DISCUSSION.

The major aim of this project was to provide experimental evidence that parathyroid hormone bound to bone cells.

The reason for examining this aspect of bone function was that parathyroid hormone had been shown to be involved in the metabolism of bone (see Introduction) but there was no evidence that the hormone interacted directly with bone cells. Over the past forty or fifty years evidence has been accumulated which suggested that PTH could be involved with all bone cell types (see Introduction) but until it could be proved that the hormone actually bound to any cell type, any theory proposed for its mechanism of action at the cellular or subcellular level had to be presumptive. Therefore, the results of this project were considered to be essential for a full understanding of parathyroid hormone function in the metabolism of bone.

To fulfill the aim of this project it was necessary to obtain a parathyroid hormone preparation that :

had been biosynthesised and secreted,

was biologically active,

was chemically pure, and

was heavily labelled with radioactivity.

It was decided to use bone and PTH from the same species because it is likely that there are variations in the amino acid sequences of PTH molecules from different species.

It was decided to use PTH that had been biosynthesised because, at the time of starting this project, the structure of PTH from any species was not definitely known. The amino acid sequence of rat PTH has still to be determined.

It was decided to use secreted PTH because it was not known whether the hormone was secreted in the same form as it was synthesised (Sherwood et. al. 1970; Potts et. al. 1971 a).

It was necessary to show that the PTH was biologically active because if it were inactive it would probably

have a different conformational structure and/or a different state of oxidation and it might react differently with its target cells.

It was necessary to use a radioactive hormone preparation that was chemically pure, or else the autoradiography would be meaningless.

It was necessary to produce hormone molecules that were heavily labelled with radioactivity to have any hope of localizing them in bone by autoradiographic means.

It had been reported that rat parathyroid glands synthesised and secreted PTH in organ culture (Raisz et. al. 1965; Au et. al. 1970), so the technique of organ culture was used in the experiments reported here.

As the PTH used here was radioactive, it was obvious that it had been synthesised during the period of culture, when the glands were exposed to radioactive amino acids. Also, it was concluded that the PTH used with bone had been secreted during the period of culture and was not

the result of cellular disruption. This conclusion was based on the results of a thorough histological examination of all glands after they had been removed from culture. As was to be expected, all cultures were not 100% successful and cell death, followed by the loss of intracellular components, occurred in some instances. The media from these unsuccessful cultures contained a greater range of radioactive polypeptides than media from healthy cultures. This was probably caused by the action of hydrolytic enzymes, released by the disrupted cells, on radioactive molecules that had been secreted by healthy cells. Also, cell death would have allowed the release of partially synthesised proteins and of complete molecules that otherwise may not have been destined for secretion. When media from these cultures were fractionated on G50, the usual, single peak in the fractionation range was replaced either by several radioactive peaks or by a broad peak exhibiting one or more shoulders. These results indicated that such media contained a greater number of molecular species, within the molecular weight range 1,500-30,000, than media from healthy cultures and that it would not be profitable to try to separate PTH from such a mixture. Accordingly, material from these

media was discarded and never used with bone for subsequent autoradiography.

The culture medium used in the initial incubations was supplemented with 20% horse serum, because organ culture methods always seemed to include serum additives of some kind. This system seemed to work very well in that the glands appeared to be healthy by histological criteria and hormone activity was demonstrable in the culture medium. It was very difficult to separate the PTH from all the other molecules that were present, and it was obvious that the serum was the cause of this problem. To overcome this difficulty, parathyroid glands were incubated in a simple medium that contained no serum additives. (It was interesting to note that the Rochester group (Au et. al. 1970) lowered the serum content of the media from 50% to 5%). The absence of serum from the medium probably resulted in much lower yields of PTH, when compared to results of other workers (Au et. al. 1970; Martin et. al. 1971), but it proved to be a more satisfactory technique as it made possible the recovery of PTH in pure form. The glands appeared to be healthy, morphologically, and the cells synthesised and secreted PTH so, apart

from the initial cultures, glands were incubated without serum additives.

Under the conditions of these experiments, only one molecular species, similar in size to PTH, was recovered from culture medium. These molecules had been newly synthesised and had been secreted by the cells. Other investigators (Raisz et. al., 1965; Au et. al., 1970; Licata et. al., 1972) recovered more than one type of molecule (similar in size to PTH) from parathyroid gland culture medium. These glands were cultured with serum and it may have been that the serum aided the secretion of a greater range of molecules by the cells. On the other hand, these workers apparently did not look at all their material histologically, so they could not have been certain that cell disruption had not occurred. In support of the evidence in this thesis, primary column chromatography of media (free of serum additives) from human parathyroid adenoma cell cultures (Martin 1971) produced PTH that was pure by polyacrylamide gel electrophoresis.

Carbon - 14 was used to label the parathyroid hormone

because this did not increase the number of atoms in the molecule. Also, the label could not be lost without disruption of the molecule, and this could readily have been detected if it had occurred. It was thought that radioactive PTH produced in this manner would more closely resemble natural PTH than if extra atoms (e.g. $^{131}_{1}$) were used to label the hormone. The amino acids used were uniformly labelled with ^{14}C in an attempt to produce a highly radioactive protein for use with autoradiography. The incorporation of so much radioactivity probably increased the possibility of radiolysis, but chromatography on Sephadex G25 indicated that the molecules were stable for at least two weeks when stored dry at - 70°c.

In 1962, Rasmussen and Craig reported the use of Sephadex G50 for primary column chromatography of parathyroid gland extracts. The samples applied to these columns were very heterogeneous, and subsequent work (Aurbach and Potts, 1964) indicated that G100 was more suitable at this stage of the purification procedure. G100 has also been used for column chromatography of parathyroid gland culture media containing serum proteins

(Au et. al., 1970). However, the samples fractionated by column chromatography in the work reported here were more homogeneous than those referred to above, and Sephadex G50 offered the most suitable fractionation range. When parathyroid gland culture medium, free of serum additives, was subjected to column chromatography on G50, only one peak was found in the fractionation range, the material in this peak was similar in size to PTH, and it exhibited calcium - mobilizing ability. The material which eluted in the void volume of the G50 column did not appear to have PTH activity and its molecular weight was probably in excess of thirty thousand. Its nature was not examined further.

The ion exchange chromatography was based on the advice of Dr. L.G. Raisz (personal communication) whose team at Rochester had found a carbohydrate containing molecule which ran with rat PTH on Sephadex. In one of their recent papers (Licata et. al., 1972) they described the incorporation of D-glucosamine into macromolecules by rat parathyroid glands in tissue culture. When medium from these cultures was examined by Sephadex chromatography, one of the sugar containing molecules contaminated the rat PTH. When this mixture was subjected

to anion exchange chromatography on D.E.A.E. Sephadex, the sugar containing compound was retarded at low ionic strength while the PTH was not. When a cation exchanger (carboxymethyl cellulose) was used, the PTH was retarded at low salt concentrations while the labelled sugar was not. The authors were not certain whether the sugar containing protein had been secreted by the parathyroid cells or whether it had been liberated into the culture medium by disruption of some of the cells. Unfortunately, they did not examine the glands histologically after the cultures were terminated. In my experiments, it was not possible, by either anion or cation exchange chromatography, to separate any other radioactive molecules from the rat PTH recovered from the second radioactive peak from gel filtration of serum-free parathyroid gland culture medium. There are at least three explanations which may account for these conflicting results. First, Licata et. al. (1972) used 5% heat inactivated serum from thyroparathyroidectomized rats in their culture medium, and it may be that parathyroid glands secrete a wider range of molecules under these conditions. Secondly, all the glands used in the serum-free cultures were examined histologically.

Evidence of cellular disruption usually paralleled an altered elution profile on G50, and such material was discarded. This would indicate cell death as the reason for the appearance of the sugar molecule in the medium. Thirdly, Dr. Raisz (personal communication) said that the labelled glucosamine was readily incorporated but that labelled amino acids did not show up in the molecule till some time later, suggesting that there was a lot of preformed protein available. Under the conditions of the serum-free cultures, it is possible, but unlikely, that new protein moieties for the sugar-containing molecules were not synthesized. In a recent paper, Nakagami et. al. (1971) reported experiments employing electron microscope autoradiography on rat parathyroid cells. They interpreted their results as suggesting that carbohydrate may be incorporated into a prohormone molecule or that a carbohydrate moiety may be involved in the secretion of PTH. If their interpretation is correct, then it is possible that carbohydrate is associated with PTH before secretion, but not secreted with it.

As mentioned in "Results", the polyacrylamide work

must be regarded as a preliminary study. However, these results were certainly suggestive that the second radioactive peak from G50 was homogeneous, and they received support from Martin (1971).

The work with Sephadex G25 and paper chromatography was done mainly to look for contamination of the PTH by small molecules or for evidence of radiolysis. These methods indicated no inhomogeneity of material used for autoradiography.

Therefore, within the limits of the techniques used, the rat PTH incubated with rat bone for autoradiographic purposes was substantially pure. The only indication of inhomogeneity of the second G50 radioactive peak occurred with material recovered from gland culture medium which had been incubated with disrupted parathyroid tissue. Whenever this situation arose, the material was discarded and was not used with bone.

Various methods have been used for assaying parathyroid hormone activity (Munson, 1961; Raisz, 1963;

Berson et. al. 1963; Caunton et. al. 1965; Amar 1968; Parsons and Robinson 1971a, 1971b). In the experiments reported here there was no attempt to assay the amount of rat PTH that was recovered; all that was required was a simple, rapid technique which could be used to detect calcium-mobilizing ability. Initially, an *in vivo* technique was tried but did not seem to be sensitive enough for the quantities of rat PTH that were recovered. This left *in vitro* techniques: using either radioimmunoassay or some type of organ culture of bone. The latter method was chosen for its simplicity and because organ culture techniques were well established in this laboratory. Also in the interests of simplicity, the technique described above was used in preference to the use of ^{45}Ca and embryonic bone (Raisz 1963). As was pointed out earlier, no attempt was made to relate the activity of rat hormone to other PTH preparations, because the large number of experiments required to set up an assay was outside the scope of the project. Although the number of experiments was small the second radioactive peak from G50 consistently stimulated the removal of calcium from bone in the *in vitro* system and the calcium concentrations

of test media were substantially above those of control media (Table 1). Of great importance were the results of those experiments where the rat protein had been incubated with antibodies to bovine PTH before addition of the bone shaft. In these cases, the calcium mobilizing ability of the rat protein was blocked, and this was a strong indication that the protein was actually parathyroid hormone.

Once a reliable system for the synthesis and purification of biologically active rat PTH had been established it was necessary to introduce the hormone to bone and then to try and localise its binding sites. If the very small quantity of hormone available had been injected into an animal it would have been diluted out so much that the chances of finding it again would have been remote. Therefore, it was administered to bone <u>in vitro</u>.

The next problem to be overcome was the choice of a technique that would show what type of bone cell was associated with the radioactivity. Bone cells can be classified by their morphology and their relationship

to the calcified matrix. There is no successful method for separating bone cells from matrix and then dividing them into various types. Therefore, it seemed necessary to use a morphological method, and autoradiography was the obvious choice.

The times of incubation of radioactive PTH with bone were chosen arbitrarily and these times could not be related to the in vivo situation because of the lack of circulation, and other differences, in vitro. The aim was to allow sufficient time for the PTH to bind to bone, but not to leave it so long that the hormone could be broken down and the radioactivity spread around non specifically. Autoradiographs were done with bone that had been incubated for ten minutes with PTH but the results did not suggest that any radioactivity had been bound to the bone. Incubation times of thirty and sixty minutes were suitable because the bone cells bound radioactivity, and as the labelling patterns were similar in both these instances, it was presumed that hydrolysis of the hormone had not occurred. Autoradiographs were done with bone that had been exposed to radioactive amino acide to see how rapidly they were incorporated. After an incubation of only four

minutes, these small molecules were taken up by bone cells and silver grains were found overlying the protein synthesising areas of the cells. From these results it was concluded that the autoradiography, which showed grains at the cell periphery, demonstrated the binding of PTH and not its breakdown products to bone cells.

After incubation, the tissue was fixed and then embedded in araldite. This method of embedding was used because it was possible to cut very thin sections of tissue and thus to improve the resolution of the system (the thinner the section used, the better the autoradiographic resolution (Caro et. al., 1962). After these results were presented at "Endocrinology 1971" (O'Grady and Cameron), Dr. E. Kodicek (Dunn Nutritional Laboratory Cambridge, England) suggested that the resolution with ^{14}C was not good enough for this type of work. However, Dr. Maureen Owen (M.R.C. Bone Research Laboratory, Churchill Hospital, Oxford, England) who has used autoradiography a great deal, disagreed with Dr. E. Kodicek. She stated, after the meeting, that the resolution of ^{14}C in 0.5 micron sections was at least as good as that for ^{3}H in 5.0 micron sections. Also, a detailed study

by Salpeter and Salpeter (1971) demonstrated that ^{14}C was suitable label even for high resolution autoradiography.

