The Microscopic, Submicroscopic Structure and Histochemistry of the Inclusion Body seen in Myoclonus Epilepsy*

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In the central nervous system of so-called Lafora type myoclonus epilepsy, the globular inclusion bodies in the cytoplasma of the nerve cell exists. The origin of this inclusion body is supposed by Wakui, Harriman and Seitelberger as the metabolic anomaly. In order to clarify the order of the mechanism of the generating of the inclusion body the studies on the biochemistry during the life[14], but also the structure and the histochemical analysis of the inclusion body, and the relation between the cellular element and the inclusion body must be made.

The reported cases hereto for are similar in their clinical signe and they are all the same as in the finding of the inclusion body in the brain, but reported the pathological signe in the liver by Marchand[12], in the liver and the heart only by Harriman et al.[4], Imai et al.[7], Seitelberger[25], Heycop ten Ham et al.[5] and Namba et al.[15]. And the more certain family onset was proved by the autopsy carried on by Namba et al.[13] and Heycop ten Ham et al. only, so the re-investigation is necessary from the histopathological and the histochemical standpoint, as to the question wether the reported case so far belongs to the same disease.

This report clarifies the structure of the inclusion body and the histochemical view which were found in the author's examples, then comparison is to be carried on with the reports heretofore, and is to contribute for the interpretation of the various points mentioned above.

The Clinical Symptoms

Cases are the first daughter and the second son of a family[13]. The first is 19 years old girl. The change of personality occurred at the year of 10. The grand mal at 14.10 months, after that appeared the petit mal, and the myoclonic jerks of the lower half length. And further more difficulty in walking, dysarthria, ataxia, rigidity, functional disturbance of the autonomic nervous system and pain gradually strengthened by the stimulation to every part of the body appeared, and in the end death occurred in the state of the decerebration with

* This thesis is dedicated to Prof. Michitomo Hayashi, M. D. in celebration of his 80th birthday.

the excessive dementia and the severe wasting.

The second one was the younger brother; 15 years old. He showed the poor result from the first or the second year of the primary school. The change of personality occurred at 11. Dysarthria occurred at 12. Hypersalivation, powerless feeling of limbs, ataxic gait, dysdiadochokinesia, tremor of hand, myoclonic jerks of fingers, sick and vomiting comparatively rapidly appeared alternately. The remisson and the aggravation is repeated but the speed of the sick is slower than hissister, and attending school was possible till death. In the summer while taking bath, the grand mal occurred and drowned to death.

Macroscopic Observations of the Brain

The brain weight of the first daughter is 1020 g. while that of the second son is 1429 g.. The common point to the sister and the brother is that the cerebral cortex is white, and the difference between the white matter is not clear. The brain surface of the elder sister is rather hard, and the cerebral gyri show slightly atrophy.

Microscopic Observations

Ten per cent formol-alcohol fixed paraffin section and 10 per cent formol fixed frozen section is used for the examination. For the sample of the electron microscope, the above-mentioned materials washed after the removal of paraffin and refix in the liquid of the same amount of veronal acetic acid mixed in 2 per cent osmium tetroxide and after that process embedded in metachril resin. Slices were treated an hour in 1 per cent potassium permanganicum and then treated in 5 per cent citric acid and then examined through the electron microscope.

Here the author described the brain on both cases. The relation between the inclusion bodies and the cellular element, the structure of the inclusion bodies are mentioned.

I. The relation between the inclusion body and the cellular component.

As the most numbers of the inclusion body lie in the gray matter of the brain, so when the specimen is stained by Best's carmine stain, the gray matter is seen so very red that the border to the white matter is very distinct.

The inclusion body in the gray matter is divided clearly the one which has the relation with the cellular element and the one which has the indistinct relation. The former is generally large and the layer structure is vivid and the latter is generally small and the inner structure is not vivid. The few inclusion bodies are just in the contrary. The inclusion body which has the relation with the cellular component is found in the cell body of nerve, its dendrite and in the cell body of astrocytes (Figs. 1, 2, & 3). The number of the inclusion body in the nerve cell body is from one to several and in such case the cell body become huge and the

cytoplasma is observed traceably around the inclusion body and sometimes on one side of the inclusion body adheres the nucleus transformed in the kidney form. In Cajal's silver nitrate pyridine method, in a part of the small inclusion body which seems to exist in the extracellular space the rest of the cellular component is often found. Sometimes the rosary-like structure is recognized in accordance with the flow of dendrite of cell in P A S reaction. Whether or not this is the same with the above-mentioned small inclusion body is not clear yet.

Axon, brain, and peripheral nerve and the nerve cell in Meissner's and Auerbach's plexus the similar inclusion body is unrecognized.

II. The structure of the inclusion body.

The optical microscopic and the phasencontrast microscopic observations are here written.

1. The optical microscopic observations on the nonstained frozen section.

The middle-large inclusion body of about 30μ in diameter which is found mainly in intracellular is round and has the concentric layer structure. In the outermost part there is the narrow belt of the cytoplasma of the cell which shows the granular state and this is connected with the cell nucleus that adheres in kidney-like on one side of the inclusion body. Generally, the outer side edge is flat and smooth, and the inner edge intersects cogwheel-like with the outer phase of the external layer which is the proper section of the inclusion body. The one part of cellular body goes deeply into the "external layer" of the inclusion body. The external layer is generally wide, there are a few triangular or square sections. Each of these is called the "triangular part". In the triangular part are many fine granules and their gaps which are somewhat not transparent. In the triangular part of a few inclusion bodies the tear-like structure is seen. Between each triangular part the clear and the narrow "aperture" is sometimes seen. This shows the radiating structure wholly. These are connected to the narrow and clear ring which forms the boundary between the external and the "internal layer". These are called the external layer. Inside the external layer, the ring structures and the dark thin band exist. In the inside of it is the round and rather cloud-like homogeneous part exists. This occupies the main part of the internal layer. In the center of the internal layer of a certain inclusion body the star-like structure with the faint surrounding is seen (Fig. 4). These are called the internal layer. The inner structure of the small inclusion body is not clear.

2. The phasencontrast microscopic observations on the non-stained frozen section.

In this process the small inclusion bodies can distinctly be observed from about 1.5μ, if compared with the optical microscopic observation, but it is nearly homogeneous. In the slightly larger one both the internal and the external layers are distinguished. In the external layer the base of the radiation structure is observed

but in comparison to the external layer of the middle-large inclusion body as mentioned later it is dim and near the homogeneity. The internal layer shows dark, and sometimes a small bright part exists in the center (Fig. 5). The layer structure of the inclusion body which is slightly larger than the small inclusion body comes near to the optical microscopic image as mentioned above (Fig. 6). The surface of the middle and the large inclusion body covered with the gross granular cell body (Fig. 7). The cut surface which slightly to the center from the surface of the middle and the large inclusion body appears the triangular structure as seen in Fig. 4. A part of the gross granular cell body adheres to the external side of the inclusion body and the boundary between the external layer is complicatedly intermingled and is not clear. In the triangular part the fine granule and the slightly transparent ground substance which fills the gap of the fine granule. On the side margin of the triangular part there are the dark rosary-like grain, and they are supposed to be a part of the cell body. The thin radiating structure which exists between each trinangular part and inside of it the same thin clear and non structured ring appears most clearly in the phasencontrast microscopic observation (Fig. 8.). In the cut surface of nearly the center of the inclusion body, the aperture between the triangular parts are vivider, and at the same time a rather dark and homogeneous part appears in the inside of it. This agrees with the internal layer of Fig. 4. In this plan the dark thin band is faintly appears surround the internal layer (Fig. 9). In Fig. 10, the cut surface which is more nearly to the center than Fig. 9, and in Fig. 11 shows the cutting phases which pass the center. Here the triangular parts show square. Though the number of the aperture is different according to the inclusion body ordinary from 10 to 20 are counted. The more outwardly the apertures go the thinner they become. In Fig. 10, many apertures are seen radially. It is the characteristic that the width of the aperture is mostly narrower than the triangular part, and this becomes a good mark of discernment of each structure while the histochemical studies are being carried on. The enlarged internal layer shows the typical structure and can distinguish the star-like structure in the center and five layers including the dim light part around the star-like structure. All five layers are not always found, however, except the widest, the cloud-like or homogeneous part, and the differentiation of each layer has not always any relation with the size of the inclusion body. When the inclusion body becomes large extremely, the external layer becomes flat and tracing, in contrary to this the internal layer occupies the inclusion body mostly, at the same time, the star-like structure becomes more notable (Fig. 12). As mentioned above, in the phasencontrast microscope, the clear structural characteristic than the image through the optical microscope can be got. In the globoid inclusion body, as above-mentioned, fairly different statue according to the cut surface and to the maturity of the inclusion body can be seen. These are to consider well in the histochemical studies which will be

mentioned later on.

Electron Microscopic Observations.

Same as the optical microscopic image, it is possible to classify the small and the middle-large types, and the former has an ambiguous relation with the cellbody and the latter is clear.

The cut surface of the small inclusion body shows the net structure which is consisted of the girder of 100–200 Å in width. But it is solidly supposed the grobular which has the honey-comb-like contents. The girder divides two outer-walls that have the high electron density but narrow, and the center line whose the electron density is lower than that. The former has the cloud-like attachment. The cave has the diameter 400–2000 Å, and shows the round or irregular ellips. Something like shell around the outside of the inclusion body could not be found (Fig. 13).

When the inclusion body becomes slightly large, appear the metamorphosis in the center of the honey-comb-like structure. The typical one is the middle-large inclusion body (Fig. 14). The inclusion body is surrounded by the gross granular cytoplasma that has the high electron density. On one side of the inclusion body adheres the nucleus of the cell that has the large nucleolus. A part of the cell body intrudes into the external layer of the inclusion body; the part which seems the gear in the optical microscopic image shows the clear boundary with the inclusion body.

The external layer has the high electron density; compared with the internal layer. In the structure about four elements can be distinguished: they are: the first one is a part of cell-body which intrudes into the above-mentioned external layers, the second one is the honey-comb-like structure corresponding to the triangular part in the optical microscopic image. The honey-comb-like structure crowds in to the innerhalf of the triangular part. In the 50000 enlarged electron micrograph, the honey-comb-like structure in the external layer is recognized, but the girder has been cut down (Fig. 15). The third one is corresponded to the aperture in the optical microscopic image. It seemed the bubble-like structure which has the low electron density; but it is not so clear as in the optical microscopic image to the surrounding. In 50000 enlarged electron micrograph (Fig. 16) circumference of the cell fragment in about the middle of Figure shows the mentioned aperture. The right upper corner of Figure is the triangular part as mentioned above; about the left lower corner is the internal layer. The bright ring in the inner part of the external layer in the optical microscopic image, agrees with the totally destructed part in the honey-comb-like structure which has the low electron density near the center of Fig. 17.

Again the honey-comb-like structure appeares in the internal layer but when

compared with that of the external layer, it shows the strong morphological change (Fig. 18). The girder of the honey-comb-like structure shows three kinds of the transformation. The first is the expansion of the girder and shows the fine granule (left side of Figure). If the morphological change is strong the honey-comb-like structure is nearly lost, and is recognized the indefinite group of the fine granule. The second variation is the bubble-like structure which is consisted of the bunches of the grapes structure. Each bubble is consisted of the thin but the high electron density surrounding, and the interior is lower than that, but has the higher electron density than the cave. It is supposed that the bubble-like structure is based on the expansion of the girder itself. The third variation is hard to distinguish from the second one, morphologically, but its electron density is the highest and forms the part whose the endoplasmic reticulum is not recognized yet. This is supposed as the variation of the cell fragment in the external layer. The star-like structure in the electron micrograph (Fig. 19) is accordant with the structure of the same name in the optical microscopic image, and it shows high electron density. In it fused the proper portion of the inclusion body and the trace which is supposed to vary of the cell fragments. The traceable honey-comb-like structure is faintly seen around the star-like structure.

When the electron micrographic image is viewed, the small inclusion body is the nearest to the matrix of the inclusion body, and shows the pretty honey-comb-like structure. The external layer of the middle-large inclusion body and then from the internal layer to the center the honey-comb-like structure gradually varies. The above-mentioned complicated structure of the middle-large inclusion body shows the processes of the morphological breaking with a series of the chemical change which could not be found in the small inclusion body.

Histochemical Tests of the Inclusion Body.

The Table shows the results of the histochemical tests of the inclusion body. Some explanations have to be added.

