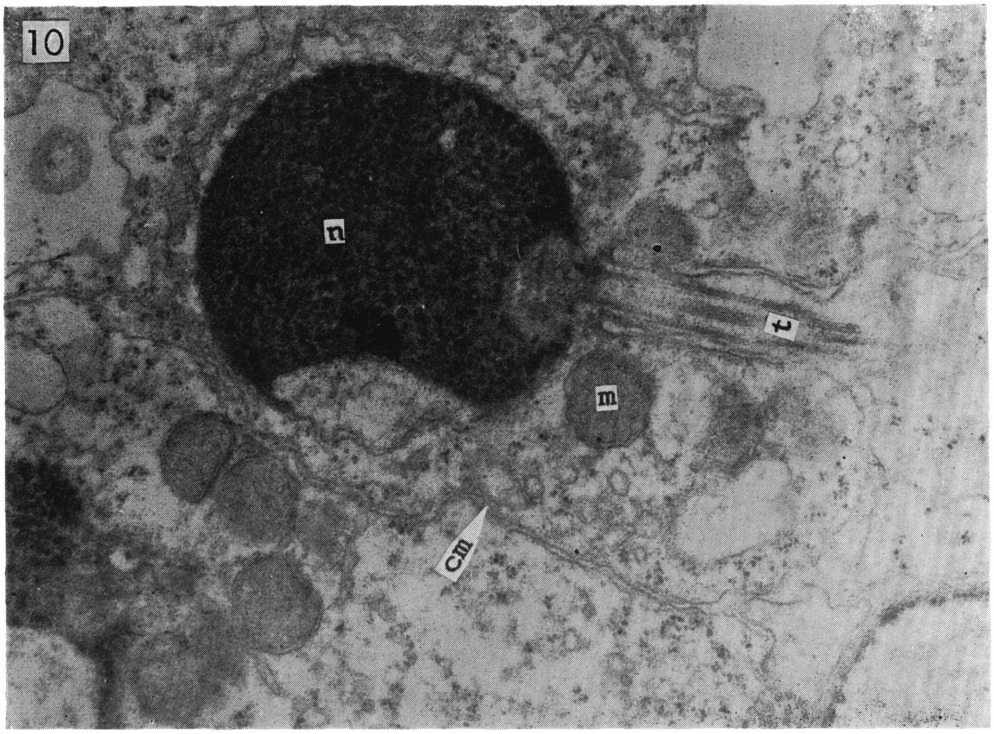
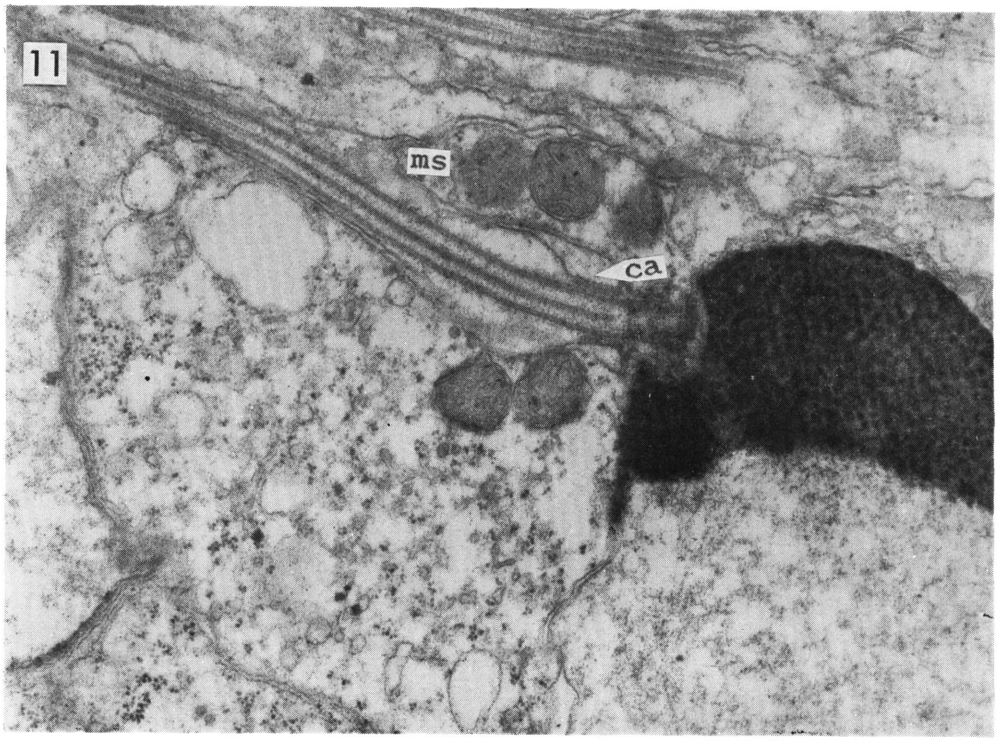