Over all, the use of ^{14}C in very thin tissue sections is considered to be suitable for autoradiography and the claim is made that rat PTH binds to osteoblasts and to osteoprogenitor cells in rat parietal bone. Further, this binding occurs close to the surfaces of the cells; that is, it is very likely that the hormone is bound to the plasma membranes. These results were interpreted to mean that the cells had specific binding sites for PTH and the fact that the grain distribution was similar whether the bone had been incubated for thirty or sixty minutes indicated that the hormone was fairly strongly bound.

The silver grains located over osteocytes were viewed with some degree of suspicion. They may have been caused by beta particles emitted from the radioactive PTH but on the other hand they may have represented non-specific grains caused by uneven distribution of emulsion over the transition area from calcified matrix to osteocyte. Further work may clarify this

dilemma, or autoradiography may not be a suitable technique for the localisation of a radioactive tracer near the periphery of an osteocyte. However, these results do not preclude the possibility that PTH may have an effect on osteocytes.

As pointed out by Professor D.H. Copp (Department of Physiology, University of British Columbia, Canada) at Endocrinology 1971, the osteoclast was prominent by its absence from these results; the more so because many workers would have had it as the prime candidate for PTH binding. There was a simple answer to this question and that was that osteoclasts were rare in the areas of parietal bone used. Although a couple of osteoclasts were found underlying silver grains, no conclusions could be drawn from this material. An attempt to clarify the position of the osteoclast with regard to PTH has been made using tissue rich in these cells (9 day fracture callus) but positive results have not been achieved. It is quite possible that existing osteoclasts are unaffected by parathyroid hormone; that the hormonal effect is to stimulate other cell types to form new osteoclasts. However, if the osteoprogenitor cells bind PTH, and if

these cells coalesce to form osteoclasts (Young, 1962), it seems likely that osteoclasts would still be capable of binding the hormone unless the projected plasma membrane receptors are altered on the formation of the multinucleate cell.

It is apparent that PTH binds to the periphery of at least two types of bone cell, but what is the significance of this? What effect does the hormone have on these cells at the molecular level, and, if PTH causes different cell types to react differently (Owen and Bingham, 1967), is this because of the initial reaction between hormone and cell? It is possible that the initial binding of PTH is the same, but that different reaction sequences are set in motion in different bone cell types.

In recent years, research workers have been concentrating on the relationship of PTH to the adenylcyclase/cyclic AMP system (Wells and Lloyd, 1967, 1969; Chase et. al., 1967, 1970; Vaes, 1968; Aurbach et. al., 1969a, 1970; Melson et. al., 1970; Rasmussen, 1970, 1971). Adenyl cyclase is a plasma membrane bound enzyme which converts

adenosine triphosphate (ATP) to adenosine 3', 5' monophosphate (cyclic AMP). Another enzyme, cyclic nucleotide phosphodiesterase, found in the particulate and soluble fractions of the cell, catalyses the hydrolysis of cyclic AMP to adenosine 5' - monophosphate, and this enzyme is inhibited by theophylline. The relationship of this system to hormone action was suggested by Sutherland and Rall (1958) when they reported that cyclic AMP mediated the effects of epinephrine on liver phosphorylase. Since that time, people have been examining adenyl cyclase activity in relation to a variety of hormones. The theory is that an increased concentration of cyclic AMP affects other cellular processes such as activation or secretion of enzymes, active transport of ions across cellular membranes, or release of other hormones. Activation of the particular system involved causes the physiological responses attributed to the hormone (Aurbach et. al. 1969b). In 1967, Wells and Lloyd reported that in rats parathyroidectomised for four days, a single injection of theophylline (120 mg kg^{-1}) caused a rapid, marked, and relatively long-lasting elevation in serum calcium. Theophylline also retarded the fall in serum calcium which followed parathyroid-

ectomy. Bilateral nephrectomy did not alter the effect, suggesting that the drug did not depend on a renal action for its hypercalcaemic effect. They concluded that since theophylline altered the metabolism of cyclic AMP in many tissues, cyclic AMP might be involved in the action of PTH. Also in 1967, Chase and Aurbach demonstrated that the administration of bovine PTH caused an increased renal excretion of cyclic AMP in rats. In 1970, Aurbach and Chase, Chase and Aurbach, and Nelson, Chase and Aurbach reported a variety of findings. PTH stimulated the formation of cyclic AMP by renal tubules *in vitro*. They prepared homogenates of fetal rat calvaria and measured the response of adenyl cyclase to a variety of agents. PTH stimulated this enzyme while calcitonin apparently did not and in turn the enzyme was inhibited by calcium. They prepared homogenates of brain, heart, spleen, thyroid, adrenal cortex and isolated adipose tissue cells. PTH had no effect on cyclic AMP production in these tissues. Again *in vitro*, they used calvaria isolated from full term rats. Addition of PTH to these cultures caused a marked increase in cyclic AMP within one minute and was maximal in five minutes. The response was a direct function of the log concentration of PTH from 0.1 to 1.0 ug ml^{-1}. Addition to these cultures of corti-

cotropin, thyrotropin, glucagon, insulin, luteinising hormone or growth hormone had no effect on adenyl cyclase activity. Epinephrine also caused a rise in cyclic AMP but this effect could be blocked by propranolol. The PTH effect was not blocked by this agent. These workers interpreted their results as suggesting that cyclic AMP may be involved in the action of PTH.

It seems possible then, that cyclic AMP may be involved in the subcellular mechanism of action of parathyroid hormone. Even if the cyclic nucleotide does mediate the action of parathyroid hormone, does the latter stimulate adenyl cyclase directly or is the enzyme one or more steps removed from the initial reaction? The results of Parsons and Robinson (1971a) were interesting because these workers have produced very convincing evidence that the initial effect of parathyroid hormone in various species is a fall in the level of serum calcium. In previous reports, this effect had been attributed to contamination of the PTH by calcitonin. This is no longer tenable and the earliest observed effect of PTH <u>in vivo</u> is a fall in serum calcium. This effect was interpreted to mean that

there was an influx of calcium into cells of bone during the first five to ten minutes after administration of PTH *in vivo*. This initial loss of calcium from the circulation would then be followed by the removal of calcium from osseous tissue as reflected by an increase in serum calcium.

In summary at this point, the action of PTH on bone cells is probably involved in the trans-membrane movement of calcium ions, perhaps involving adenyl cyclase. The action of PTH on ion translocation is likely to be related to the hypercalcaemic effect of the hormone, but despite attempts to relate PTH, calcium, adenyl cyclase and bone (Rasmussen, 1970; Rasmussen *et. al.*, 1971), the importance of adenyl cyclase and cyclic AMP in the action of PTH is still obscure. In fact, until the present results were reported (O'Grady and Cameron, 1971) there was no evidence that PTH interacted directly with bone cells.

The evidence presented in this thesis demonstrates that PTH is bound by bone cells, that it is bound on, or close to, the periphery of such cells, and that it

remains bound for a considerable period of time. This knowledge supports the ideas that PTH may be directly involved in ion translocation across the plasma membrane and that adenyl cyclase, a plasma membrane bound enzyme, may be directly involved in the action of PTH on bone cells. It is hoped that the results reported here will contribute to a fuller understanding of parathyroid hormone function.

CONCLUSIONS.

Using the techniques described above, it has proved possible to biosynthesise rat parathyroid hormone that :

1) is in the secreted form
2) is substantially pure
3) contains a covalently bound, internal, radioactive label
4) has biological activity, that can be blocked by antibodies to bovine PTH, and
5) is strongly bound to osteoblasts and osteoprogenitor cells in rat parietal bone.

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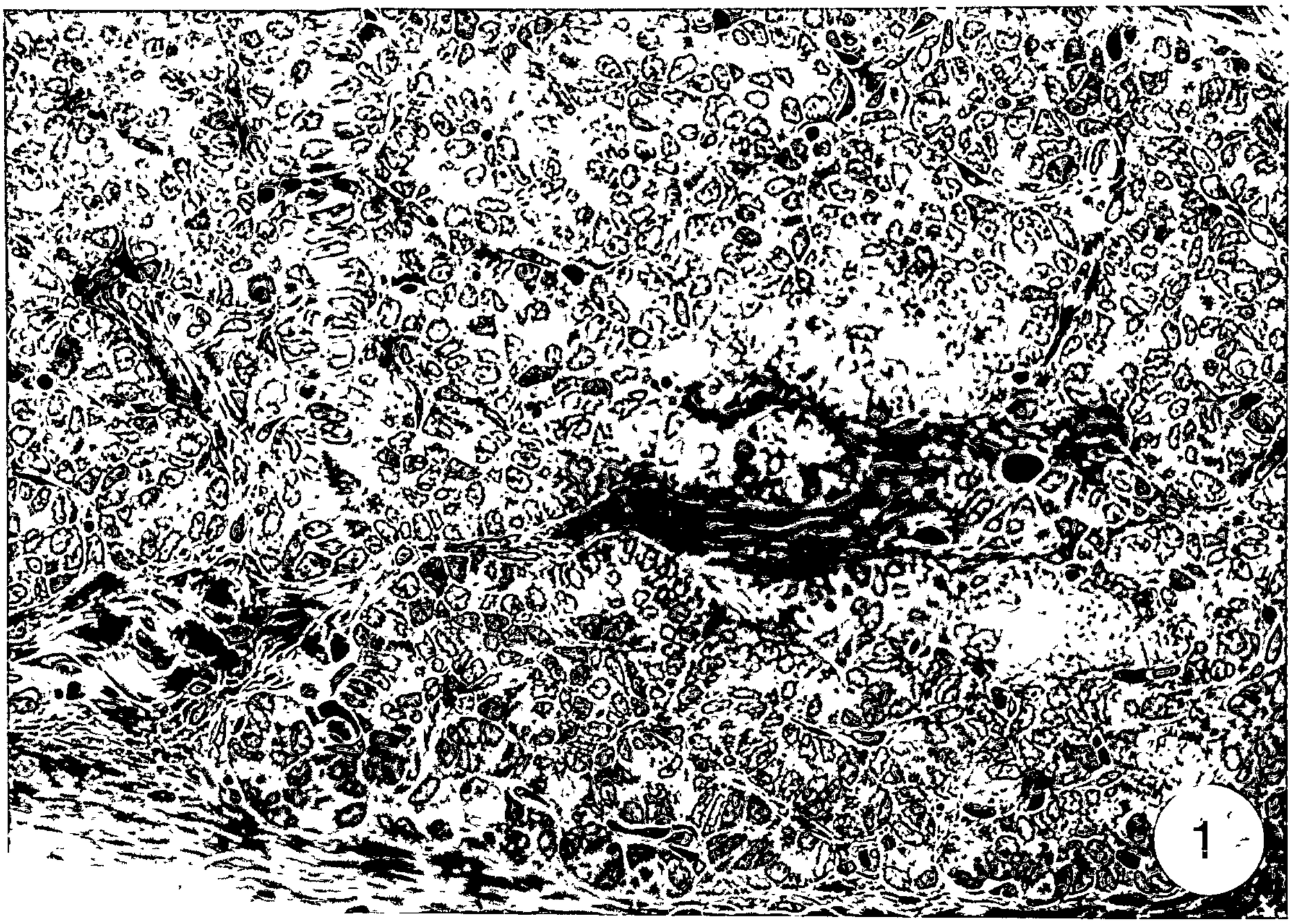
LEGENDS.

Fig. 1. Photomicrograph of a 0.5 micron section of rat parathyroid gland cultured for one day in normal Eagle's medium.

X460

Fig. 2. Photomicrograph of a 0.5 micron section of parathryoid gland cultured for five days in normal Eagle's medium.