1. P A S reaction. The formol-alcohol fixed paraffin section. The colour of both haematoxylin stain and P A S reaction are taken up for the small inclusion body faintly. The contents are homogeneous in the most small inclusion bodies, but some of them show the thin radiation structure in the center, and this is dyed more densely than the other parts. In the middle-large inclusion body the ground substance in the triangular part of the external layer shows faint positive and the small particles are slightly dark purple. Generally the outerhalf of the triangular part has the weak colour when compared with the innerhalf, and this matches well with the electron micrographic image. The aperture between each triangular part is in strong positive in P A S reaction and connects with the thin ring in the inner side of the external layer. After 24 hours of the

acetylation, P A S reaction in the small inclusion body and the external layer of the middle-large inclusion bodies are completely blocked but the internal layer has the incomplete blocking. P A S reaction in both inclusion bodies after the acetylation and the saponification is recovery incompletely. In P A S reaction after the methylation 3 hours, 6 hours and 6 days the inclusion body is weak in its colour than the contrast and the block is incomplete and the recovery of colour is not recognized after the saponification. P A S reaction in the most part of the internal layer is strong positive and the boundary of the external and the internal layer as mentioned before, this is not clear. The star-like structures are positive in middle degree. As the special observation at the outside of the inclusion body the P A S positive corona-like structures are recognized (Fig. 20). These appear positive in Best's carmine stain but the number is few. The inclusion body that has such thing, whether or not it is the inclusion body larger than the middle type; the relation between the cell is not clear.

2. Alcianblue stain.

The small inclusion body is faint positive. Its center shows often strong positive just as the boundary between the internal and the external layer or the internal layer of the middle-large inclusion bodies. The triangural part of the external layer of the middle-large inclusion body is slightly dark blue wholly but the grains in it are negative; the ground substance is weak positive. The aperture of the external layer is strong positive and it connect to the boundary of the external and the internal layers is the same colour (Fig. 21). The internal layer is almost homogeneous and strong positive and the star-like structure in the center is faint positive. The internal and the external layers of the inclusion bodies are completely blocked out after 25 hours methylation and are not recovered after the saponification.

3. Best's carmine stain.

The small inclusion bodies are weak or strong positive and their interior seems almost homogeneous. The degree of stain of the external layer of the middle-large inclusion body has the difference of tints and it shows red uniformly, but generally the small grain in the triangular part is positive in middle degree and its ground substances are weak positive. The aperture and the boundary section of the internal and the external layers are strong positive, and the former shows the radiation structure. The internal layer can also be stained in haematoxylin.

4. Hale's method.

In this specimen, as seen in Best's carmine stain, non-stained or pale blue thorn-like crystal is sometimes observed on the surface of the inclusion body.

5. Tannic acid methylen blue picrofuchsin method (Kawase & Sunahara's method[8]).

This method, same as Hale's method, appears combined iron with acid mucopolysaccharide as prussian blue, so if iron is involved in the inclusion body as

mentioned later on, the positive results shown in these two methods must be discreet in the judgement.

6. Mucicarmine reaction (Clara's method).

The small inclusion body is faint or dark blue. The triangular part of the external layer of the middle-large inclusion body is faint blue and sometimes can be stained in dark blue but as to the fine structure this is obscure. The aperture which seem to be radiation is dark blue and is the darkest and it connects to the narrow ring which is in the inside of it.

7. Mucicarmine reaction (Mayer's method).

The small inclusion body and the triangular part of the middle-large inclusion body is faint red brown. The portion which is considered as the aperture is radiating and dark reddish brown. The internal layer is reddish blue and the center is pale purple. In the internal and the external two layers the fine structure cannot be recognized.

8. Amyloid reaction (Mayer's method).

The small inclusion body is pink. The fine granule in the external layer of the middle-large inclusion body is negative; and its ground substance is pink. The aperture is pink. The internal layer is also pink, and sometimes is pinker than the external layer, and the center is weak pink.

9. Amyloid reaction (Lendrum's method).

The fine structure of the small inclusion body, the middle-large inclusion body is not clear.

10. Iodine reaction (Lugol's method).

The small inclusion body is not clear. The external layer of the middle-large inclusion body is slightly yellow. The internal layer is pale purple, and the center is yellow (Fig. 22). In the brother's case both the external and the internal two layers, are sometimes brown.

11. Toluidine blue metachromasia (Ono's method[22]).

On pH 7.0 many inclusion bodies are homogeneous and show the light gamma metachromasia and sometimes the center is stronger than the other parts and has the traceable radiating structures are recognized. The external layer cannot be stained in pH 4.1. The small inclusion body can not be stained. In pH 2.5 the same observation is seen.

12. Phosphatide reaction (the method of Okamoto et al. [11]).

The small inclusion body is positive and can be stained homogeneously. The external layer of the middle-large inclusion body can be stained positively and is homogeneous. The internal layer is strong positive. In the formol fixed meterial five years after death the colour of both the internal and the external layers became greatly faint and the samll inclusion body cannot be dyed.

13. Cerebroside reaction (the method of Okamoto et al.[20]).

The small, the middle-large inclusion body is negative, and the existence of

cerebroside is denied.

14. Sudan black B stain (Sudan BB).

In the formol-alcohol fixed paraffin section, the small inclusion body is homogeneous and weak positive. In the frozen section from the old formol fixed material 5 years after death, same as Okamoto's method for phosphatide, the internal and the external layer can be stained remarkably weak. The small inclusion body and the middle-large inclusion body after Kauffmann's postchromation method can be stained more powerfully (Fig. 23). If Baker's pyridine extraction method is applied the small inclusion body and the internal and the external layers of the middle-large inclusion body become negative (Fig. 24). But there remains the granular structure of the external layer and not all the materials an dissolved away.

15. Nile blue sulphate stain.

The formol-alcohol fixed paraffin specimen. A part of the small inclusion body is the slight blue or slight blue red. In the middle-large inclusion body the triangular part, same as the small inclusion body can sometimes be stained from dense to slight blue or reddish blue (Fig. 25). It is difficult to distinguish the small granule from the ground substance. The internal layer can be stained with fairly dense blue and homogeneous. When Baker's pyridine extraction method is applied the small inclusion body and the external layer of the middle-large inclusion body can not be stained or is seldom pink (Fig. 26). The internal layer unusualy has light blue than the nontreated specimen.

16. Sudan III stain.

Every part of the inclusion body is negative.

17. Metachromasia (Feyrter's Einschlussfärbung).

The small, the middle-large inclusion bodies are all positive (Fig. 27). When Baker's pyridine extraction method is carried on, the small inclusion body and the external layer of the middle-large inclusion body is negative, and the internal layer shows slightly faint positive than the non-treated specimen (Fig. 28).

18. The electron microscopic observations after Baker's pyridine extraction method.

From the paraffin embedding which is fixed by formol-alcohol, the small lumps whose side is 2 mm each are taken out, and pyridine extraction method is carried on in Baker's method after the removal of paraffin. The electron microscope is then used in the mentioned way after the wash with alcohol and aqua. To the contrast specimen only picrinic acid treatment of Baker's method was carried on. The same experiment was carried on with the formol fixed tissue.

The view in the contrast specimen of the formol-alcohol fixed tissue is almost nearly the same with the view of the non-treated specimen. But the girder and the cave both show minute granular.

Now to this contrast specimen above-mentioned, the electron micrograph after

pyridine extraction the cloud-like substance melts away compared with Fig. 13 and after that the girder appears thickly and vividly, and the contrast with the cave which the contents melted away is also vivid (Fig. 29). The same view is seen in the specimen of the material of the formol fixation kept for a long time and the nontreated material of Baker's method.

19. Sulphulic acid and acetic acid anhydrid method for cholesterol and cholesterol esters (the method of Okamoto et al.[21]).

The small inclusion body is unknown. The external layer of the middle-large inclusion body is light yellow-gray. The reaction to cholesterol and to its esters is not recognized, but its result shows the existence of lipids. Besides, in the frozen section in fructose sirup has no crystal.

20. Carbolic fuchsin method for ceroid.

The internal layer of the middle-large inclusion body is homogeneously pink.

21. Millon's reaction.

The external layer of the middle-large inclsuion body is sometimes faint positive, and sometimes negative.

22. Coupled tetrazonium reaction.

The small inclusion body is not clear. When performic acid treatment is carried on, the positive part of the external layer in the middle-large inclusion body mostly faded away and is recognized as the light brown feine granule. The radiation structure and the ring which is connected in the inside of it have weak colouring. The internal layer is about the same as the non-treated one. In the benzoylated specimen the radiation structure and the ring in its base show middle positive. The view of the other parts in the external and the internal layers are the same with the non-treated specimen.

23. Mercury bromphenol blue reaction.

There is no distinction between the external layer and the internal layer of the middle-large inclusion body, and their inner structures are honey-comb-like and show weak positive. The small inclusion body shows the same view.

24. Feulgen's reaction.

All is negative.

25. Haematoxylin eosin stain (H.E.).

The small inclusion body is generally not clear, but in a little larger inclusion body the center is reddish purple, and the most part is basophilie relative. Sometimes the reddish brown particles are seen in the margin. The ground substance of the triangular part in the external layer of the middle-large inclusion body is acidophile, but sometimes all the external layer is basophile.

26. Holzer's stain.

The image in Holzer's stain lacks the histochemical value, but shows the characteristic view morphologically. The small inclusion body stained in purple and that margin is surrounded by the dark blue particles. In the external layer

of the middle-large inclusion body the dark blue granule are arranged within it. The internal layer is homogeneous and stained in purple.

27. Luxol fast blue stain.

The small inclusion body is unclear. This corresponds to the small inclusion body is not recognized in the myelin sheath. The external layer of the middle-large inclusion body is pale reddish brown and the radiation structure is seen. The internal layer is the same colour with the external layer, but fainter than that and is homogeneous. The center can not be stained.

28. Cajal's silver nitrate pyridine method.

As stated following the image of the small inclusion body is the same as that of the external layer of the middle-large inclusion body. In the external layer of the middle-large inclusion body exist the dark brown small grains. They are especially distinct in the deep side of the triangular part. In a few inclusion bodies the radiation structure appears clearly. The layer structure of the internal layer is not clear and shows homogeneously palish brown.

29. Kóssa's reaction.

All is negative.

30. Iron reaction (Prussian blue reaction).

The small inclusion body is almost equally from weak positive to middle positive. The external layer of the middle-large inclusion body diffusely or partially shows positive (Fig. 30). There is the inclusion body which cannot be stained. Generally iron reaction appears clearly in the inclusion body in the cortex and the nucleus dentatus of the cerebellum, but appears weak in the cerebral cortex. In the nitric acid treatment colour generally increases.

Summary and Discussion

Both the macroscopic observations of the brain and the relation between the inclusion body and the cell is studied. And then in every part of the small and the middle-large inclusion body, the histochemical observations in relation to the fine structure are summarized and studied literally.

I. The macroscopic observations of the brain and the relation between the inclusion body and the cell.

Macroscopically the cerebral cortex is white and the distinction between the white matter is not clear. The elder sister's one is hard and slightly atrophies. Such observation could not be found in the reports hitherto. It has become probably form a large number of the inclusion bodies found in the gray matter. In the gray matter especially in the nerve cell bodies, its dendrite and in the cellbodies of the astroglia. Therefore as stated in the preface, if the inclusion body is due to the abnormal metabolism of the nervous system, it is the common phenomena to the above-mentioned all tissue constituents.

II. A fine structure of the inclusion body and its histochemistry.

1. The small inclusion body.

The small inclusion bodies lie scattered in disseminately in the gray matter of the brain and its numbers are overwhelmingly numerous compared with the middle-large inclusion body. Because of the fact that in these small inclusion body the existence of the cell fragments is found electron microscopically, the small inclusion bodies which are supposed to exist through the optical microscope in extracellular seen really exist in the cell. So far as the morphological studies are concerned, the phasencontrast microscope and the electron microscope are particulary excellent, and the latter shows the peculiar honey-comb-like structure. The inner structure of the small inclusion body is simple compared with the middle-large inclusion body, and it shows nearly the matrix of the inclusion body. The studies through the electron and the phasencontrast microscope have the value in finding out the similar diseases. Seitelberger et al.[26] reported almost at the same time with Namba[16] the brief electron microscopic findings, but Seitelberger et al. does not fully ascertain the existence of the cell fragments in the inclusion body. The destruction of the cell elements in the specimen from the formol-alcohol fixed paraffin material is so remarkable that the author could not elucidate the origin of the occurrence of the inclusion body. Now so far as these materials of this study are concerned, except the similarity of the cell fragments mixed in the inclusion body, as mentioned after this later on, the author think there are no normal submicroscopic elements to agree with the inclusion body.

From the result taken up in the list histochemically, and the result of salivary digestion test, it is hard to think the existence of acid mucopolysaccharides and glycogen. Sudan BB stain, phosphatide reaction (Okamoto's), nile blue sulphate stain, Feyrter's stain and Baker's pyridine extraction test all suggest the existence of lipids and its mixture with the pyridine insoluble substance. Perhaps these pyridine insoluble materials contain at the same time a little quantity of protein and iron. Besides, the blue granule of Holzer's stain and haematoxylin stain resemble the external layer of the middle-large inclusion body. Though the essential qualities are unknown, this is the noteworthy characteristic.

When the histochemical views of the middle-large inclusion body and of the small inclusion body are compared, the center of the traceable radiation structure of the small inclusion body, in accordance with the morphological view, resembles to the aperture and the internal layer of the middle-large inclusion body. To this, the most parts of the small inclusion body resembles the ground substance of the triangular part of the middle-large inclusion body, mostly. Therefore, as to the histochemical observation of the small inclusion body will be mentioned in the part of the external layer of the middle-large inclusion body.