Plate V



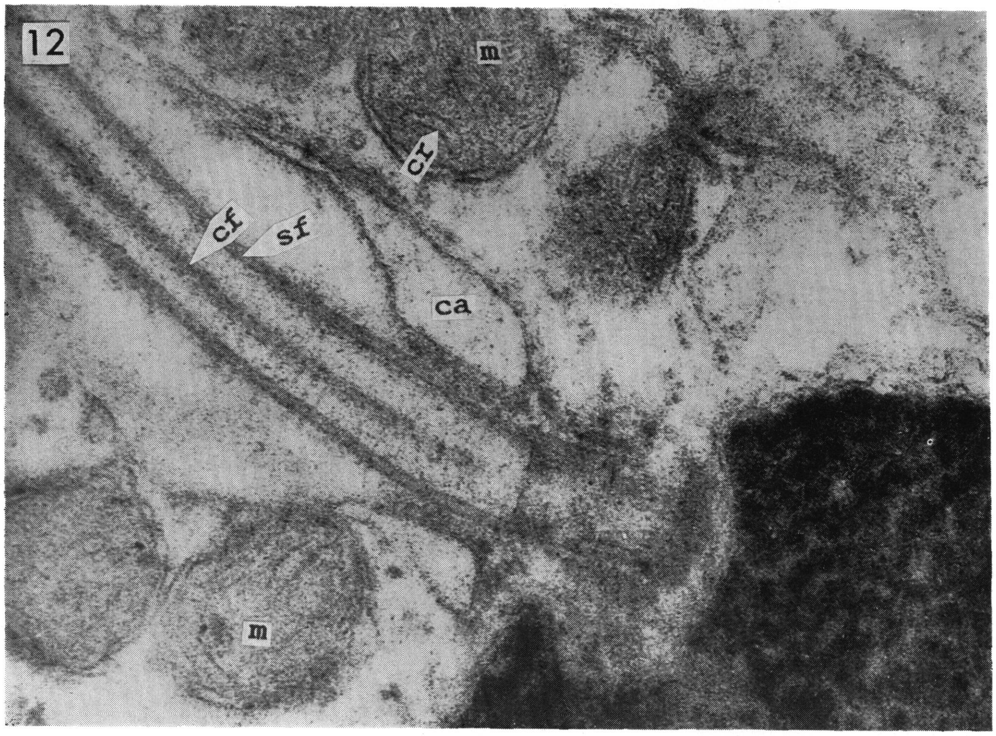
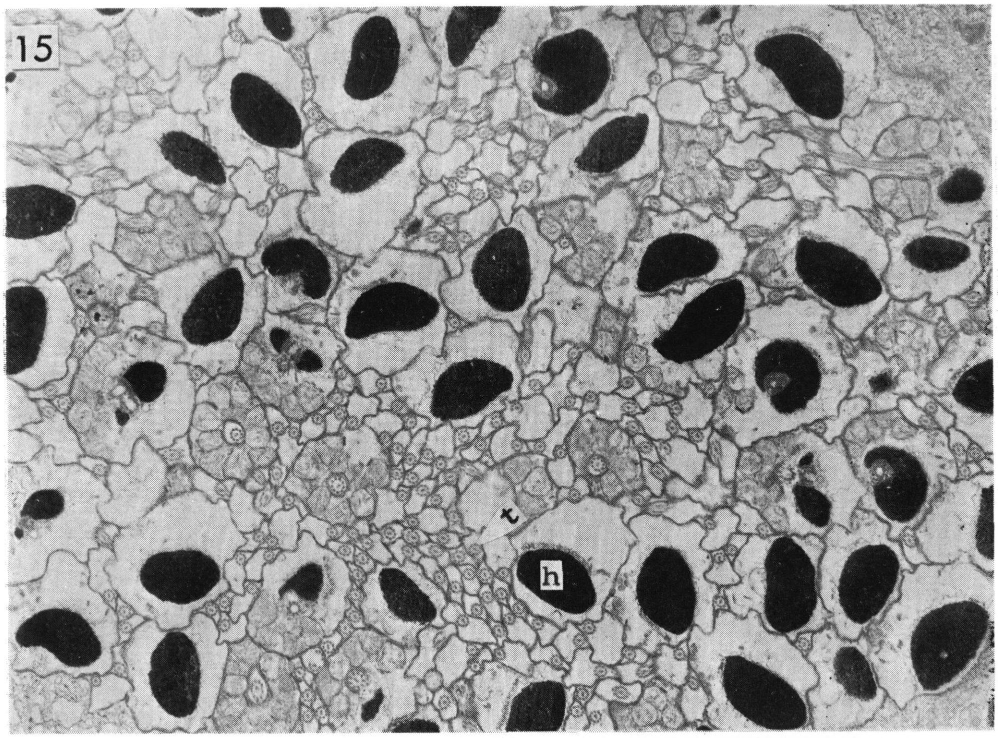