X 460



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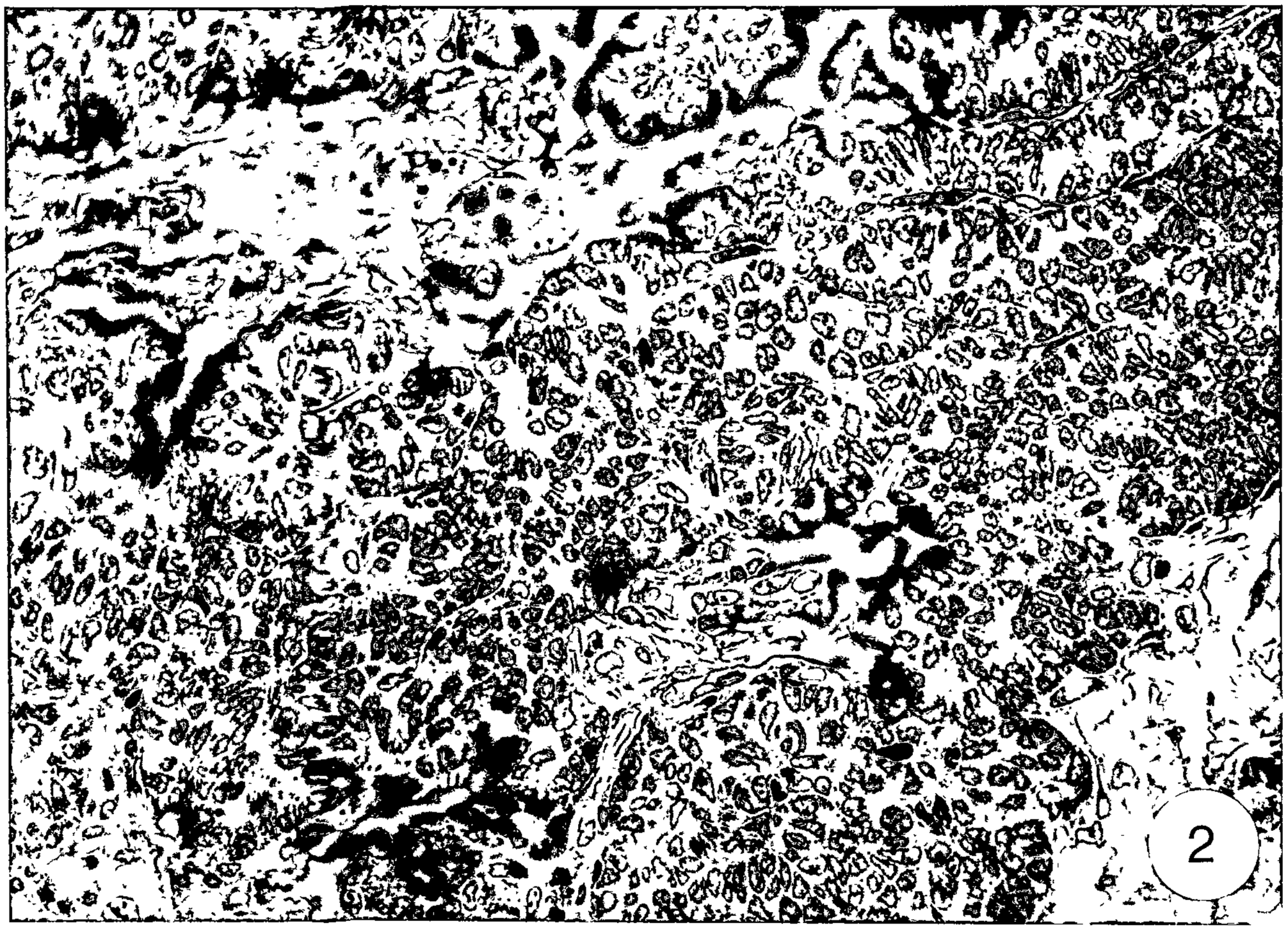


2

Fig. 3. Photomicrograph of a 0.5 micron section of parathyroid gland cultured for nine days in low calcium medium.

X 660

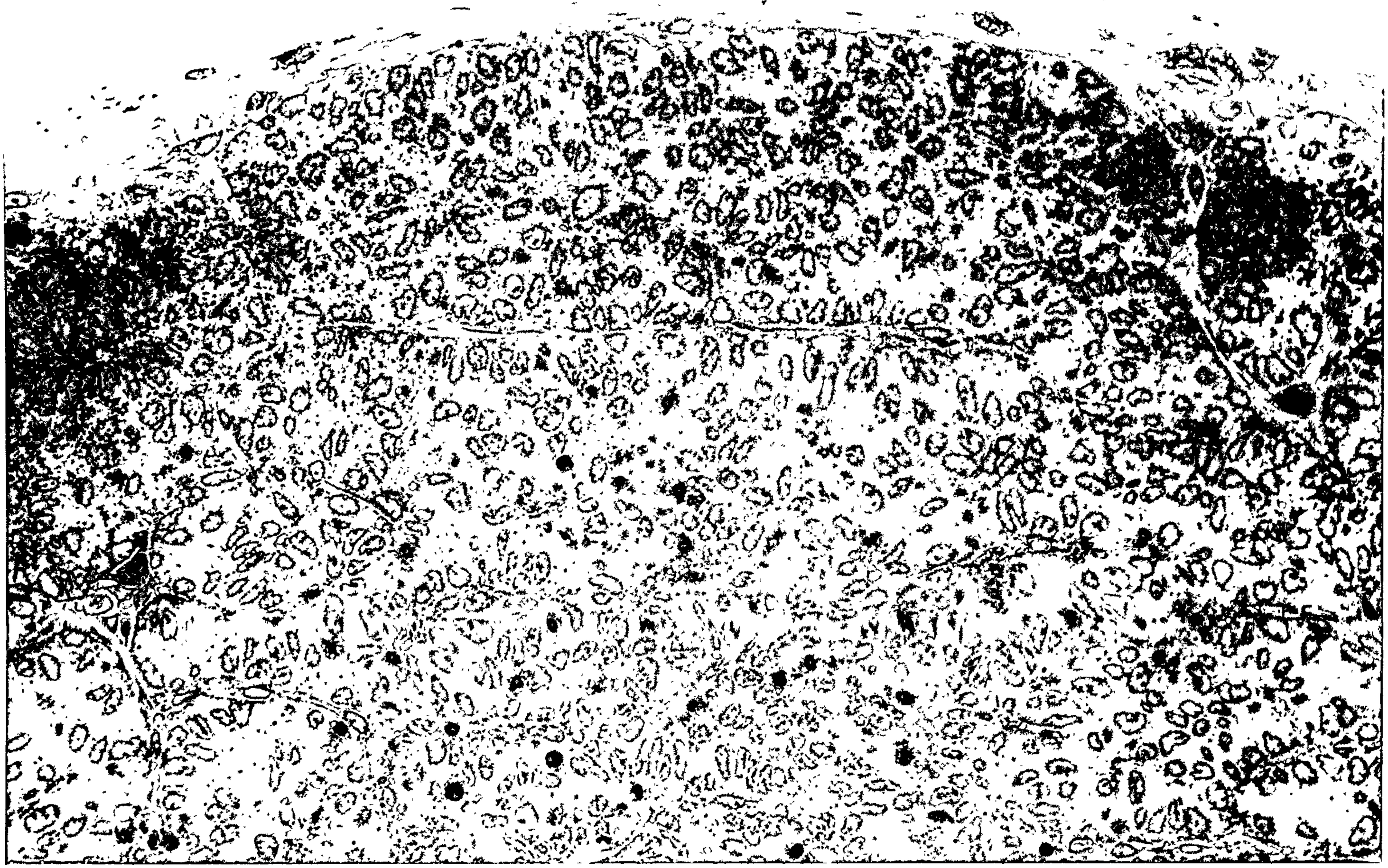


Fig. 4. Electron micrograph of parathyroid tissue cultured for five days in normal Eagle's medium. Note the presence of lysosomes, free ribosomes, active mitochondria (one in undergoing division), and interdigitating plasma membranes with desmosomes.

x 15,000

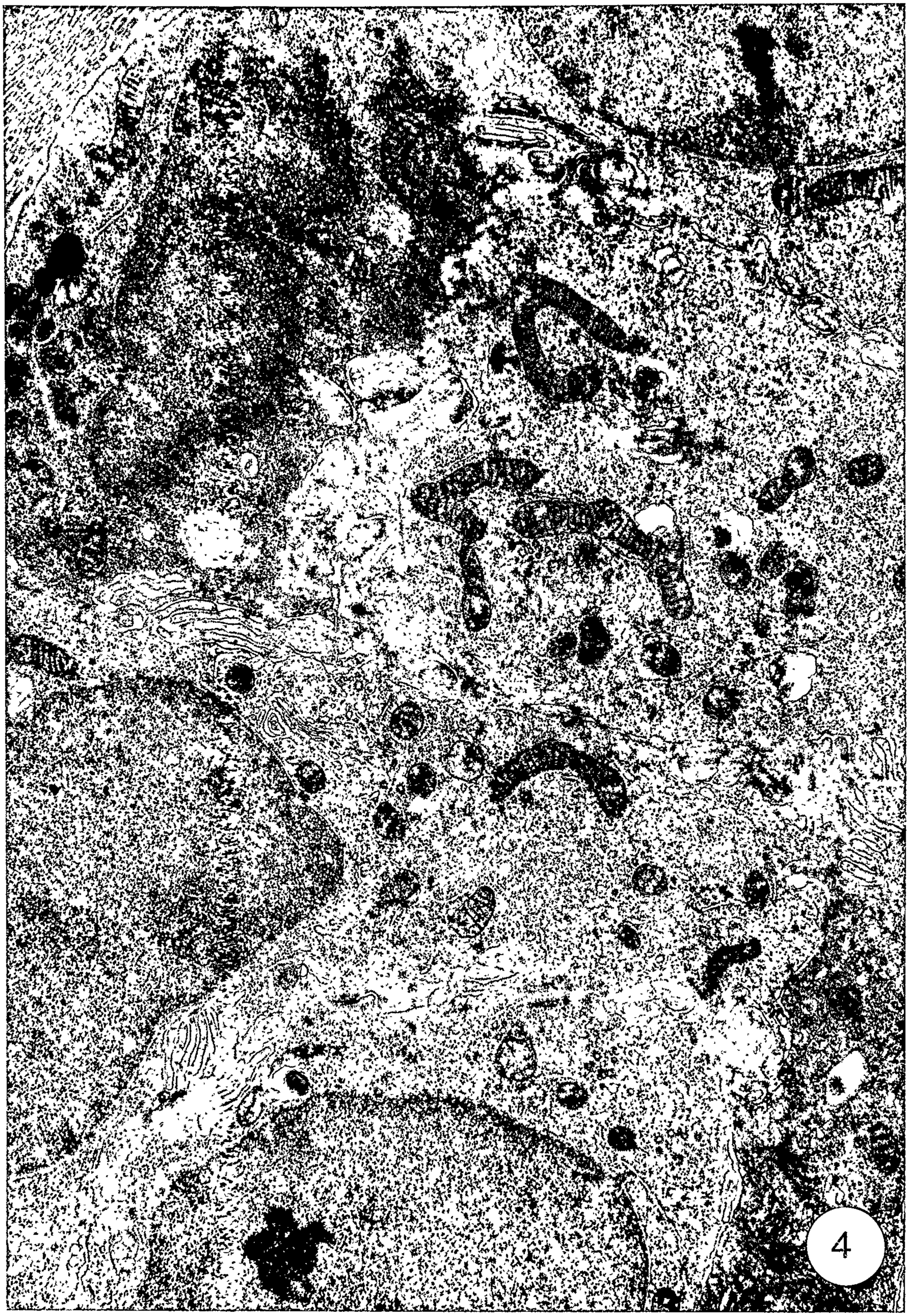

4

Fig. 5. Electron micrograph of parathyroid tissue cultured for five days in normal Eagle's medium. This tissue came from another gland in the same culture as that in Fig. 4.