2. The middle-large inclusion body.

a. The external layer.

The microscopic image - especially the phasencontrast microscopic image - the triangular part, each aperture and the homogeneous ring-structure in connection of it at the boundary of the external and the internal two layers; these parts appear. The triangular part is consisted of the fine granule, and the homogeneous ground substance between the fine granule. Sometimes there are the large and the homogeneous tear-like structure. The triangular part shows a series of the relation with the small inclusion body electron microscopically. When compared with the small inclusion body the honey-comb-like structure is although considerably destroyed. In the aperture, the minute structure shows the stronger change. A part of the dense granule in the electron microscopic image in the external layer of the middle-large inclusion body agrees with the Nissl's granule.

Therefore in the judgement of the histochemical view, the small inclusion body with no mixture of the cellular elements, or the view on the ground substance between the grains must be considered. In fact, the author often find the difficulty in the difference between the small grains and the ground substance in the triangular part, but the comparison with the small inclusion body makes the judgement possible.

According to Lison[11] the acetylation to P A S reaction does not inclease any speciality, but the methylation has the deep reliance in the judgement of acid mucopolysaccharide. After 6 days methylation result, the blockade is not carried on completely. In the saponification the recovery of colour could not be seen. And the result of alcianblue stain, methylation, saponification, toluidine blue metachromasia, and catch reaction of iron, are similar to the small inclusion body, and does not accord to acid mucopolysaccharide. The existence of glycogen is denied.

The existence of lipids, same as the small inclusion body, which is near to the matrix, Okamoto's phosphatide reaction, the sudan BB stain after the postchromation in the paraffin section, the change of positive of Feyrter's stain to negative after pyridine extraction, the electron micrograph after pyridine extraction, the opinion in Okamoto's sulphulic acid and acetic acid anhydrid method, and the soluble qualitative in the formol for a long time, although the insoluble qualitative in the formol-alcohol fixed paraffin embetting shows the existens of lipids in the inclusion body. According to Lison, Pearse[24] and Okamoto[18], lipids that have such nature are ganglioside, glyceride, cerebroside and sulphatide excepting, belong to phospholipids especially to esterphosphatides, acetalphosphatides, phosphatidylcholine and to phosphatidyl ethanolamine. The positive reaction of Feyrter's stain in the cases of Imai et al., and Harriman et al., and of nile blue stain in the cases of Westphal[33], Davison et al[3]., Ostertag[23] and Hirai[6] described. But they are not referred to the existence of lipids in the inclusion body.

The histochemical observations in the triangular part of the external layer are here summarized as follows; in the external layer of the middle-large inclusion

body and in the small inclusion body, the existence of little quantity of mucopolysaccharide or the similar substance and containment of a little quantity of protein is not denied. As more noticeable constituent the author can refer to lipids especially to phospholipids or lipids which is closely related to this.

The metabolic desease concerning the inclusion body in the central nervous system known so far, is sphingomyelin (Niemann-Pick's disease), cerebroside (Gaucher's disease) or ganglioside (Tay-Sachs's type) etc, which are included in the abnormal deposit in sphingolipids. The inclusion body of the author's cases is not only different from the metabolic disease of sphingolipids in its shape, but also histochemically it must be distinguished clearly as the abnormal metabolic disease of phospholipids.

Among the other various reactions, the examination of iron reaction is weak, except the cerebellum, so iron is necessary as the constituent of the formation of the inclusion body, or the absorption in the constituent of the inclusion body or due to the topographical speciality of the organization of the inclusion body, these three points are unknown. Among Seitelberger's cases a case of positive iron reaction is reported.

There is little histochemical significance, but the peculiar view in Holzer's stain and as Imai's case pale reddish brown colour in luxol fast blue stain, is seen. This may be a point of the distinction from the other diseases.

The aperture and the ring structure in the small inclusion body are hard to recognize. In the electron micrograph these parts are morphologically stronger changed than the triangular part of the external layer and that near the internal layer is shown. This well corresponds with the histochemical view. Generally the aperture is not clear mostly except the phasencontrast microscopic observation. In protein reaction the part which is shown in the granular structure is because of the cell elements mixed in the inclusion body, and seems different to the aperture histologically. The various reactions to mucopolysaccharide appear most vividly in comparison with the triangular part of the external layer, but putting the results of P A S reaction, the acetylation, the methylation, the result of the saponification, the various results of toluidine blue metachromasia, hyaluronidase digestion test and acidophile together, it does not accord to acid mucopolysaccharide. Phospholipids are not clear as the triangular part, but its existence can not be denied.

b. The internal layer.

In the non stained specimen, at least five layers can be distinguished in the middle-large inclusion body, but in many stained specimen, only the homogeneous part and the star-like structure in the center can be recognized. In the electron micrographs, the peculiar structure breaks strongly in comparison with the external layer. Moreover, the cell fragments in the inclusion body are destroyed or are fused with the proper section of the inclusion body. So that histochemi-

cally, they must show different view than the external layer. Histochemically, the most parts of the internal layer greatly differ from the triangular part of the external layer or from the nature of the small inclusion body. The star-like structure in the center shows the different observation than the main part of the internal layer.

P A S reaction after the acetylation, the methylation and the saponification, the results of toluidine blue metachromasia and alcianblue stain after the methylation and the saponification are summarized, the possibility of involving acid mucopolysaccharide which has the sulphate ester group is suggested. Perhaps a part of such result is due to the destruction of cellbody mixed. In addition, the difference between the cases of the sister and the brother in iodine reaction suggest the process of the desintegration of phospholipids or polysaccharides.

From the result of sudan BB stain, nile blue sulphate stain and pyridine extraction test the existence of phospholipids is expressed more strongly than the other parts of the inclusion body. From the electron micrograph of the pyridine treated material, it is also proved. And the result of carbolic acid fuchsin method makes the author think the variation of lipids. There is much difference compared with the small inclusion body and the external layer of the middle-large inclusion body, that Feyrter's stain in the internal layer is positive after pyridine extraction, is the prove existence of sulfate. Beside, haematoxylin eosin stain, it is basophile and is different from the external layer. The center is acidophile again and is opposed with morphological difference.

c. Corona- and crystall-like structure.

That corona-like structure is seen in the middle-large inclusion body which has relation with the cell body is not clear, so it may generate in different condition to the middle-large inclusion body in the cell body, but the possibility of image of the surface or the artificial product. Between corona- and crystall-like structure the Best's carmine stained image is only alike, but the rest doesn't agree to morphologically and from the point of stain.

III. Comparison with the cases of reports so far.

There are many cases written as myoclonus epilepsy clinically. But only 35 cases, in which the inclusion body was found by the autopsy. As to the clinical symptoms Namba[13, 17] reported, the personal change in the initial stage, the cerebellar symptoms and the death age are described as the remarkable symptoms. All cases except Namba's drowned case died from 17 to 24. After Namba's reports more cases were added by Seitelberger et al., and the death age was called to attention. The appearance of the grand mal is distributed from 6 to 17; but the greater part centers round 14–16: three years. Myoclonus occurred age is the same as the grand mal, or within two years of the grand mal. The author's cases, except the familial onset cannot be distinguished from the above-mentioned various reports.

The morphological stand point of the inclusion body and its many scattered

distribution in the gray matter in the reported autopsy cases are identified with the author's cases. Although, whether the inclusion body has the relation with the gliacell and the other components in the brain, there are many different opinions. When the author think of the origination of the inclusion body it is important to ascertain the relation with the gliacell, but in the reports so far, Lafora[9], Ostertag, Marchand, Solé-Sagarra[28], Tachibana[30] and Imai et al. did not write about astrocytes at least, and Buduls et al., Harriman et al., and Wakui persist that the inclusion body does not exist in gliacell. To this opinion, same as the author Westphal, Shou[27], Davison et al., Hirai and Seitelberger wrote about the inclusion body in the gliacell. The inclusion body that has no relation with the cell component is discussed by Westphal, Shou, Marchand, Buduls, Solé-Sagarra, Harriman, Hirai, and Seitelberger. In the author's cases the small inclusion body which seems to exist in the extracellular optical microscopically has relation with the cell components electron microscopically and such small inclusion body is proved to occure in the cell components. The morphological study of inclusion body is done excellently by the phasencontrast microscope and the ordinary optical microscope (non-stained), but there is no report by this method the except the author's cases. The various reports so far and their comparison including the author's cases must rely on the observation of the stained specimen, so the detailed structural discrimination becomes difficult. But the size and the inner structure which shows the layer and the radiation are almost the same as the report of the author's cases, and this characteristic structures clearly differ from the inclusion body written by Weiman[32], Bielschowsky[1], Lewy[10], Spielmeyer[29], or from the intracellular deposit by Hallervorden-Spatz's disease, Pick's desease, granuro-vacuolar degenaration, tuberose sclerosis or the intracellular deposit as seen in the above-mentioned lipidosis.

About the histochemistry of the inclusion body the other authors except Harriman et al., Seitelberger and Imai et al. described only few. The view of Best's carmine stain in Lafora's case agrees with the author's cases but there is little difference between Lugol's method and the view in toluidine blue stain. In the case of Westphal et al., there is the similarity between the author's cases in the Best's carmine stain and Lugol's method, but the difference is seen the linear structure as is seen in Kóssa's method, negative appearance in iron reaction and lipid reaction. Ostertag's case is very near to the author's cases as mentioned following points; acidophile of the margin of the inclusion body also basophilie relative, acidophile of the radiation part, the results of nile blue stain and Best's carmine stain, the denial of fatty acid, calcium oxide is negative and the result of salivaly digestion. In Marchand case the findings in Lugol's method, Best's carmine stain, and the view in Bielschowsky's and Cajal's method, are in accord with the author's cases. It is said that the case of Buduls et al. is the same view with the case of Ostertag, but the calcification of the center of the inclusion body is contrary to all cases inclusing the author's cases. The result of nile

blue stain, the sudan III stain, and the existence of the argentaffin substance in Davison's case are roughly in accord with the author's cases. In Wakui's case the external layer is acidophile, and the description to Kóssa's reaction, is in accord to the author's cases, but a part of nile blue stain and Lugol's method, and the result of iron reaction are different. In Hiriai's case, Best's carmine stain and nile blue stain are equal to the author's cases, but in H.E. stain the whole part is basophile, which is the different point from the author's cases.

The most detailed histochemical study is the reports of Harriman et al., Seitelberger and Imai et al., but the histochemical report which takes the fine structure of the inclusion body into the consideration has done by Seitelberger, but it is done after the classification of the internal and the external only two layers. Therefore, precise comparison cannot be undertaken in the observation of the above-mentioned three cases as well as the author's cases. In P A S reaction, the cases of Imai et al., Harriman et al., and Seitelberger, are almost resemble to the author's cases although there is the difference of weak and strong point to reaction. To hyaluronidase digestion test the cases of Imai et al. and Harriman et al. are the same as the author's. And the result of P A S reaction after the acetylation and the saponification, are similar to the author's cases in the cases of Harriman et al. and of Seitelberger. In the author's cases, the cases of Imai et al. and Seitelberger Best's carmine stain is positive, but only Harriman's case is extraordinary. In dialysed iron method, the cases of Harriman et al. and of Seitelberger are resembling to the author's cases. As to alcianblue stain, only the case of Seitelberger is written, and the external layer is +, and the internal layer is ±, although the author's cases are positive, but the view in the detailed part is slightly different. In toluidine blue stain, the case of Harriman et al. is little bit different from the author's cases, especially Seitelberger shows all negative in toluidine blue standard method. In iodine reaction there is no case which shows purple except the author's case and all is brown and positive. As to protein; there is little difference in the statement, but Imai et al., Harriman et al., and Seitelberger recognized its existence same as the author's cases. The result to luxol fast blue stain of the author's cases and Imai's is the same. Basophile is recognized by the three authors, but the author's cases are little bit different. Iron reaction, except the author's cases and one case of Seitelberger, all is negative. In Cajal's silver-impregnation method, both author's cases and Imai's case are resemble. The result of Kóssa's reaction is negative in the author's cases and in Imai's case. Feulgen's reaction is negative in the cases of Imai et al., Harriman et al. and of the author. The above-mentioned descriptions are the point of the similarity and of the difference in the reaction for the mucopolysaccharide and its related materials, protein, and in non special stain in each case. Except the negative result of Best's carmine stain in Harriman's case and toluidine blue stain in Seitelberger's case and negative iron reaction, the histochemical findings in all the reported cases show the similar

view,

Between the various reports on the morphological and the histochemical view of the inclusion body so far reported, there are the differences considerably, though to these differences, the method of fixation, the period of fixation, technic of stain, the difference of observation, and the error due to the complicated inner structure and the cut surface of the inclusion body must be considered. Therefore, to decide the difference between each other and the author's cases the prudent attitude must be taken.

As the conclusien of the constituent material of the inclusion body, Imai et al. said that it must be the complex of protein and saccharide and it must be amyloid substance. Harriman et al. said it belongs to the acid mucopolysaccharide and small quantity of protein is involved. According to Seitelberger the external layer is made from mucopolysacchride except glycogen. Therefore, they thought of the metabolic anomaly as mucopolysaccharide as to the outbreak of the inclusion body. Now to this the author could not deny the existence of mucopolysaccharide or mucoprotein. But more important than that, the metabolic anormaly of phospholipids must be respected.