Plate VI

Plate Ⅶ



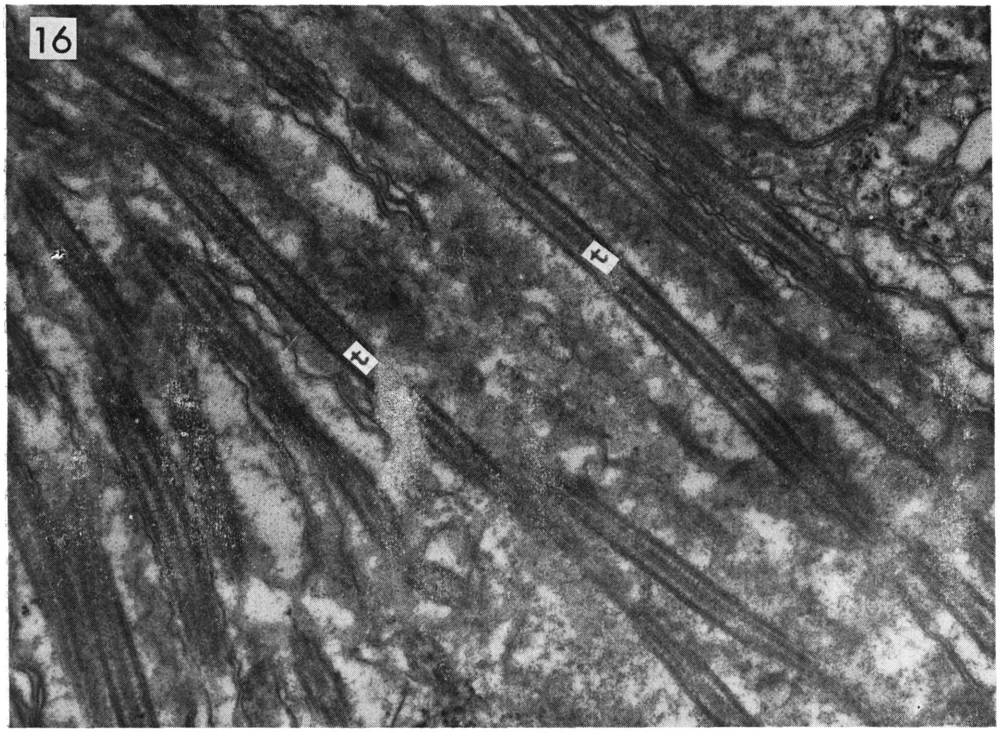
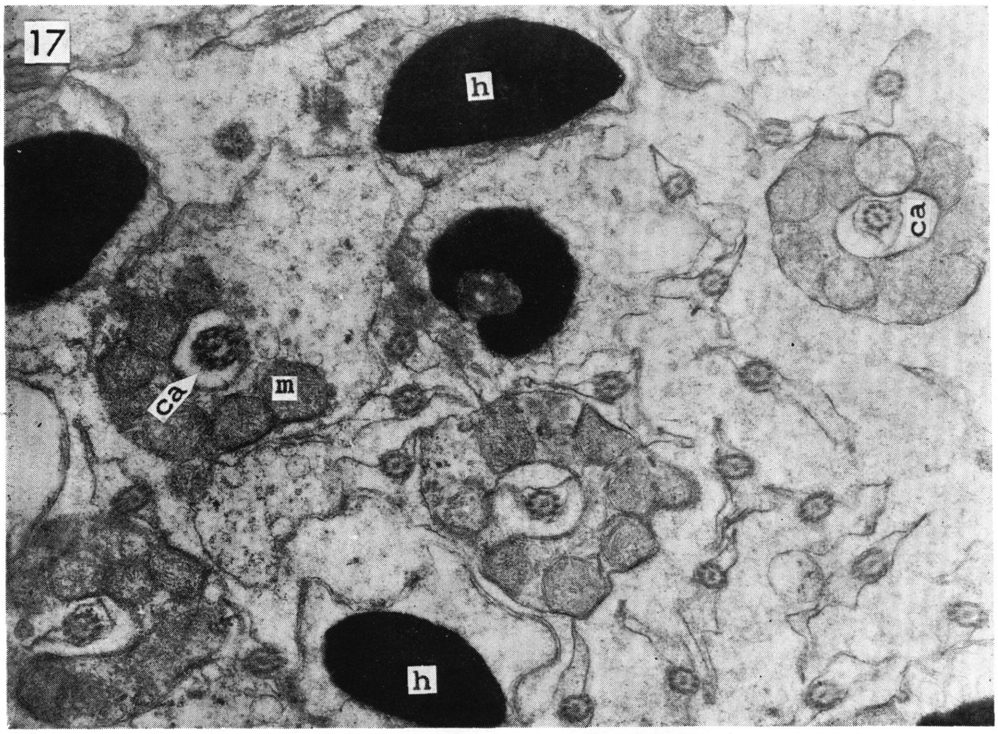


Plate VIII



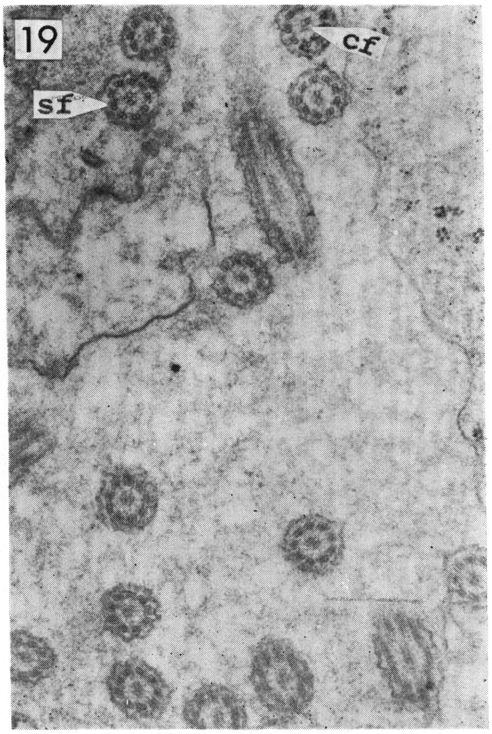


Plate IX