x 24,000

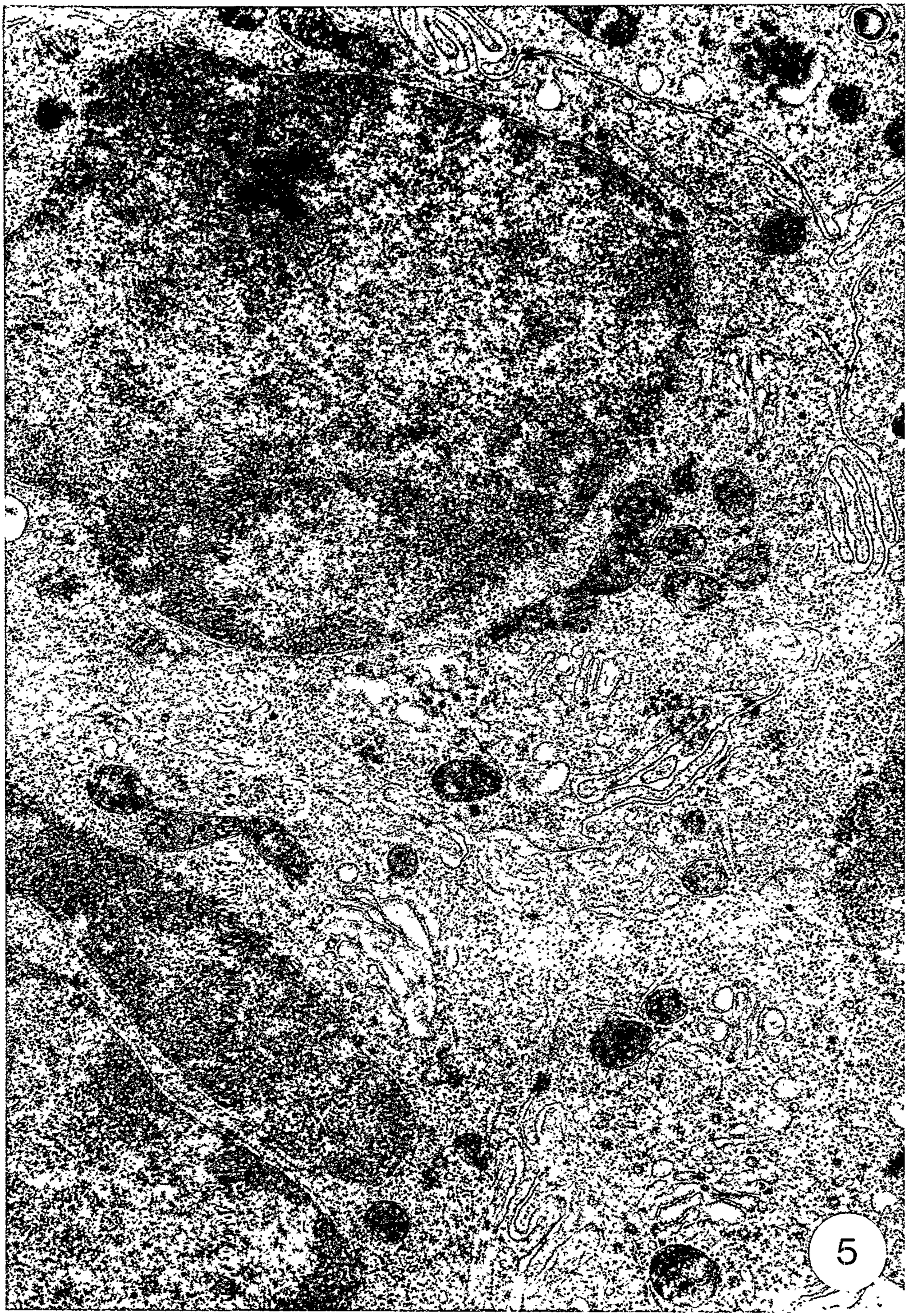
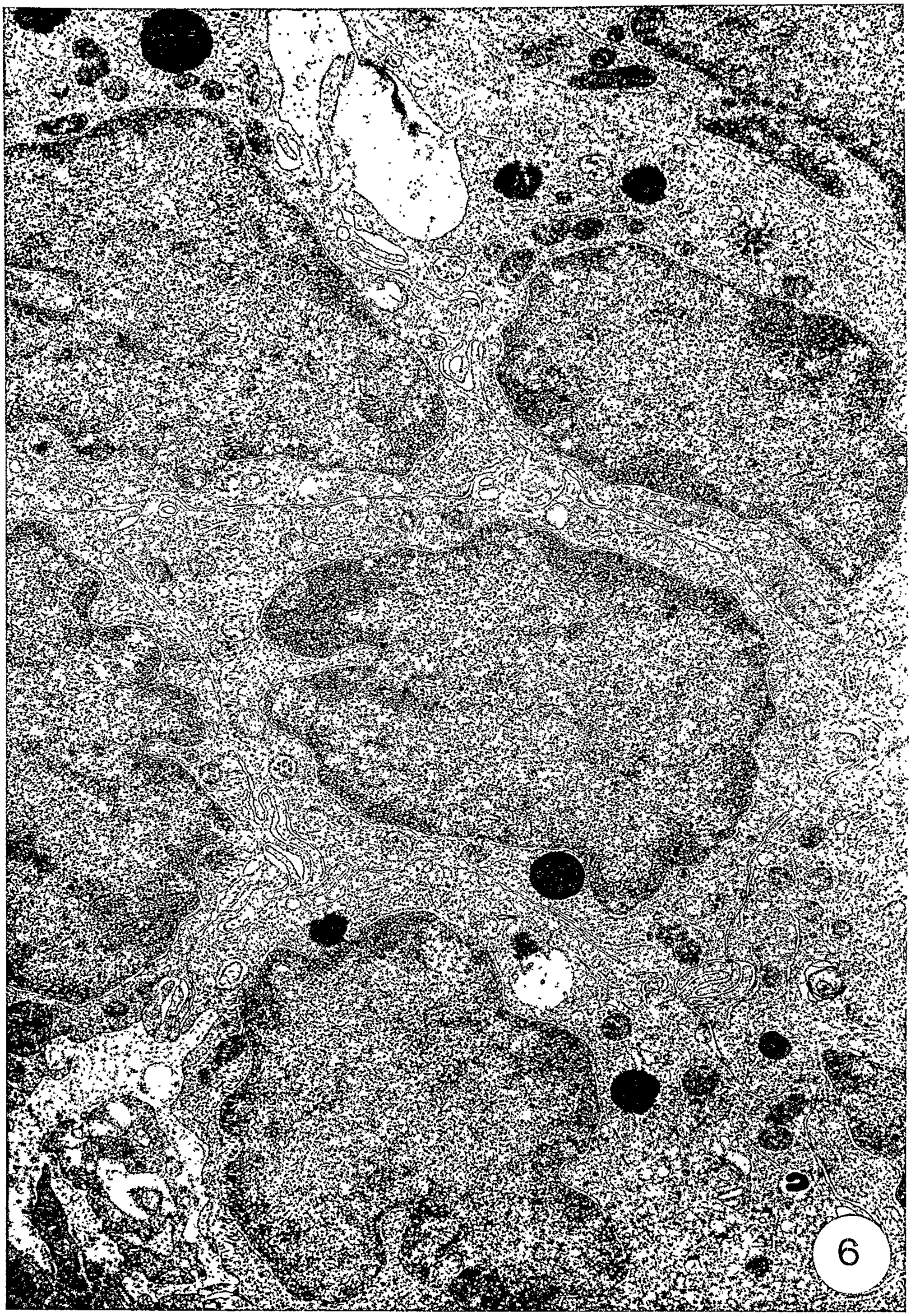


Fig. 6. Electronmicrograph of parathyroid tissue cultured for eight days in normal Eagle's medium. Note the accumulation of lipid droplets which are more evident in these longer term cultures.

x 13,00



6

Fig. 7. Electronmicrograph of parathyroid tissue cultured for three days in normal Eagle's medium supplemented with 20% horse serum.

X 15,000

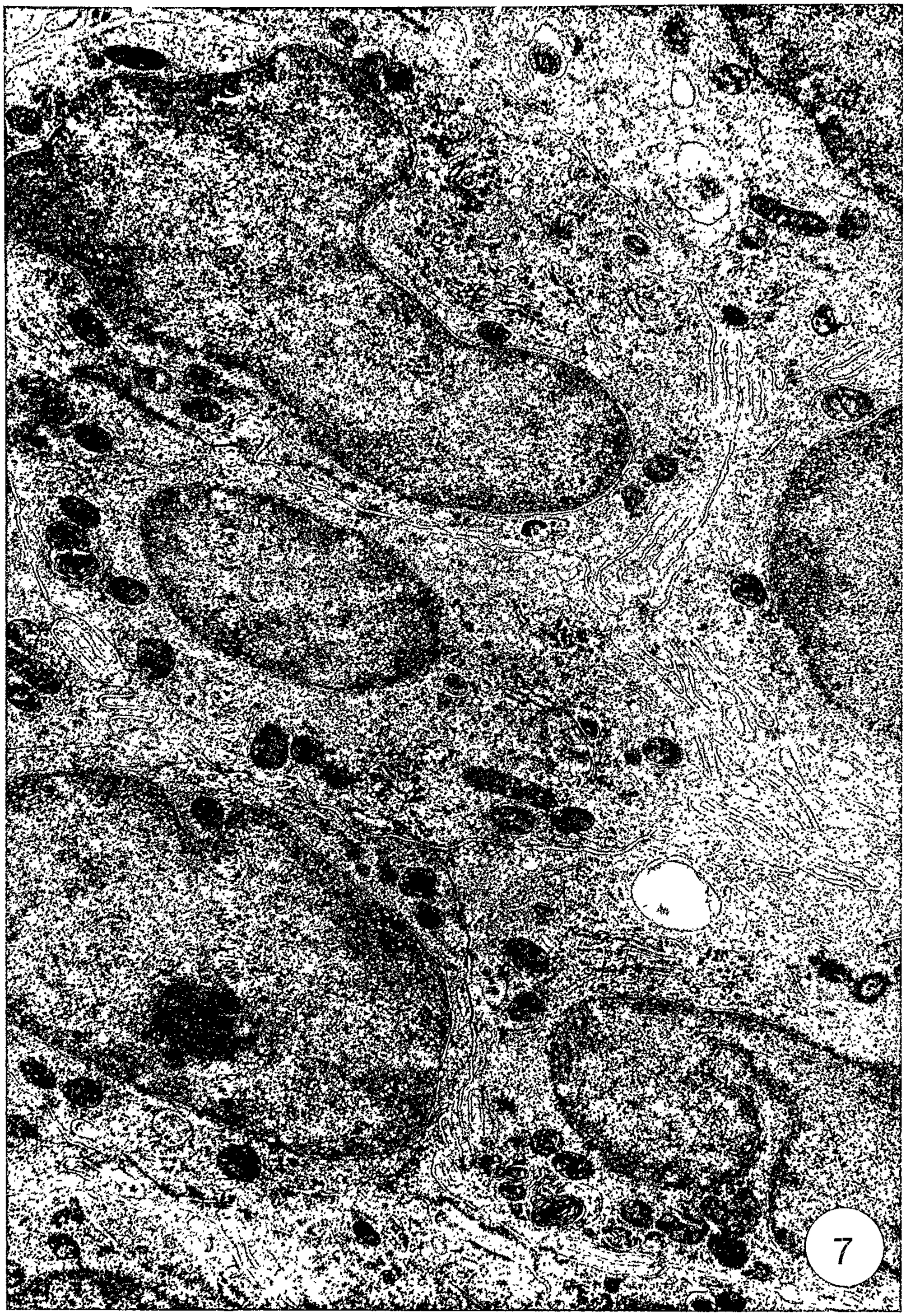


Fig. 8. Electronmicrograph of parathyroid tissue treated in a similar manner to that in Fig. 7.