Conclusion

1. In the brain of two myoclonus epilepsy patients in one siblings the peculiar inclusion body be found by the autopsy.

2. Macroscopically the gray matter in the brain is white, and the difference between the white matter is not clear.

3. The inclusion body as found in the nerve cell and its dendrite, together with the astrocyte cell body. Generally the small inclusion body has the relation with the cell elements.

4. In the brain nerve, the peripheral nerve, in the nerve cell of Auerbach's and of Meissner's plexus the inclusion body can not be found.

5. The fine structure was examined in the formol fixed frozen section and the formol-alcohol fixed paraffin section, through the ordinary optical microscope, the phasencontrast microscope and the electron microscope.

6. The great difference can not be seen in the ordinary and the phasencontrast microscope, but generally, in order to know the inner structure of the inclusion body the phasencontrast microscope is the most superior.

7. Phasencontrast microscopically, the middle-large inclusion body is consisted of the external and the internal layers. In the former may be distinguished the triangular part, the aperture and the boundary part between the internal and the external layers. The internal layer has the star-like structure in the center. In comparison to the structure of the middle-large inclusion body, that of the small inclusion body is simple.

8. In the electron micrographs a perculiar honey-comb-like structure appears. The external and the interal layers of the middle-large inclusion body show a series of the morphological alteration from the small inclusion body as the matrix.

9. In the electron micrographs, in the normal cellular elements there are nothing that falls under the inclusion body.

10. Generally, as the size of the inclusion body increases, the inner structure becomes complicated, and at the same time the histochemical variation gradually attended.

Histochemical reactions of the inclusion body

Method	External layer			Internal layer		Small inclusion body
	triangular part		aperture	homogeneous part	star-like center	
	fine granule	ground substance				
PAS	+	+	+++	+++	++	+
// after acetylation 24 hrs	−	−	−	+	+	−
// after saponification	±	±	++	++	+	−
// after methylation 30 hrs	+	±	++	++	+	±
60 hrs	+	±	++	++	+	±
6 days	+	±	++	++	+	±
// after saponification	+	±	++	++	+	±
// after hyaluronidase digestion	+	+	+++	+++	++	+
Alcianblue stain (paraffin section)	−	+	+++	+++	+	+ center +++
// after methylation 25 hrs	−	−	−	−	−	−
// after saponification	−	−	−	−	−	−
// after Baker's pyridine extraction test	−	−	−	++	+	−
Best's carmine stain	++	+	+++	+++	+	++
// after saliva digestion	++	+	+++	+++	+	++
Hale's method	−	−	+	+	−	−
// after hyaluronidase digestion	−	−	+	+	−	−
Kawase & Sunahara's method*1	−	−	+	−	−	−
Clara's method*2	?	?	?	−	−	?
Mayer's method*3	?	?	?	−	−	?
Mayer's method*4	−	?	?	?	?	?
Lendrum's method*5	−	−	−	−	−	−
Lugol's iodine method	in brother's case brown			in sister's case purple		?
Toluidine blue stain pH 7.0	β	γ	γ	γ	β	γ
4.1	−	−	−	β	β	−
2.5	−	−	−	β	β	−

Okamoto's method*6	+	+	+	+++	+	+
Okamoto's method*7	−	−	−	−	−	−
Sudan III stain	−	−	−	−	−	−
Sudan BB stain (paraffin section, postchromation)	+	+	±	++	+	+
〃 after Baker's pyridine extraction test	−	−	−	−	−	−
Nile blue sulphate stain (paraffin section)	+	+	+	+	+	+
〃 after Baker's pyridine extraction test	−	−	−	±	±	−
Feyrter's stain*8	+++	+	++	+++	++	++
〃 after Baker's pyridine extraction test	−	−	−	++	+	−
Okamoto's method*9	pale yellowish brown			−	−	−
Carbolic acid fuchsin stain	−	−	−	+	±	−
Feulgen's reaction	−	−	−	−	−	−
Millon's reaction	±	±	±	+	−	−
Coupled tetrazonium reaction	−	−	+	+	+	−
Mercury bromphenol blue reaction	+	+	+	+	+	+
Holzer's stain	dark blue grain			purple homogeneity		purple. margin is dark blue grain
Luxol fast blue stain	pale reddish brown			pale reddish brown	−	?
H.E. stain	reddish brown or blue		reddish	blue	−	blue or red. margin is reddish brown granule
Kóssa's method	−	−	−	−	−	−
Prussian blue reaction	+	+	+	+	+	+

* 1. for hyaluronic acid
2. for mucicarmine
3. for mucicarmine
4. for amyloid
5. for amyloid
6. for phosphatide
7. for cerebroside
8. for phospholipids
9. for cholesterol and cholesterol esters

11. Histochemically, in the small inclusion body, the triangular part of the external layer and the internal layer of the middle-large inclusion body, phospholipids exists.

12. It cannot be denied that in the external layer of the middle-large inclusion body, and in the small inclusion body, a little quantity of mucopolysaccharide, or the similar substance exists, but it is not acid mucopolysaccharide.

13. A part of the inclusion body has positive iron reaction.

14. Although non-special stain, but as the noteworthy observation, the small inclusion body and the external layer of the middle-large inclusion body shows the blue grain in Holzer's stain and the faint reddish drown colouring in luxol fast blue stain.

15. Now the author discussed the difference of each other, in comparison with the various cases reported of the existence of Lafora's inclusion body, there are no reports which have discussed the detailed struture of the inclusion body, and

ascertained the existence of phospholipids histochemically.

The Explanation of the Diagram

1. Inclusion body in the nerve cell. Nissl's stain. ×4000.
2. Small inclusion body in the dendrite of the nerve cell. Cajal's silver nitrate pyridine method. ×4000.
3. Small inclusion body in the astroglia cell body. ×2000.
4. Middle inclusion body. From the external side the narrow cell body, the wide granular external layer (the triangular part and the aperture) and the comparatively homogeneous internal layer can be distinguished. The internal layer is consisted of 5 layers furthermore. Optical microscope. Nonstained frozen section. ×4000.
5. Small inclusion body. The structure of the external layer in the small inclusion body is comparatively simple. Phasencontrast microscope. Frozen non-stained specimen. ×4000.
6. Slightly larger inclusion body. The structure of the external layer is a bit more complicated compared with Fig. 5. The internal layer and the ring structure appear. do. ×4000.
7. The surface of the middle inclusion body. The gross granule of the cell body that is not seen in Fig. 6 appears. do. ×4000.
8. The cut surface which passes the deep part of the external layer of the middle inclusion body. The triangular structure of the external layer appears. do. ×4000.
9. Middle inclusion body. The cut surface at the deeper part than the image in Fig. 8. The aperture between each triangular part of the external layer is clear. A part of the internal layer appears in the center of the inclusion body in this Figure. do. ×4000.
10. Middle inclusion body. The cut surface, at more inner part than Fig. 9. The triangular part of the external layer is rather square and its aperture is thinner and homogeneous than the triangular part, on one side of the inclusion body is the flat cell-nucleus. do. ×4000.
11. Middle inclusion body. The cut surface which passes the center. So the whole layer of the inclusion body is recognized vividly, but is intersects complicatedly into the boundary between the cell body and the external layer. do. ×4000.
12. Large inclusion body. The external layer is traceable. In the contrary the internal layer increases remarkably. The star-like structure in the center appears clearly. do. ×4000.
13. The cut surface of the small inclusion body. The net-like structure is shown. The cloud-like substance being exist in the cave, the boundary between

the gird and the cave is not so clear. Electron micrograph. × 100000.

14. Middle inclusion body corresponding to Fig. 11. The cell body, that circles around the margin of the inclusion body can be distinguished from the inclusion body clearly and links to the nucleus with the large nucleorus. In the external layer of the inclsuion body a part of the cell body intermingle. The internal layer is bright as compared with the external layer. The star-like structure can be seen. Electron micrograph. × 6000.

15. Partially enlarged picture of the external layer in Fig. 14. The girder of the net-like structure is considerably cut down. Nu: Nucleus. Nm: Nucleic membrane. T: Triangular part. C: Cell body. do. × 50000.

16. do. A: Aperture in the optical microscope, is equivalent to this. T: Triangular part. I: Internal layer. do. × 50000.

17. do. The boundary between the external and the internal two layers. In the inside of the external layer exists densely the girder cutting off. In the internal layer scattered the bubble-like structure. R: The boundary between the internal and the external two layers. The honey-comb-like structure is almost lost. do. × 50000.

18. do. The fine granular change of the girder. B: Bubble-like structure coused from the expansion of the girder. Cs: The portion that has especially high electron density is thought to be the change of the cell fragments in the external layer. do. × 50000.

19. do. The center part of the internal layer. St: The part that agrees with the star-like structure in the optical microscope. The trace of the endoplasmic reticulum is observed. do. × 50000.

20. Middle inclusion body. Few inclusion body have the corona-like stucture on the outer edge of the inclusion body. Formol-alcohol fixed paraffin section. P A S reaction. × 2000.

21. Middle inclusion dody. The triangular part of the external layer is faint positive in alcianblue stain. The aperture is strong positive. Formol-alcohol fixed paraffin section. × 2000.

22. Middle inclusion body. In the sister's case the internal layer is pale purple in iodine reaction. Formol-alcohol fixed paraffin section. Lugol's iodine method. × 2000.

23. Middle inclusion body staind more powerfully in Sudan black B after the postchromation. Formol-alcohol fixed paraffin section. × 2000.

24. Middle inclusion body is negative in sudan black B stain after the postchromation and pyridine extraction. do. × 2000.

25. Middle inclusion body is slight blue. Nile blue sulfate stain. Formol-alcohol fixed paraffin section. × 2000.

26. Middle inclusion body is negative or seldom pink in nile blue sulfate stain after pyridine extraction. do. × 2000.

27. Middle inclusion body is positive in Feyrter's Einschlussfärbung. Formol-alcohol fixed paraffin section. ×2000.

28. The external layer of the middle inclusion body is negative and the internal layer shows faint positive in Feyrter's Einschlussfärbung after pyridine extraction. do. ×2000.

29. The electron micrograph after pyridine extraction test. The girder is thick and in contrast with the cave, which is very clear. Electron micrograph. ×100000.

30. Middle inclusion body is positive in iron reaction. Prussian blue reaction. Formol-alcohol fixed paraffin section. ×2000.