x 15,000

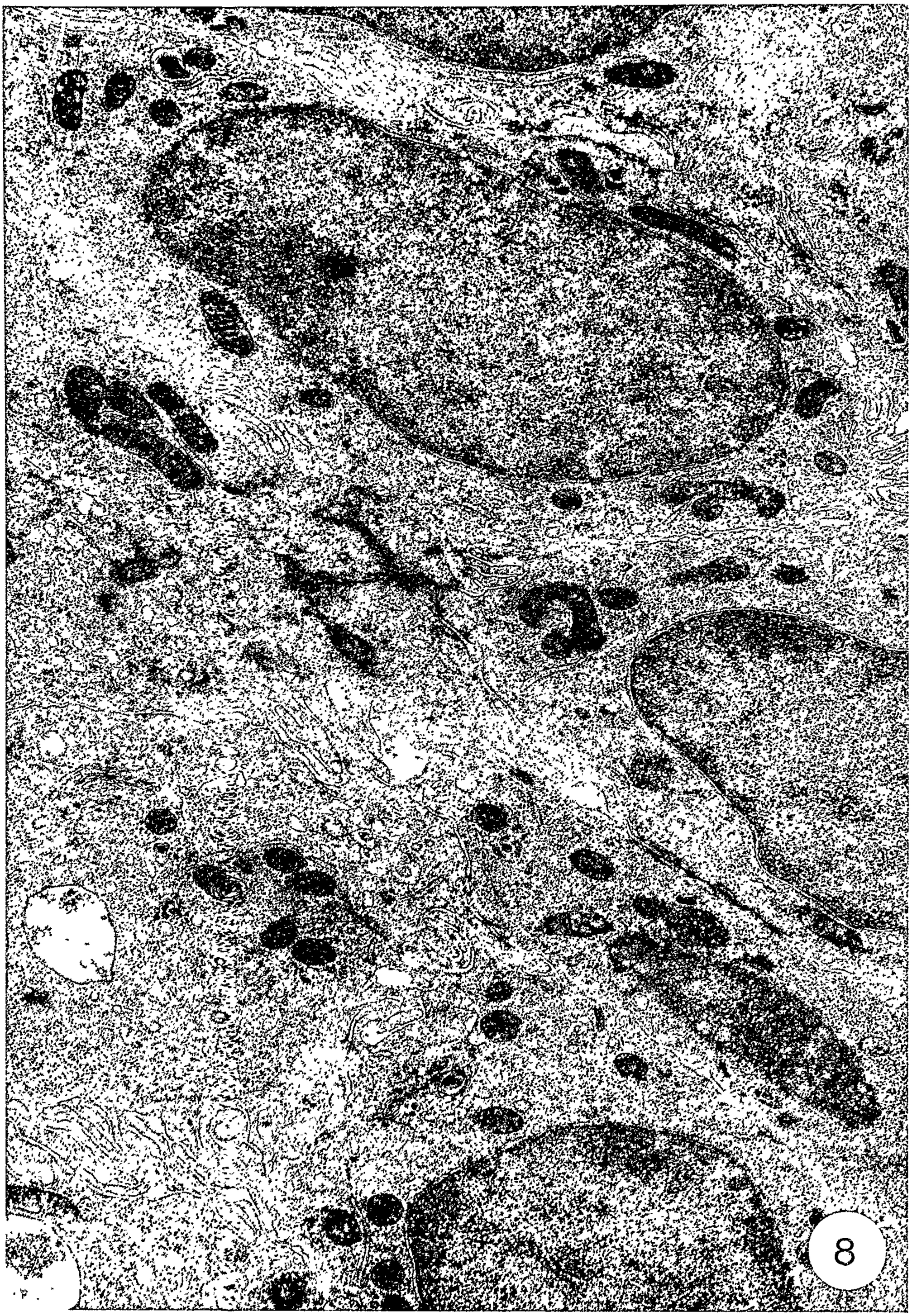


Fig. 9. Electronmicrograph of parathyroid tissue cultured for two days in low calcium medium. A few lipid droplets and lysosomes are noted. More ribosomes are membrane bound than in the previous pictures.

x 11,000

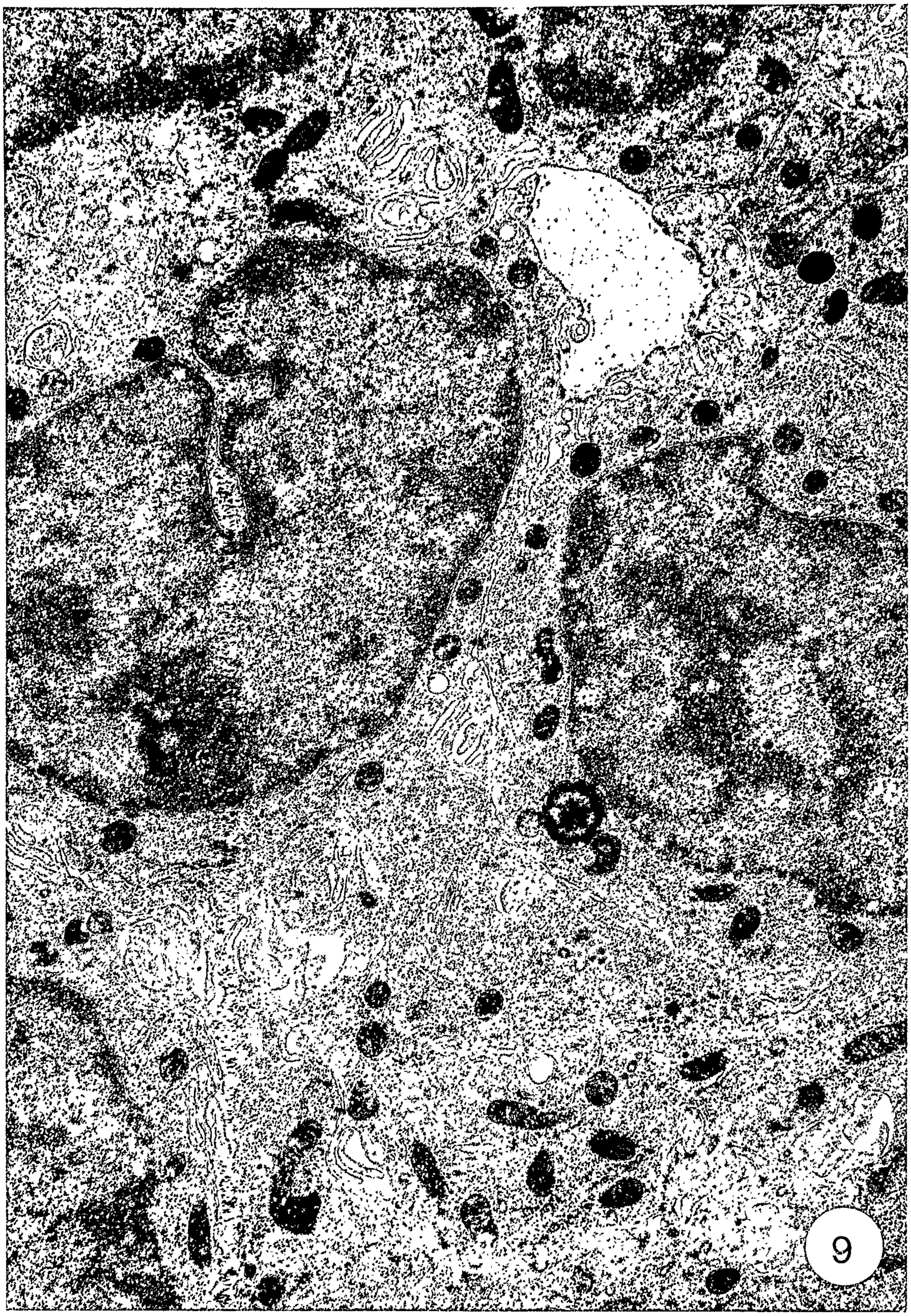

9

Fig. 10. Electronmicrograph of parathyroid tissue treated in a similar manner to that in Fig. 9.

x 11,000

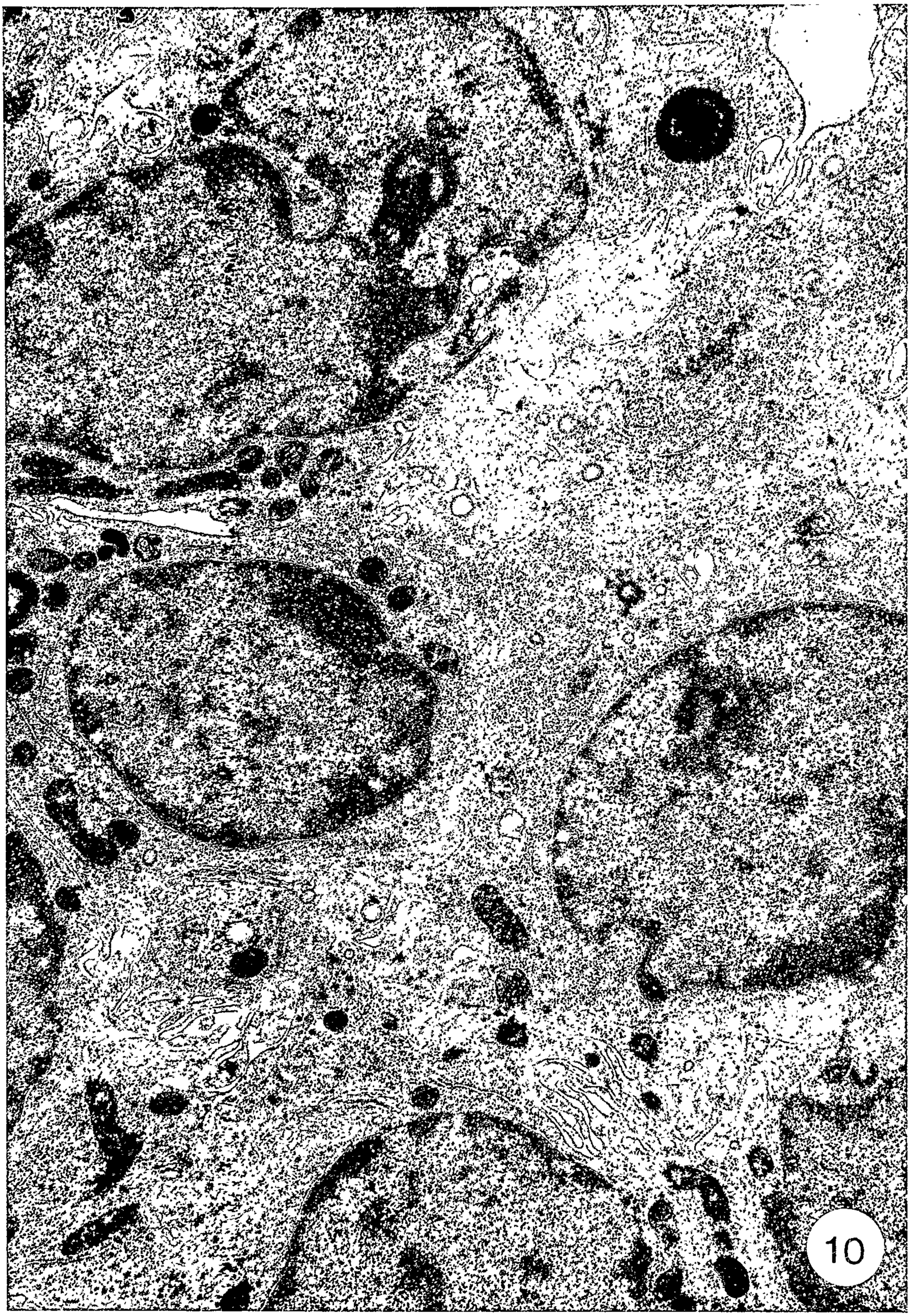
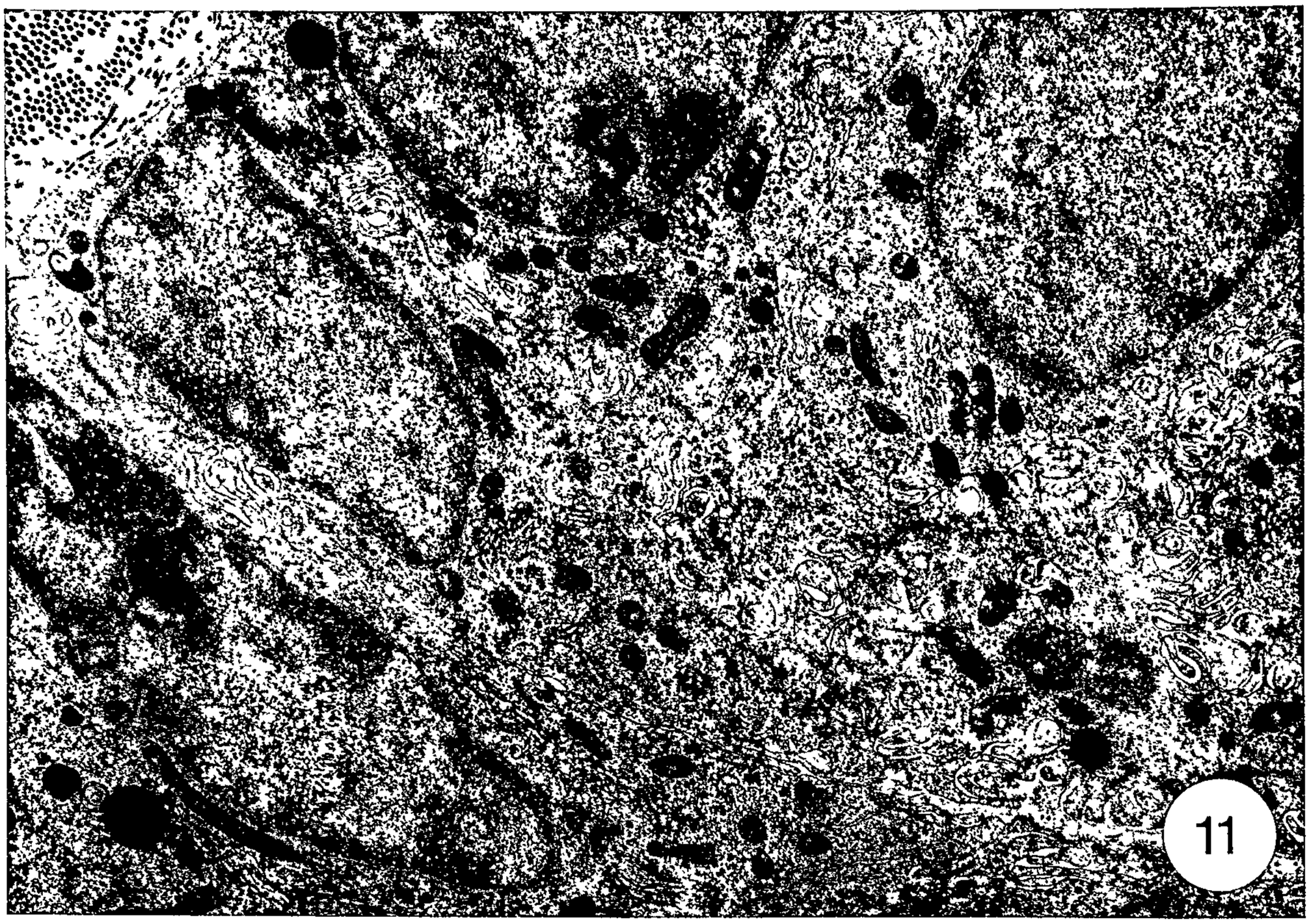


Fig. 11. Electronmicrograph of a group of paratjyroid cells cultivated for two days in low calcium medium. Note the rough endoplasmic reticulum, particularly in the cell at the base of the picture.

x 9,000

Fig. 12. Electronmicrograph of parathyroid tissue cultured for two days in low calcium medium. Note the secondary lysosomes so common in the cultured cells.

x 11,000


11

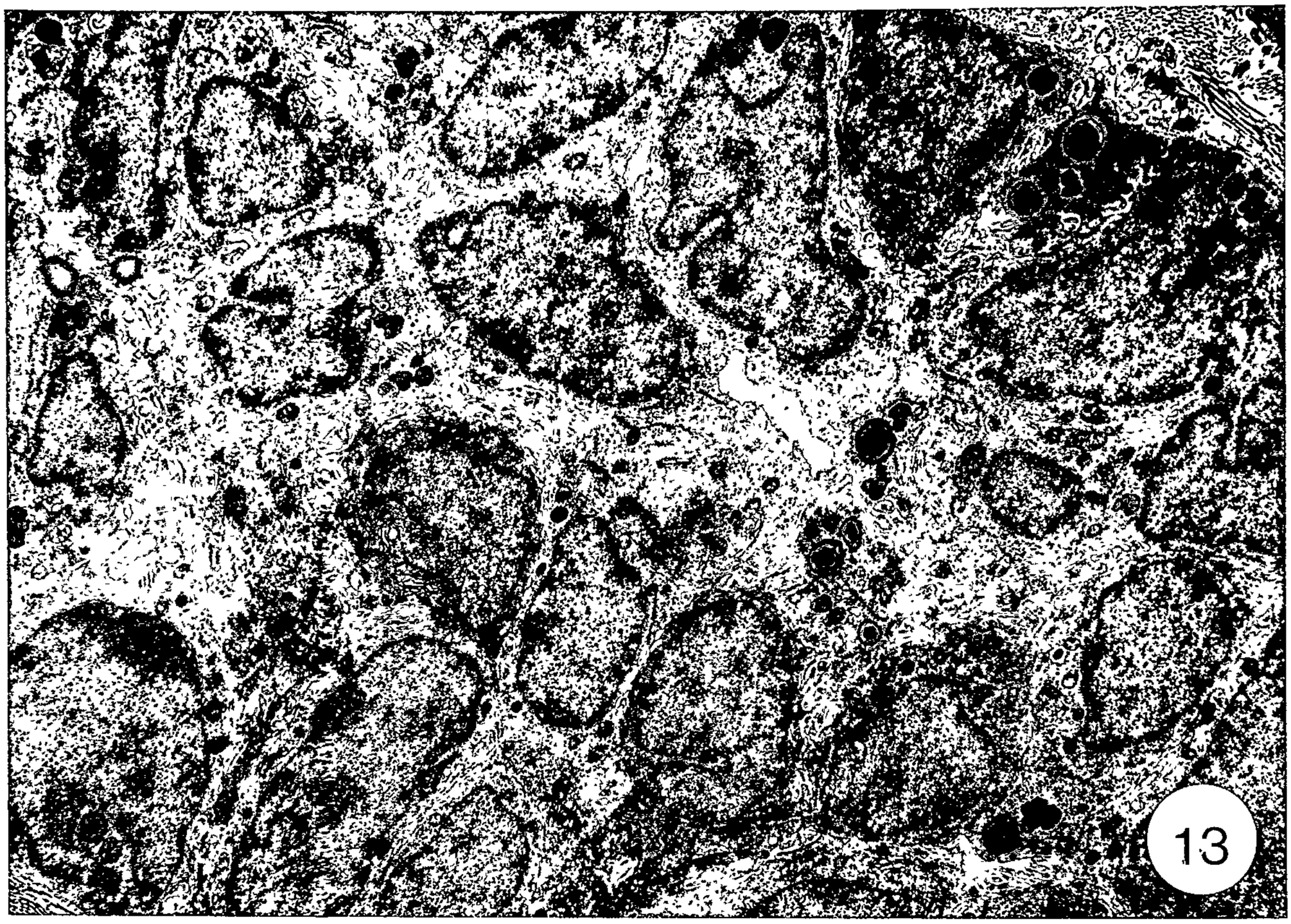

12

Fig. 13. A low power electronmicrograph of parathyroid tissue cultured in low calcium medium for six days. Lipid droplets and secondary lysosomes are very prominent and the complex pattern of plasma membrane contacts can be seen.

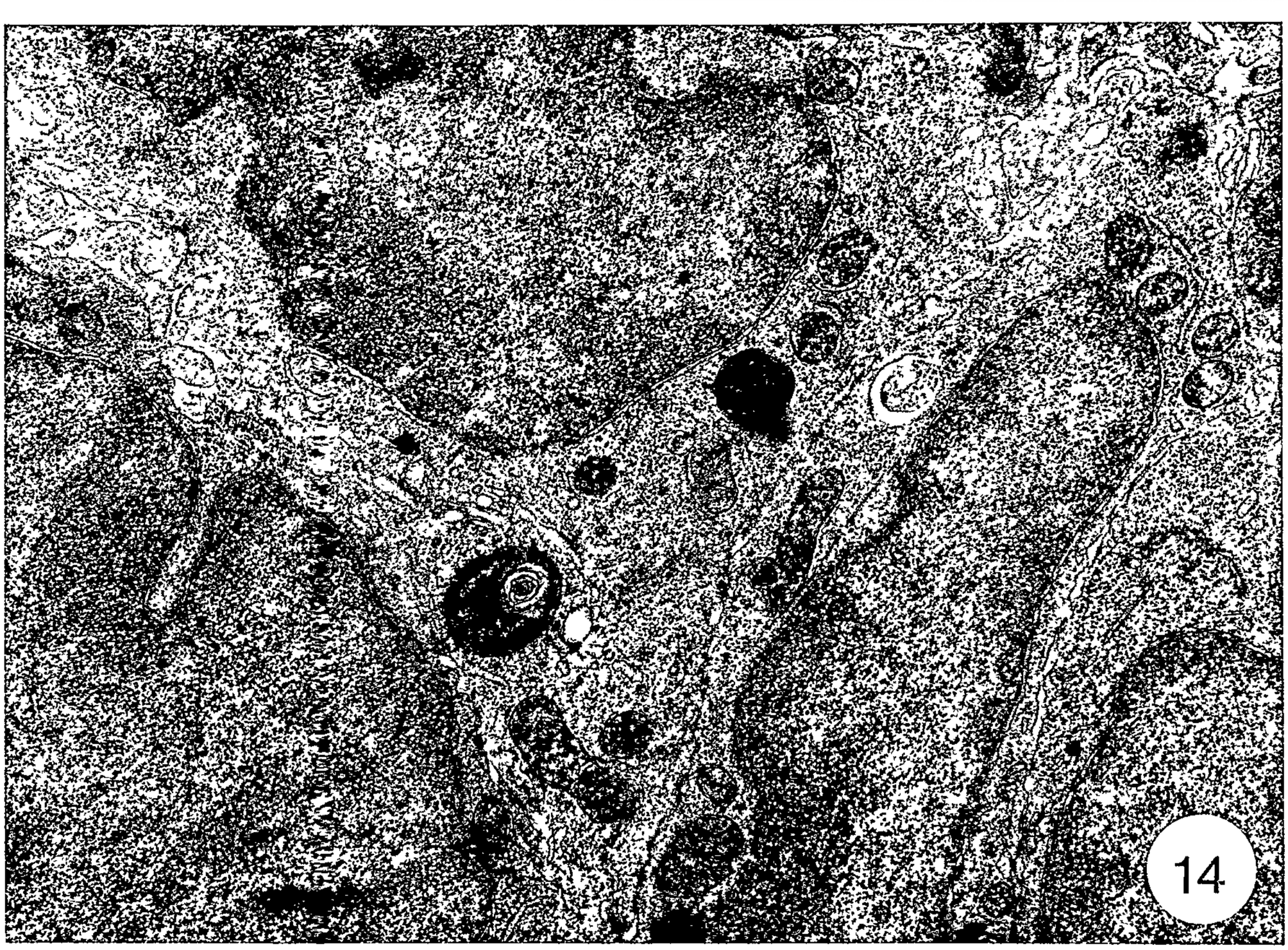
x 4,000

Fig. 14. Electronmicrograph of parathyroid tissue which had been treated similarly to that in Fig. 13.

x 16,000



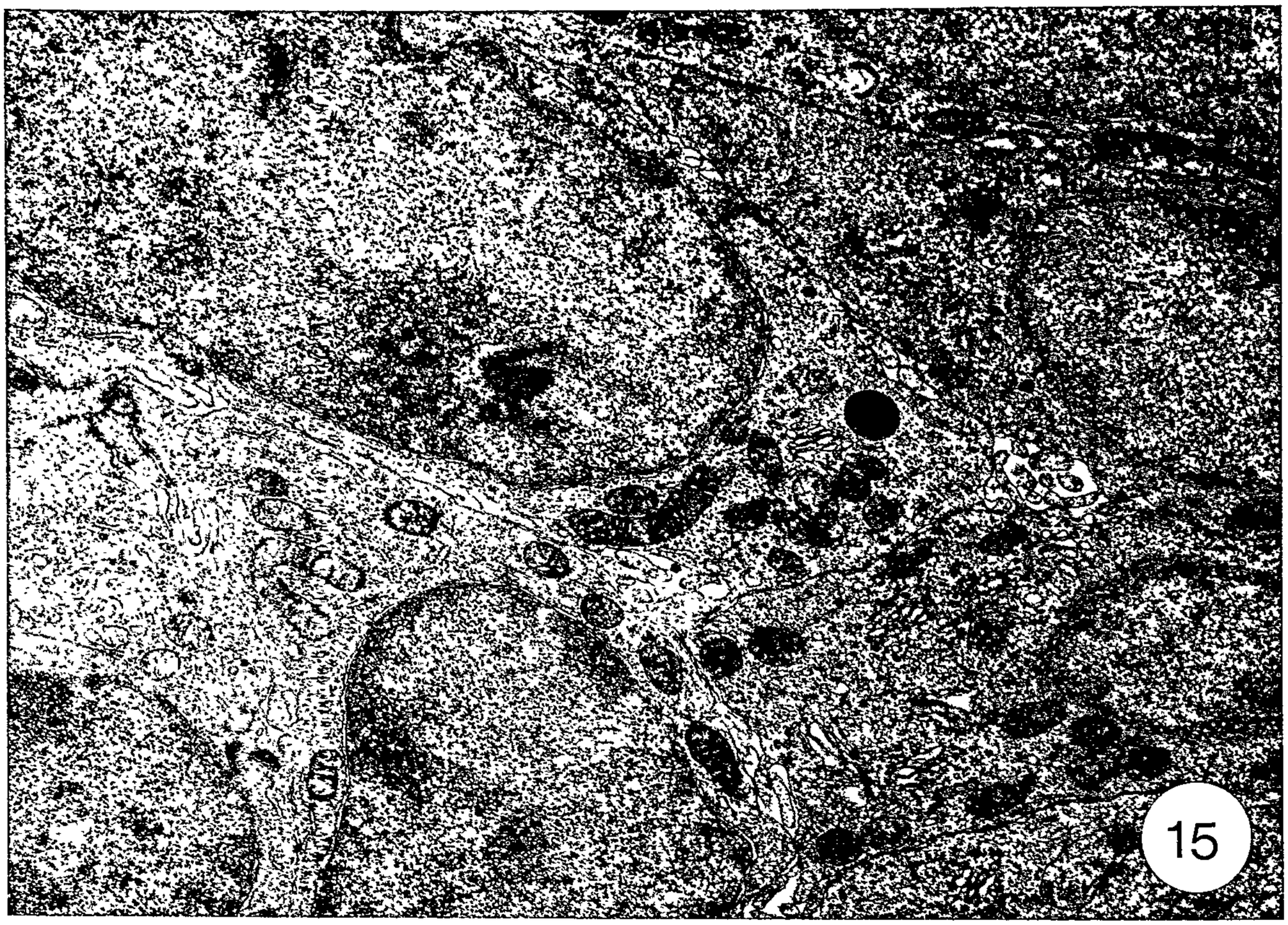
13



14

Figs. 15, 16. These are electronmicrographs of parathyroid tissue cultured for six days in low calcium medium. The Golgi complexes are well developed and there are an appreciable number of membrane bound ribosomes.