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PLATE 1

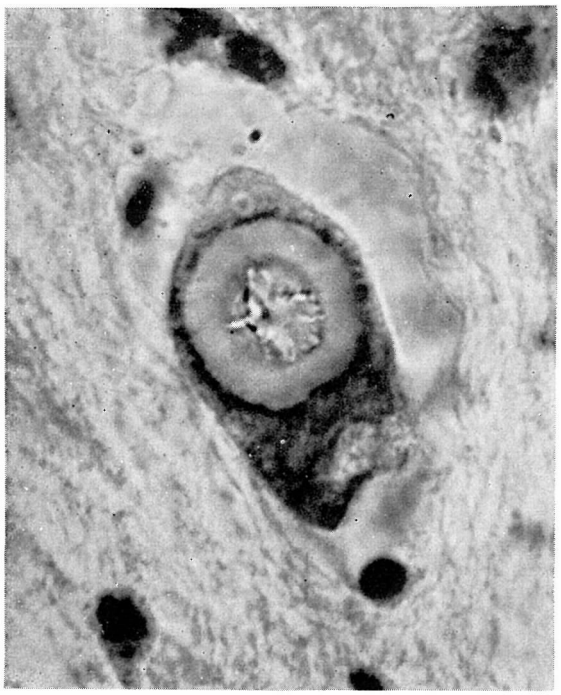


Fig. 1

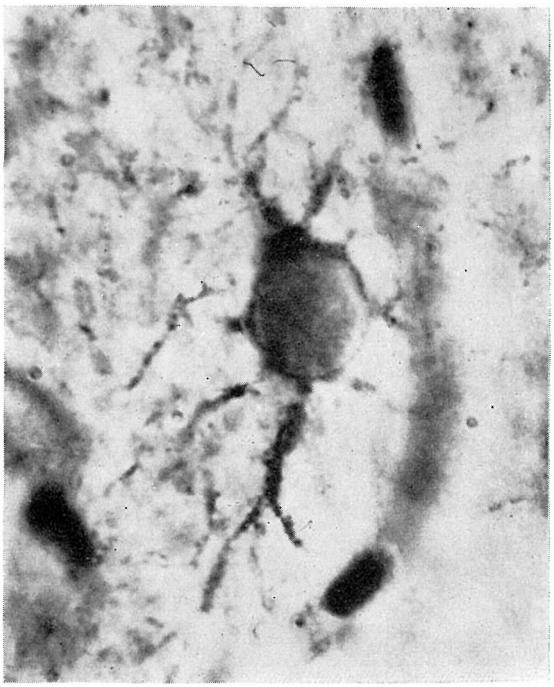


Fig. 3

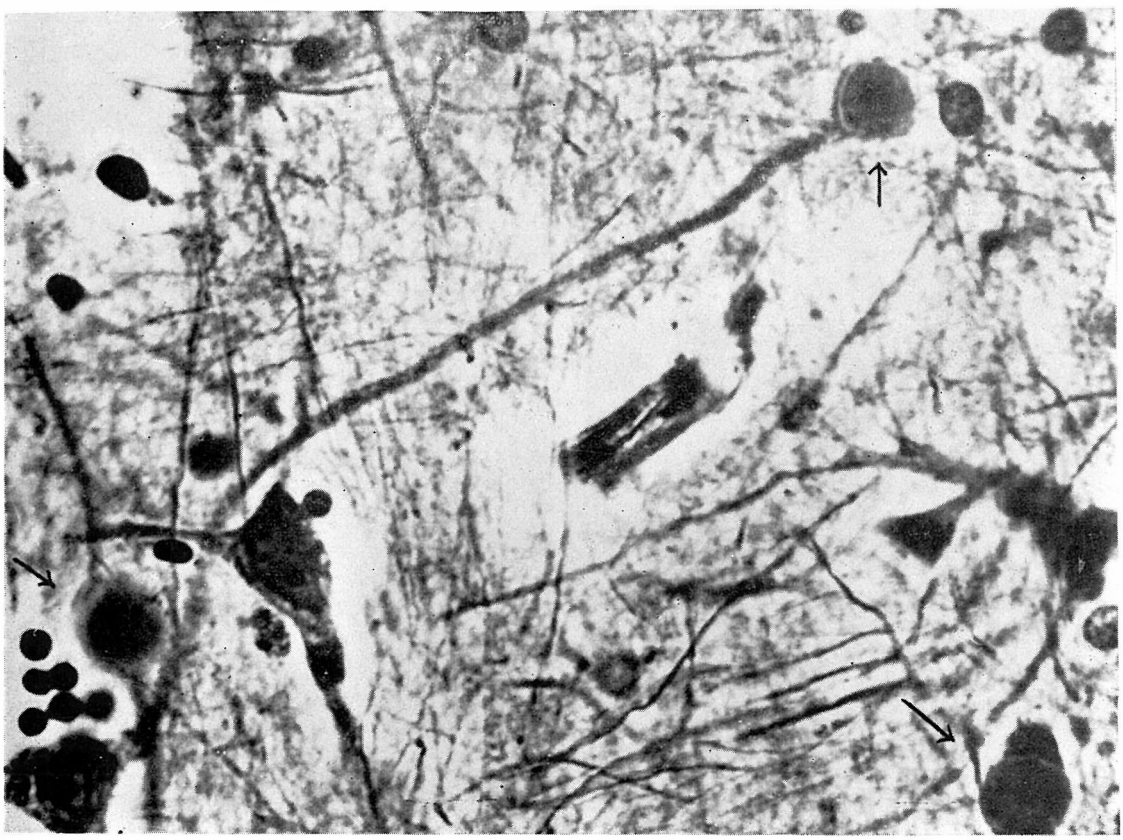


Fig. 2

PLATE 2

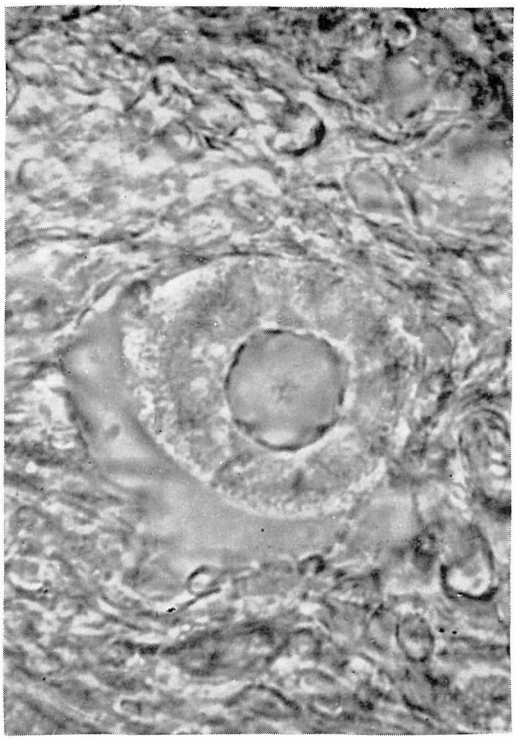
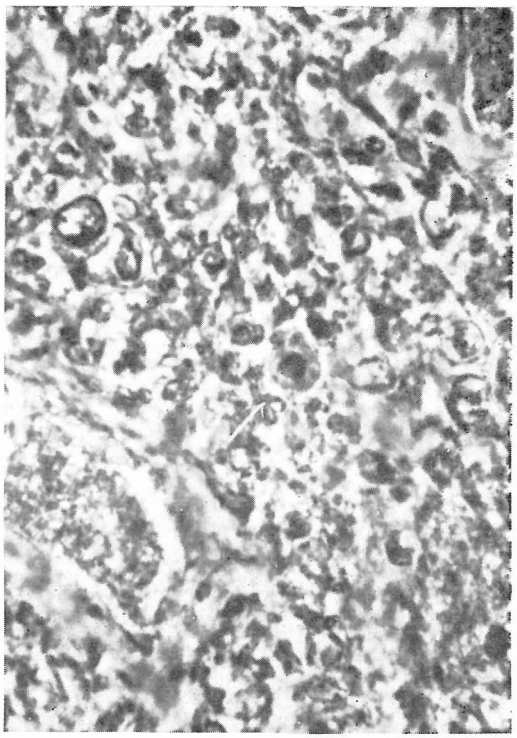


Fig. 4



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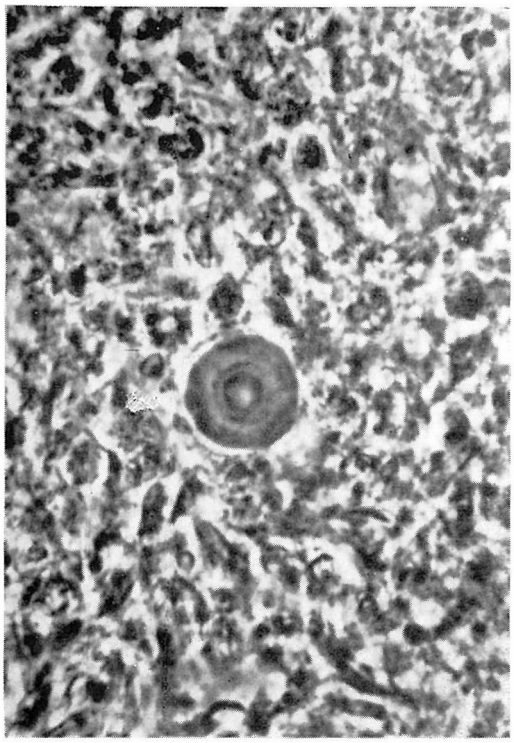

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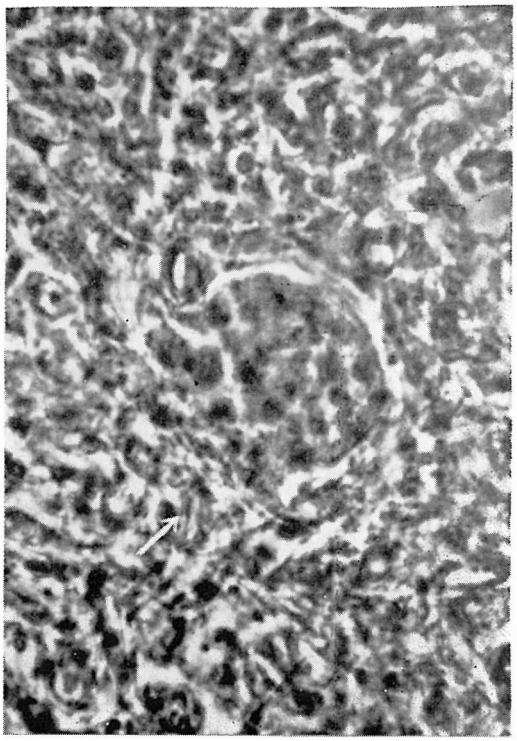


Fig. 7

PLATE 3

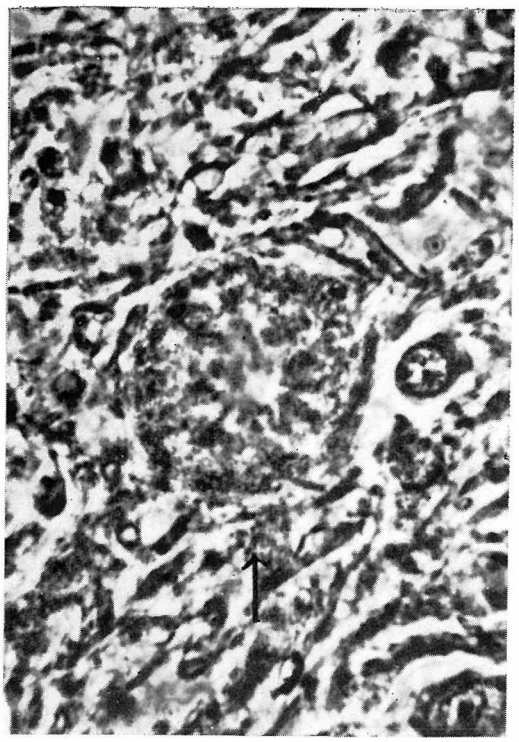
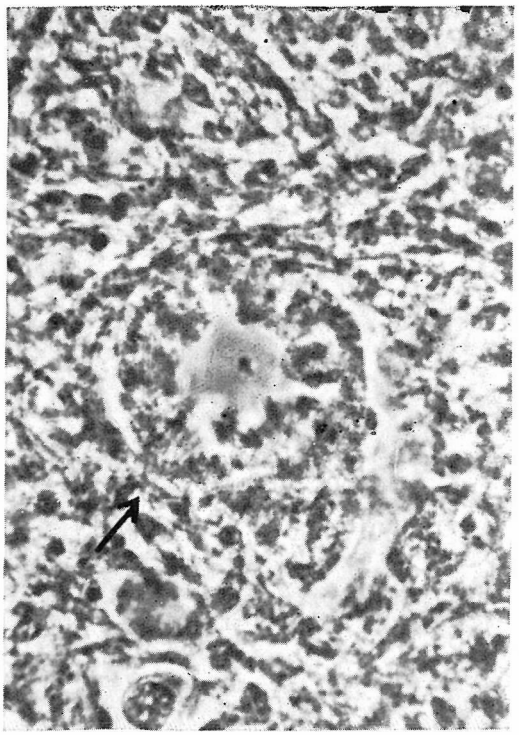


Fig. 8



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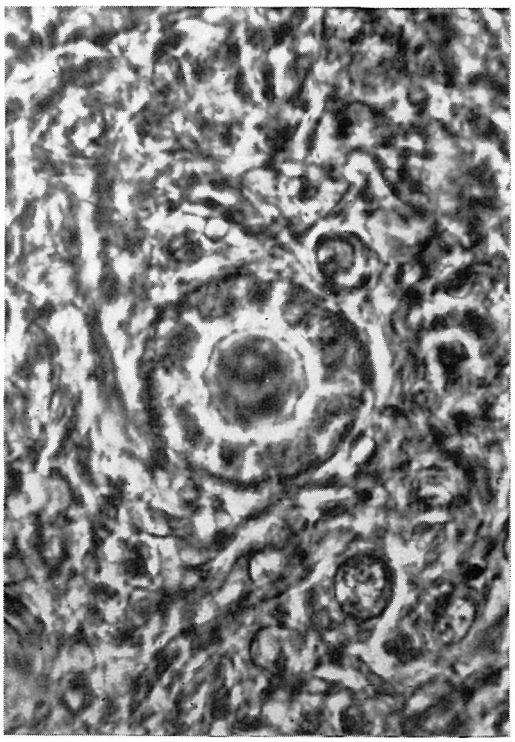