X 11,000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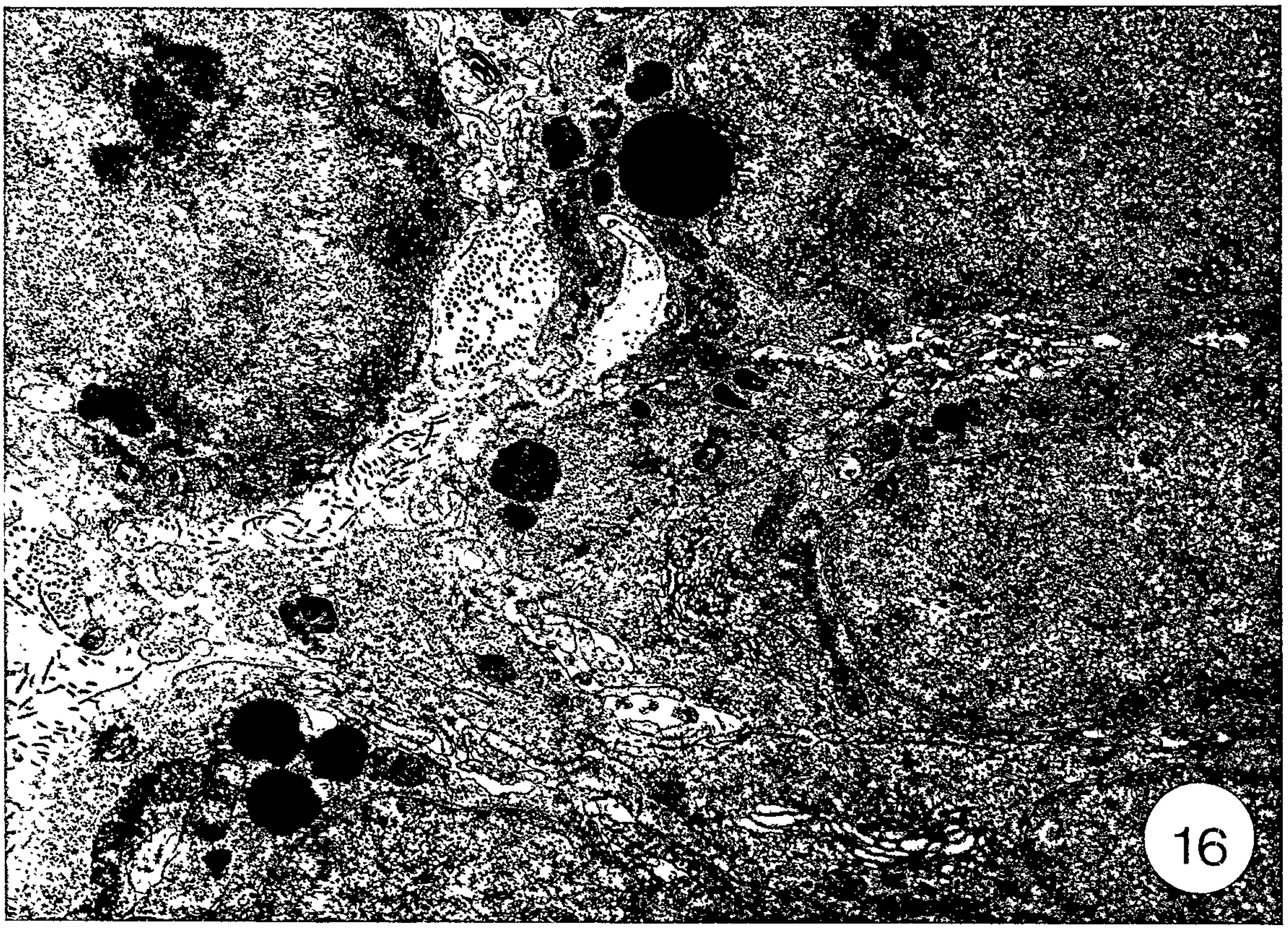
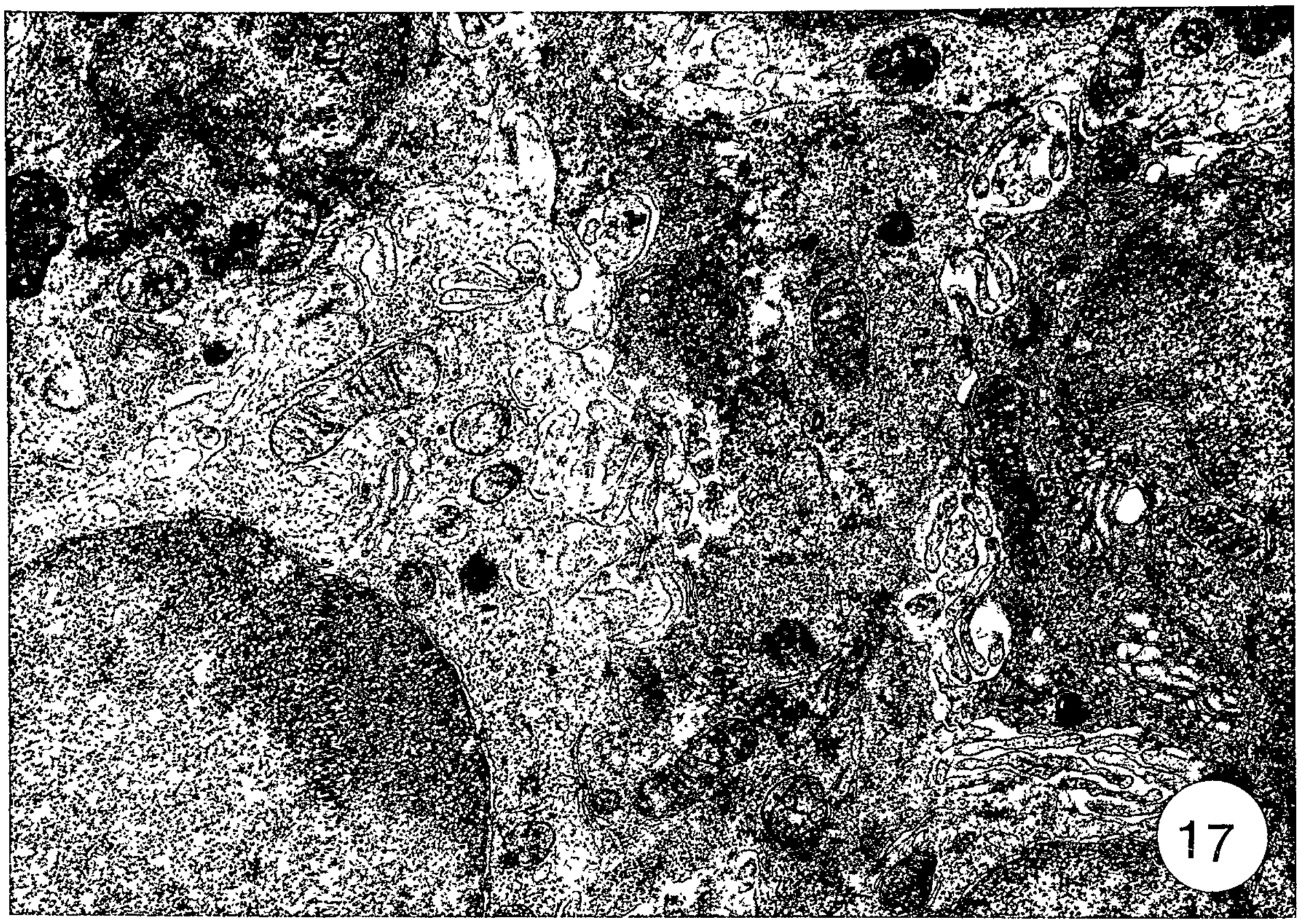
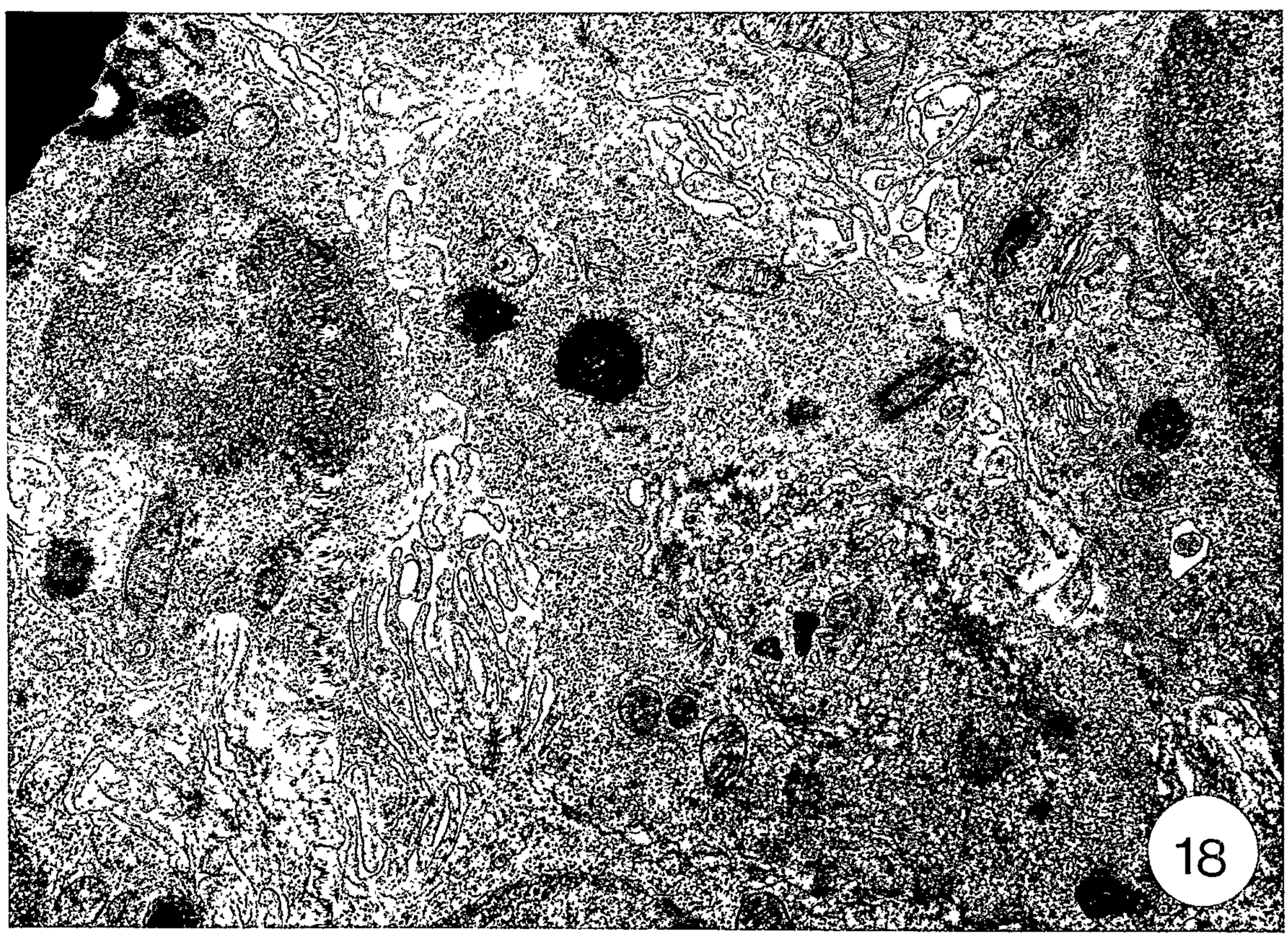

16

Figs. 17, 18. These are electronmicrographs of parathyroid tissue cultured for six days in low calcium medium. Fig. 18 shows a very large Golgi complex and in both pictures, the plasma membranes run a particularly tortuous course.

x 16,000



17



18

Fig. 19. Graph showing the elution pattern of radioactivity from parathyroid gland culture medium fractionated on Sephadex G50 (1.5 x 100 cm). Peak 1 corresponds to the void volume of the column; peak 2 (PTH) lies within the fractionation range; peak 3 represents a collection of small molecules.