Fig. 10

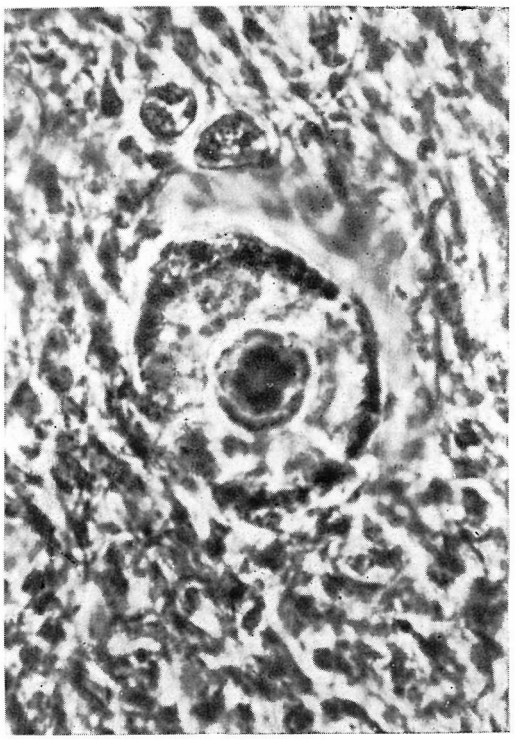

Fig. 11

PLATE 4

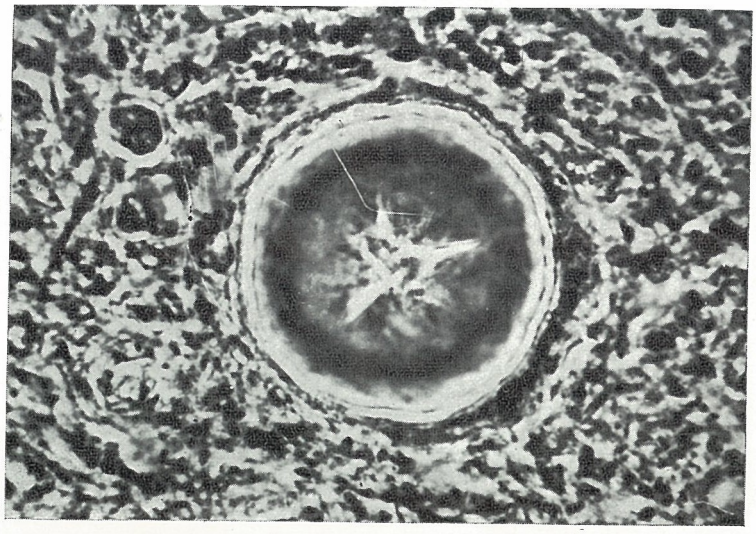

Fig. 12

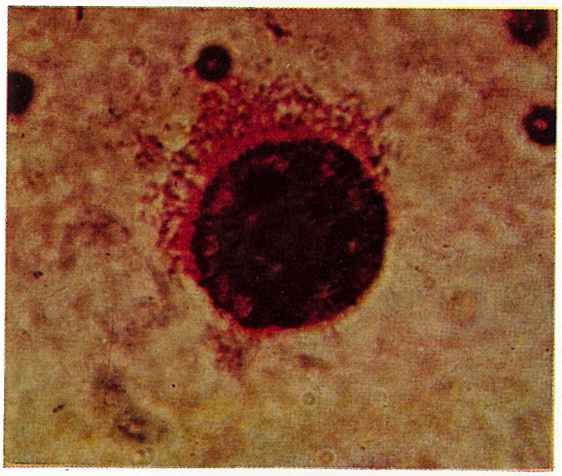

Fig. 20

Fig. 21

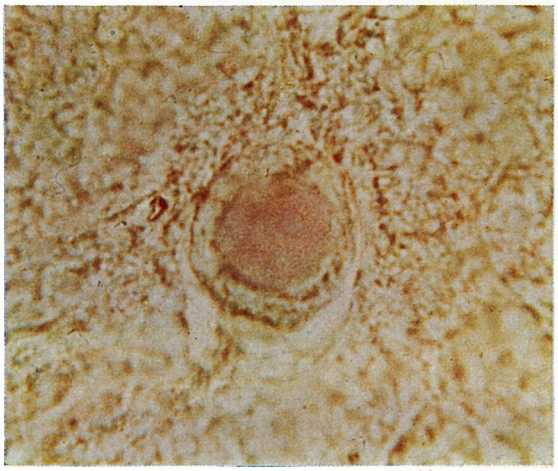

Fig. 22

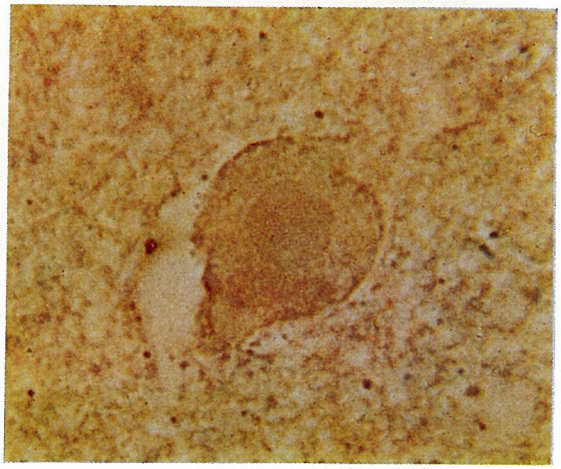

Fig. 23

PLATE 5

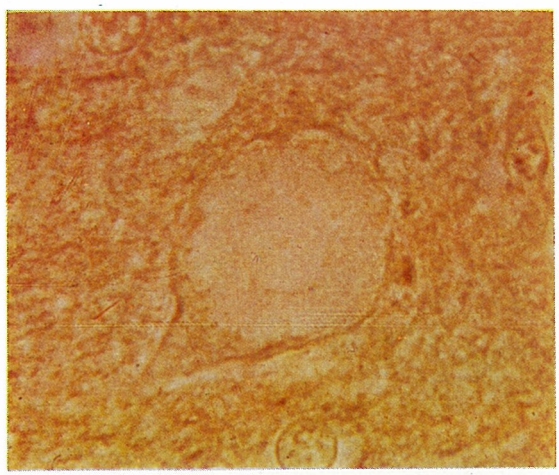

Fig. 24

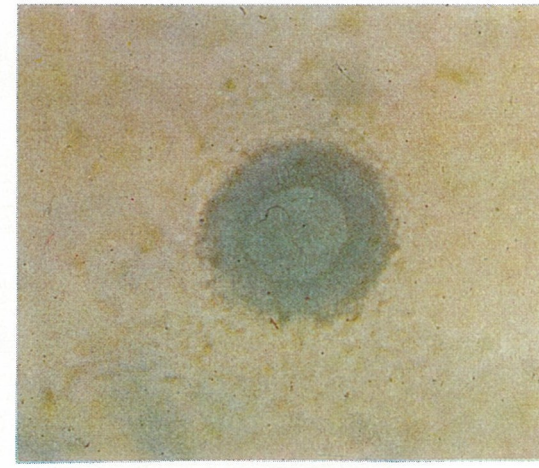

Fig. 25

Fig. 26

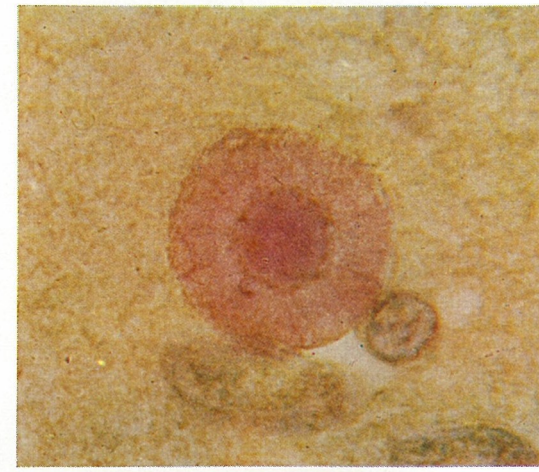

Fig. 27

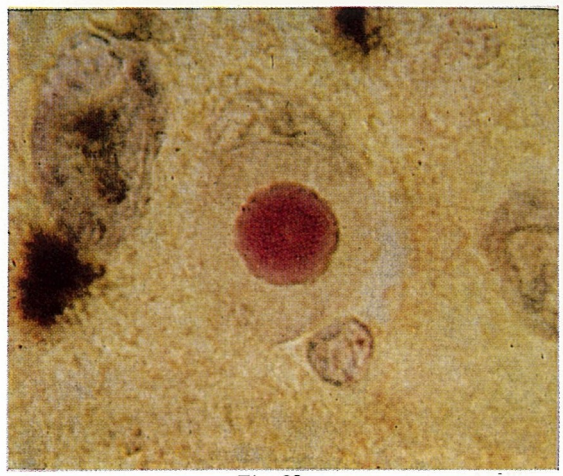

Fig. 28

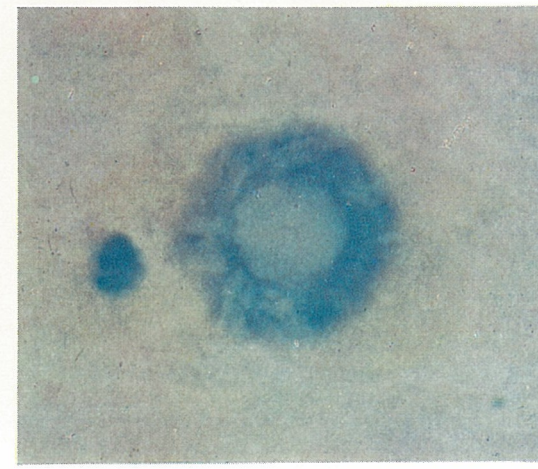

Fig. 30

PLATE 6

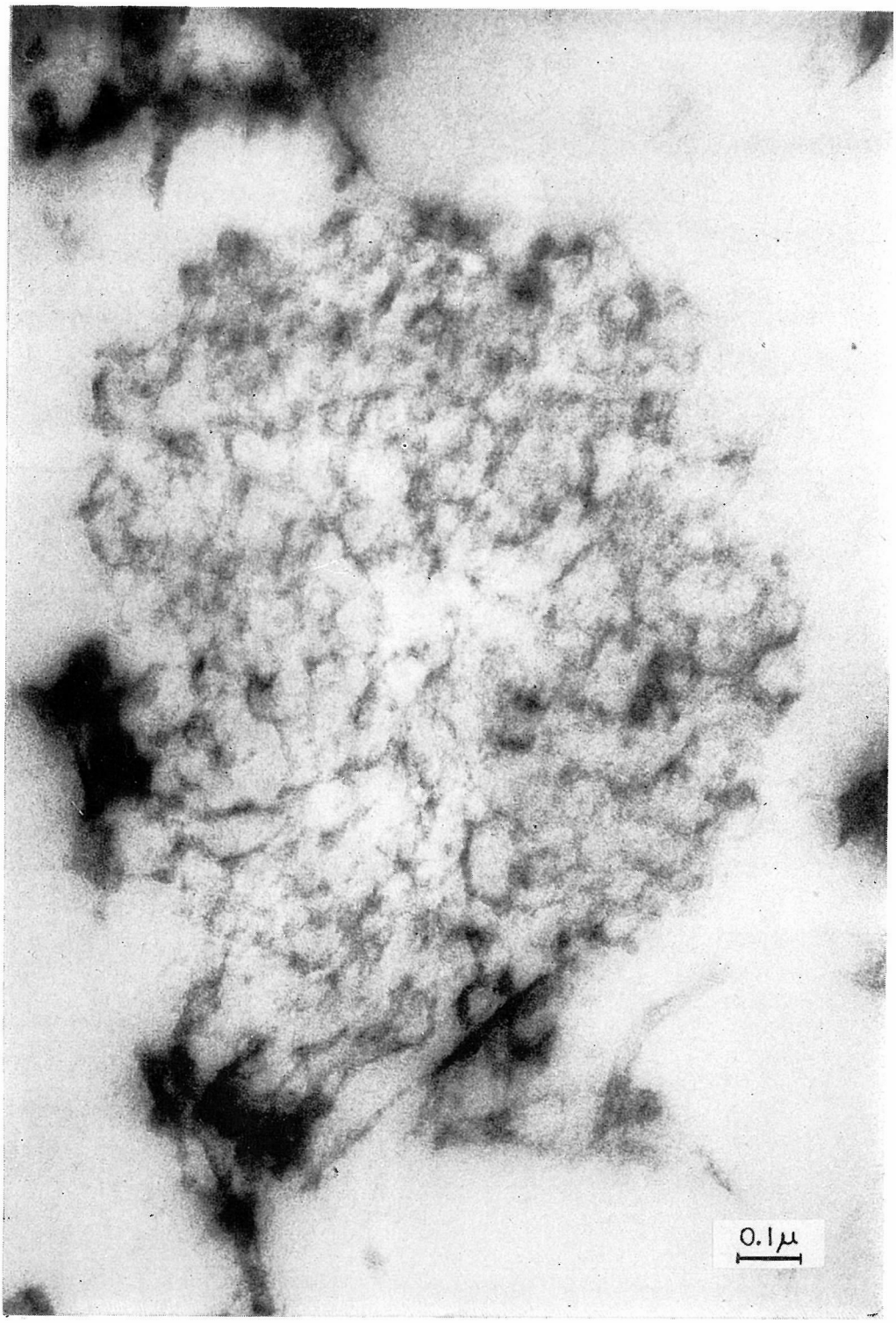

Fig. 13

PLATE 7

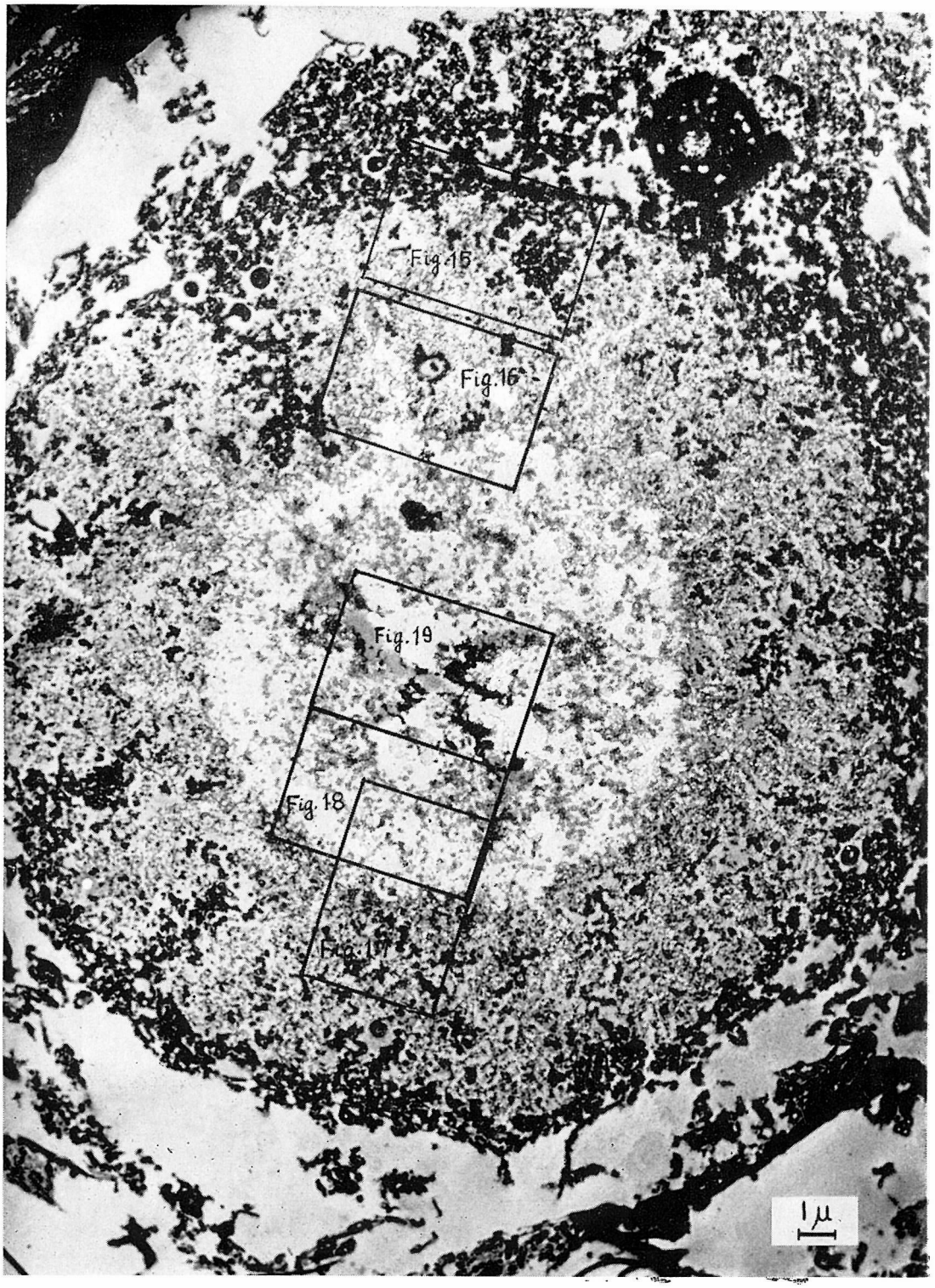

Fig. 14

PLATE 8

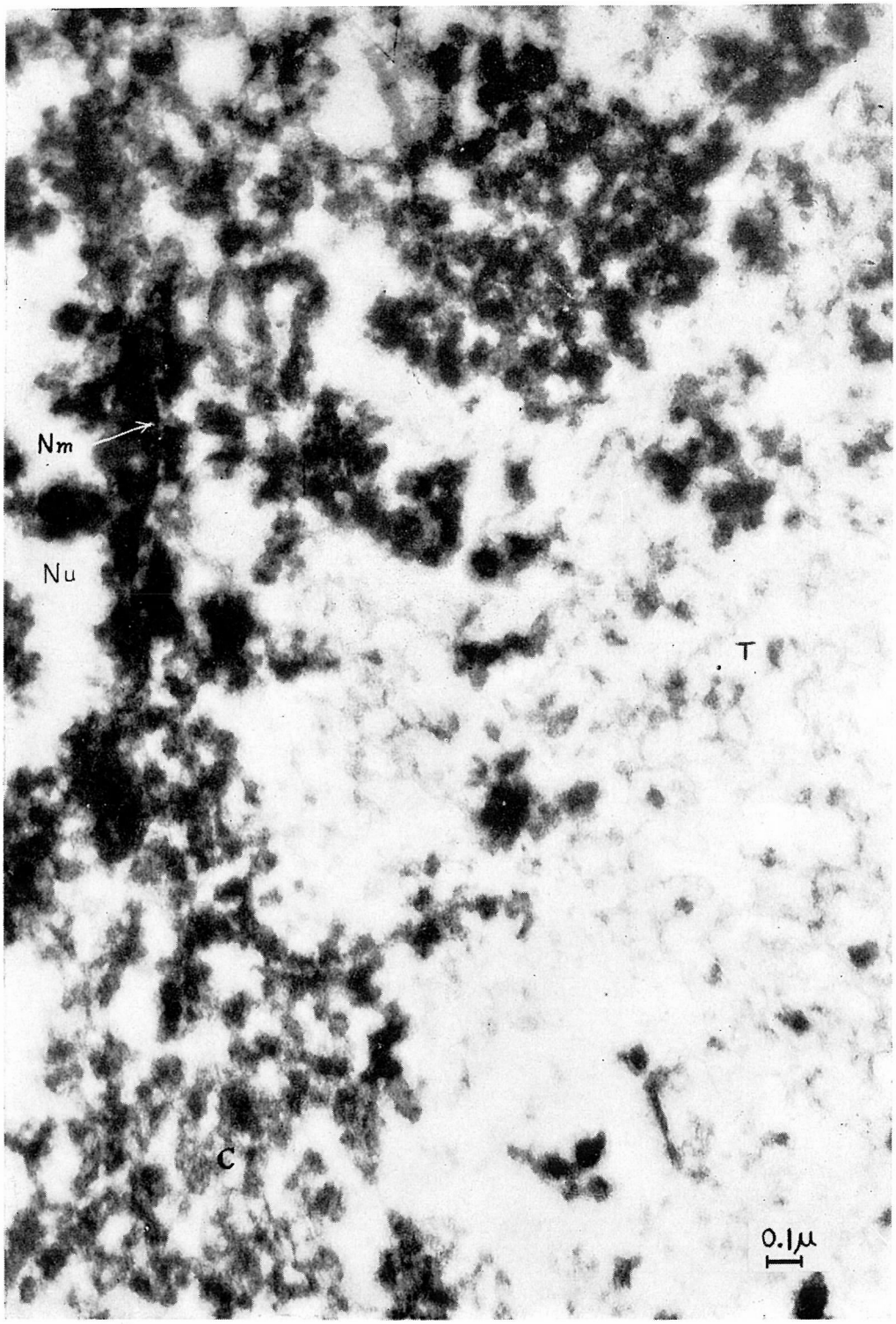

Fig. 15

PLATE 9

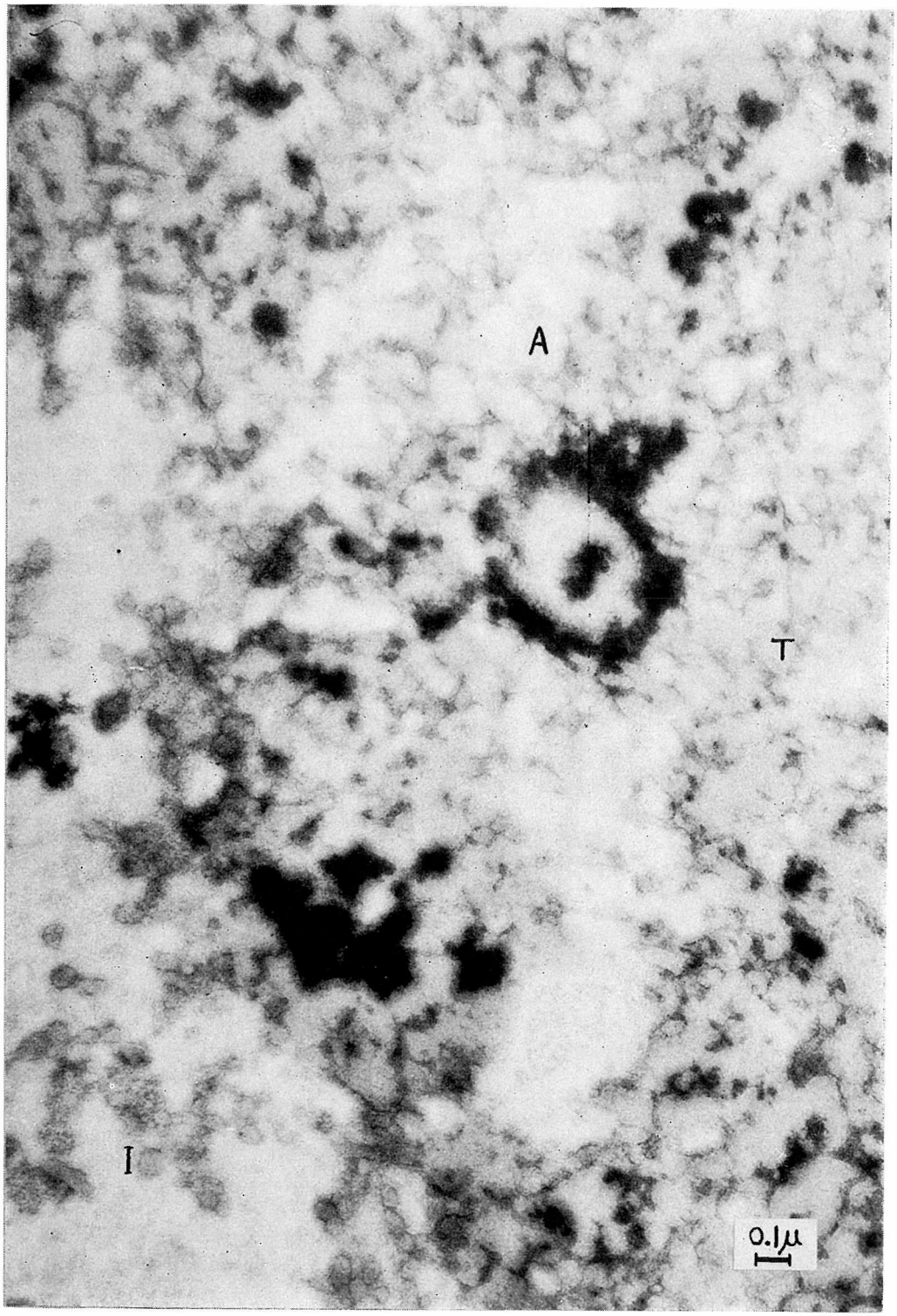

Fig. 16

PLATE 10

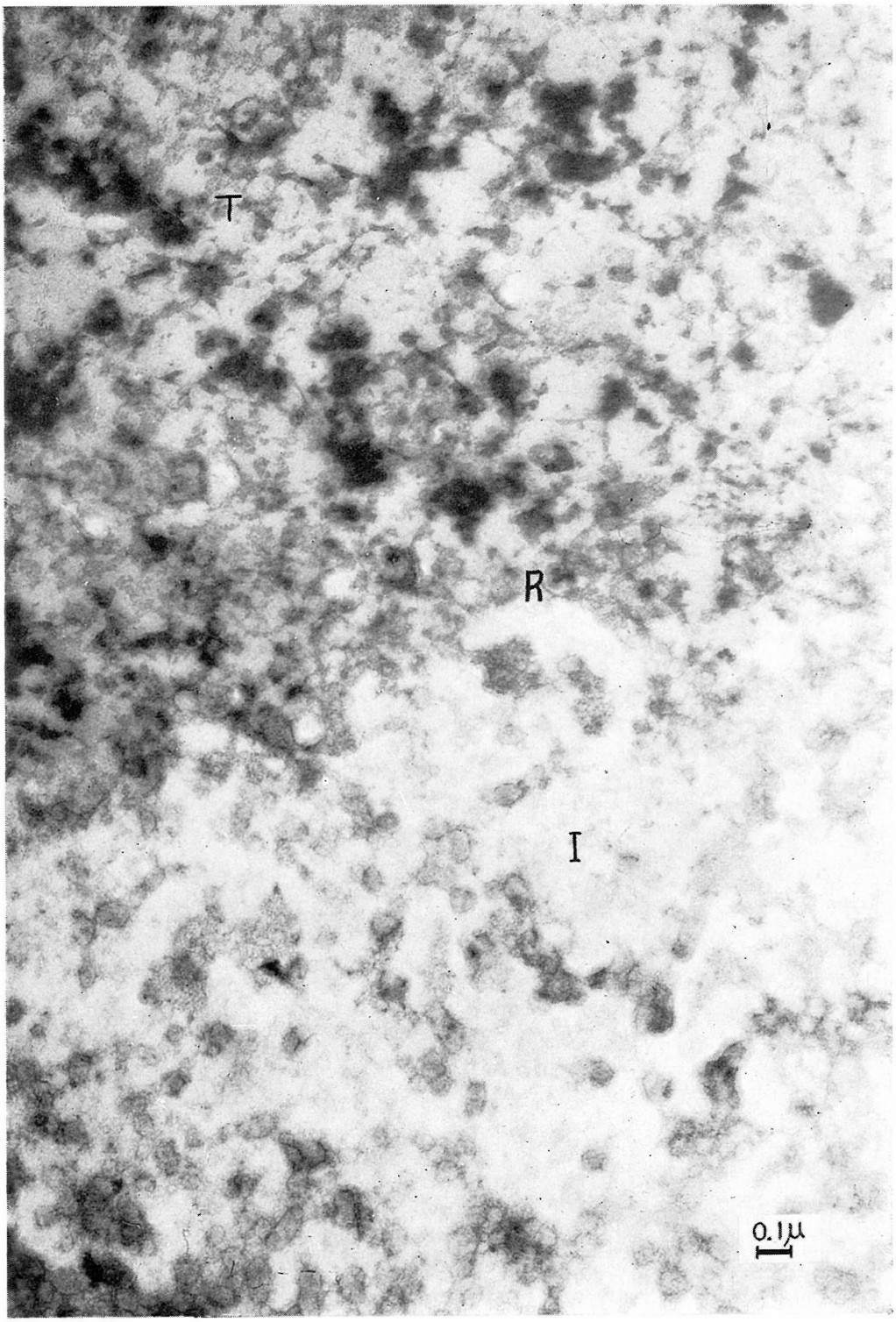

Fig. 17

PLATE 11

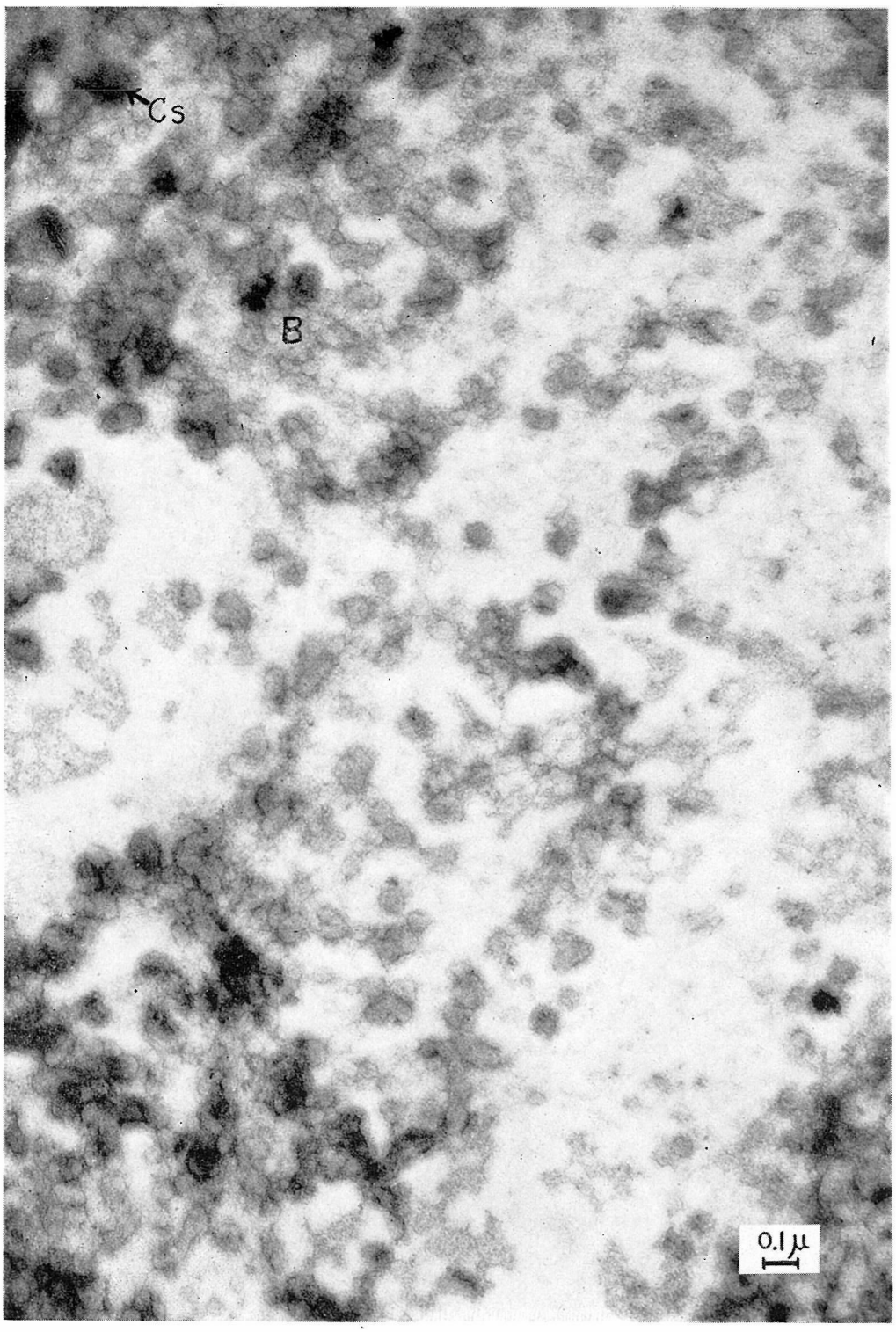

Fig. 18

PLATE 12

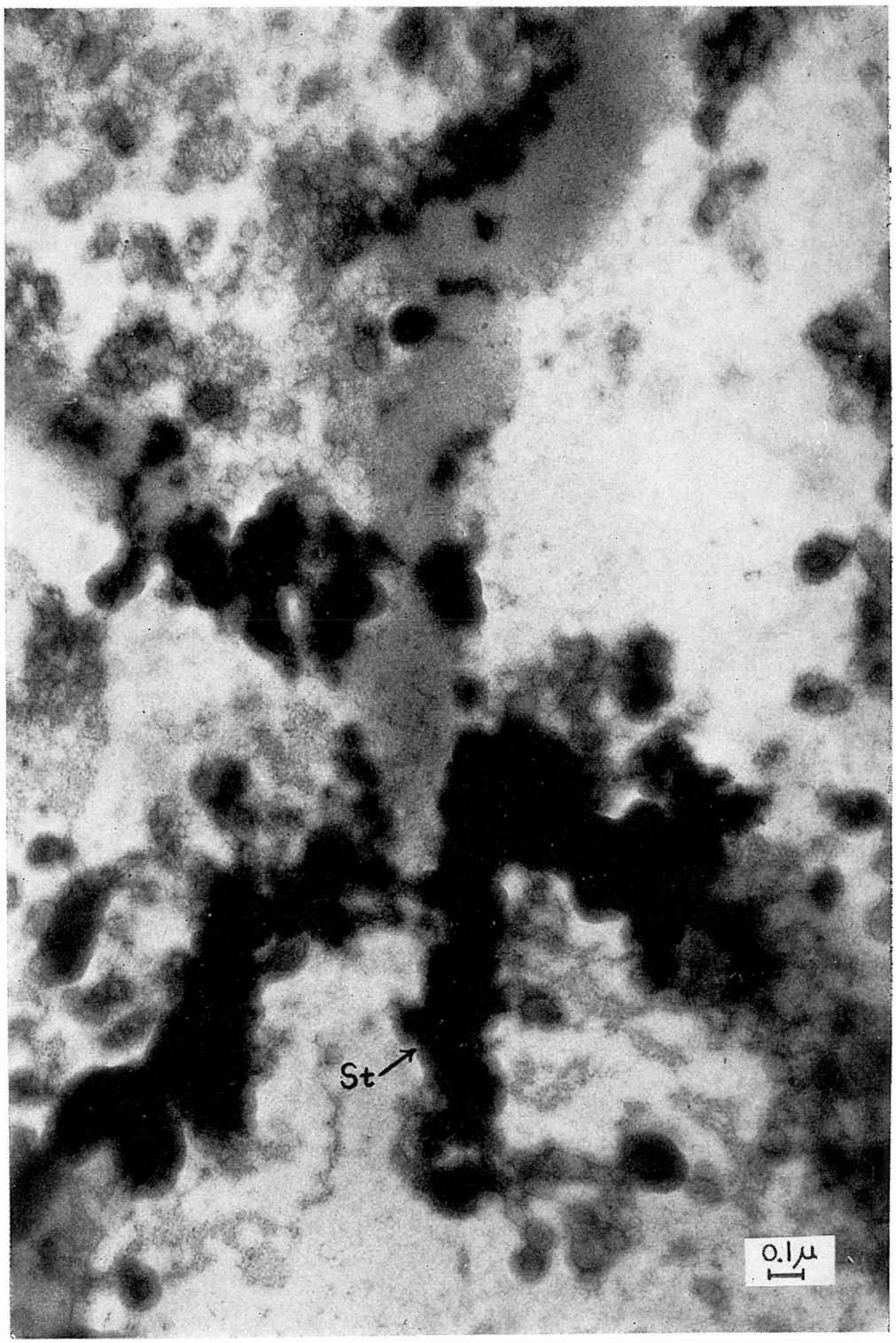

Fig. 19

PLATE 13

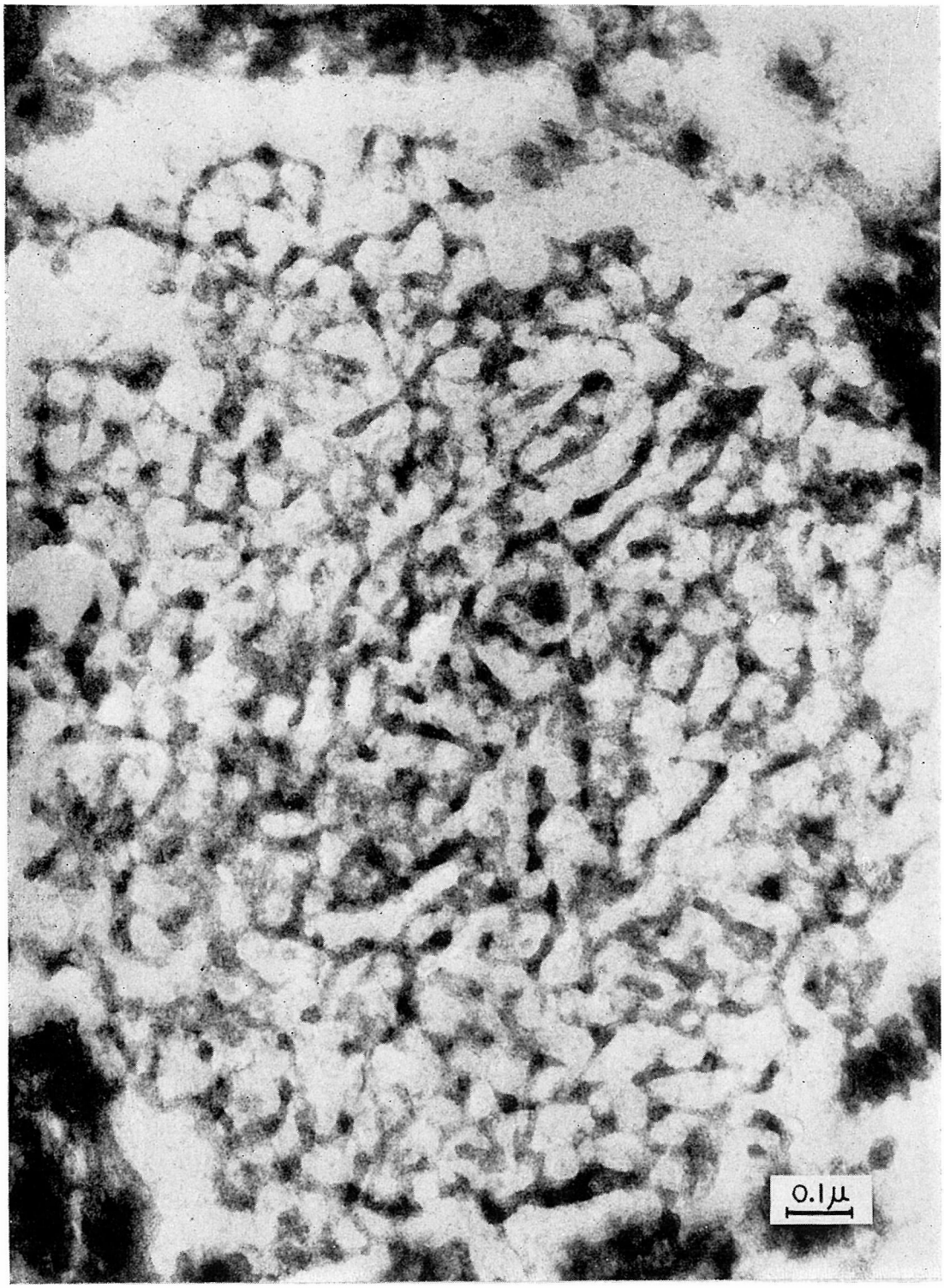

Fig. 29