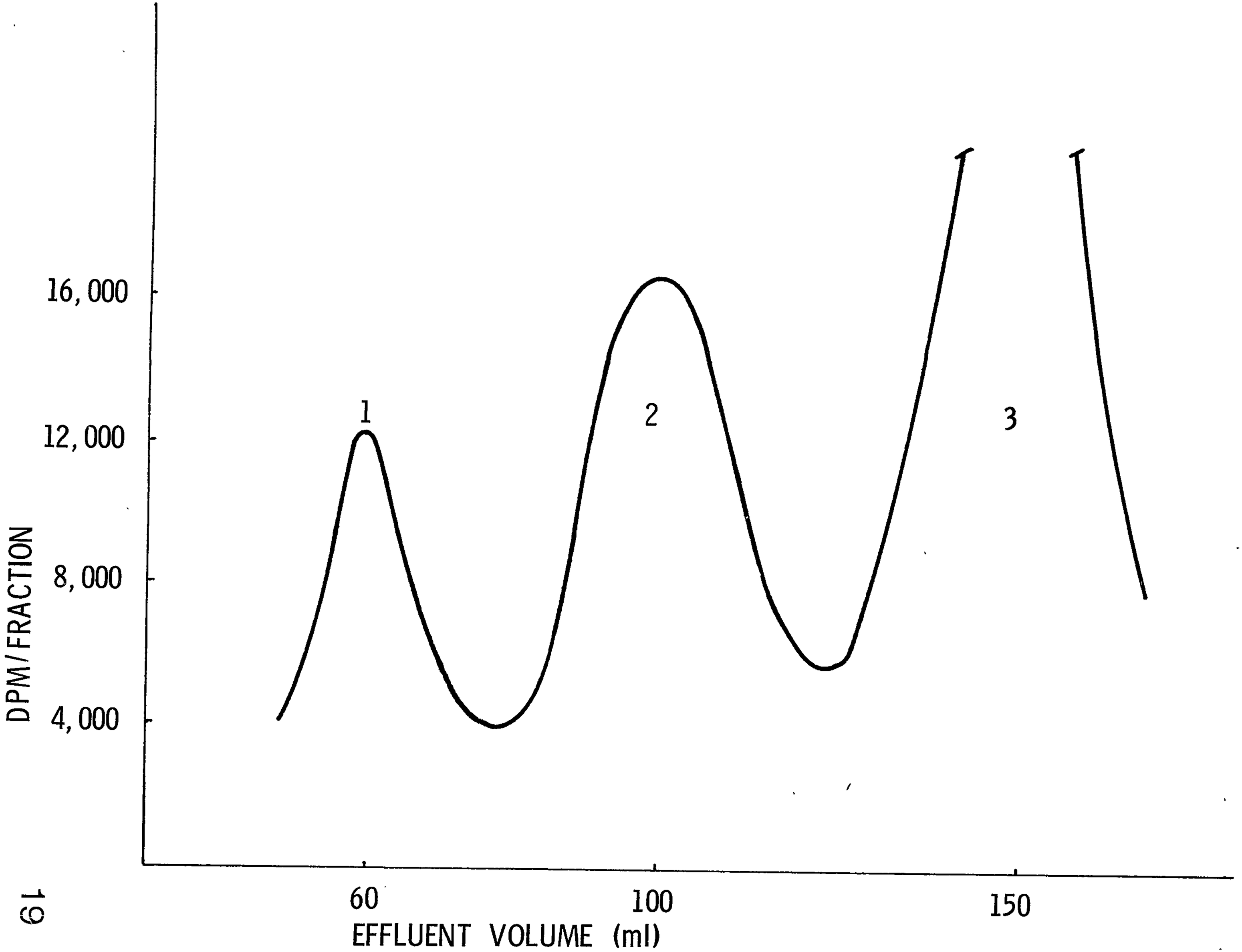

16,000
12,000
8,000
4,000
DPM/FRACTION
1
2
3
60
100
150
EFFLUENT VOLUME (ml)

Fig. 20. Graph showing the elution pattern of radioactivity when peak 2 (Fig. 19) was run on Sephadex G25 (1.5 x 30 cm).

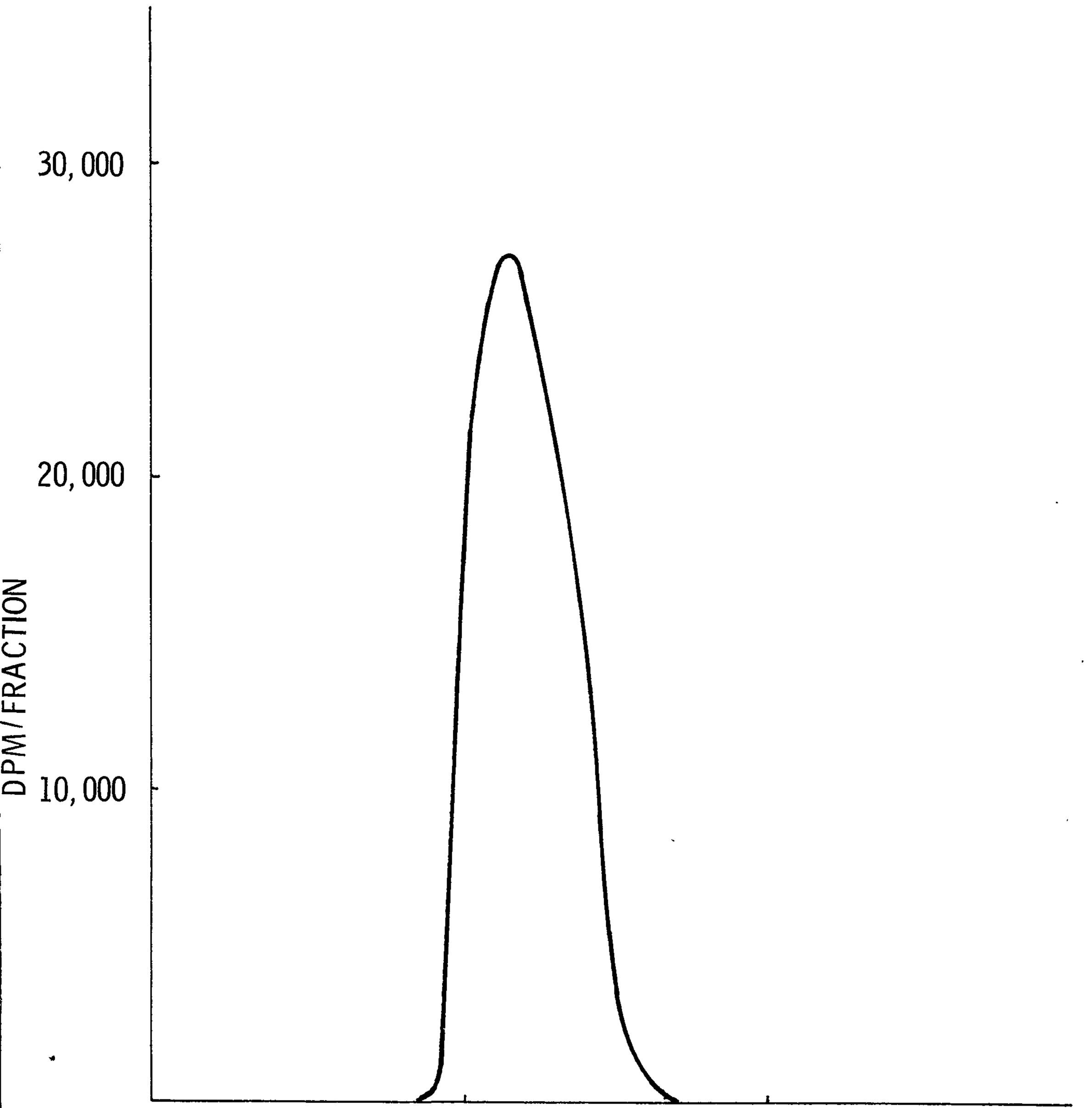
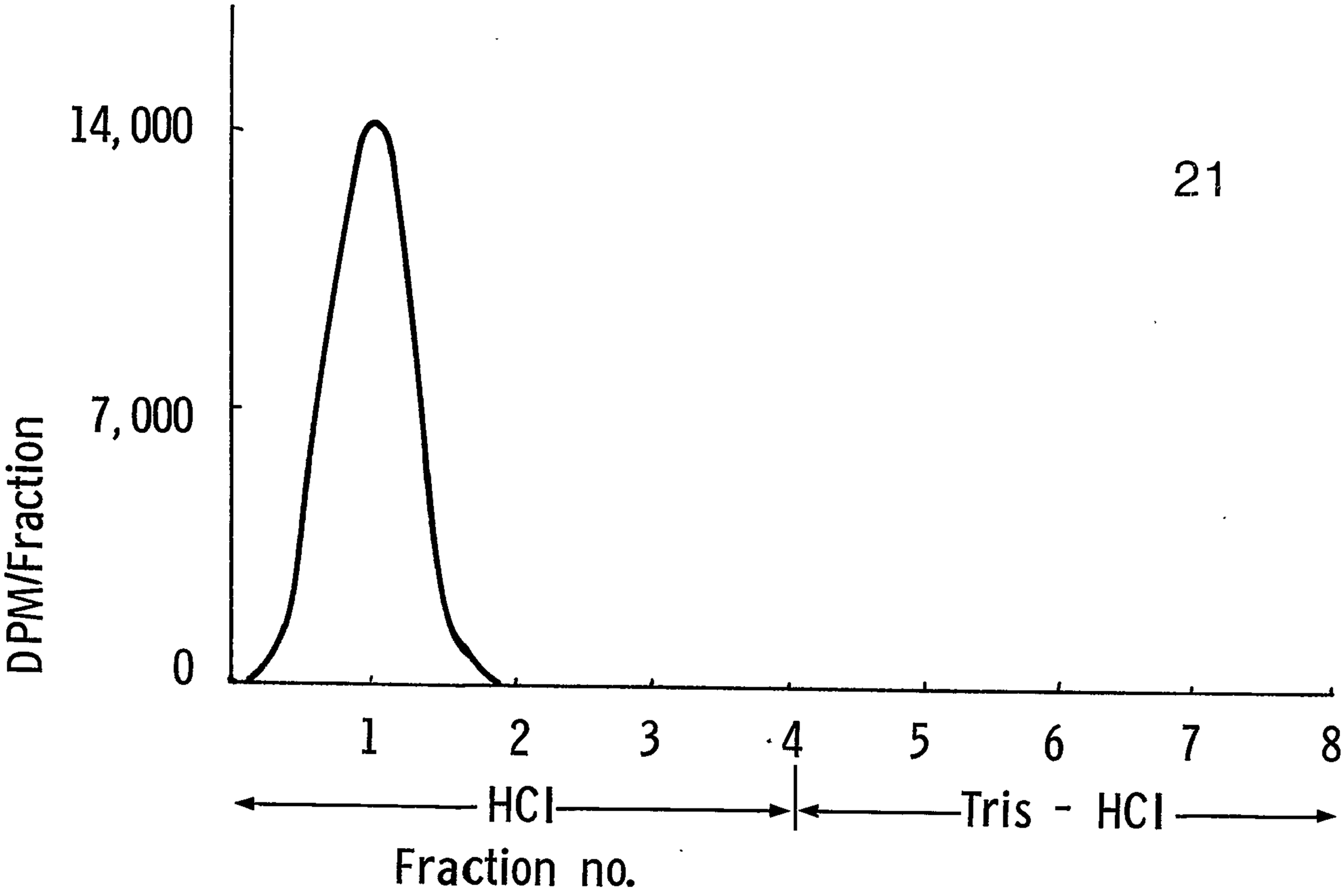

30,000
20,000
10,000
DPM/FRACTION
20
40
60
EFFLUENT VOLUME (ml)

Fig. 21. Graph showing the elution pattern of radioactivity when peak 2 (Fig. 19) was run on an anion exchange resin (Sephadex DEAE).

Fig. 22. Graph showing the elution pattern of radioactivity when peak 2 (Fig. 19) was run on a cation exchange resin (Dowex 50).

21


14,000
7,000
0
DPM/Fraction
1
2
3
4
5
6
7
8
HCl
Tris - HCl
Fraction no.

22

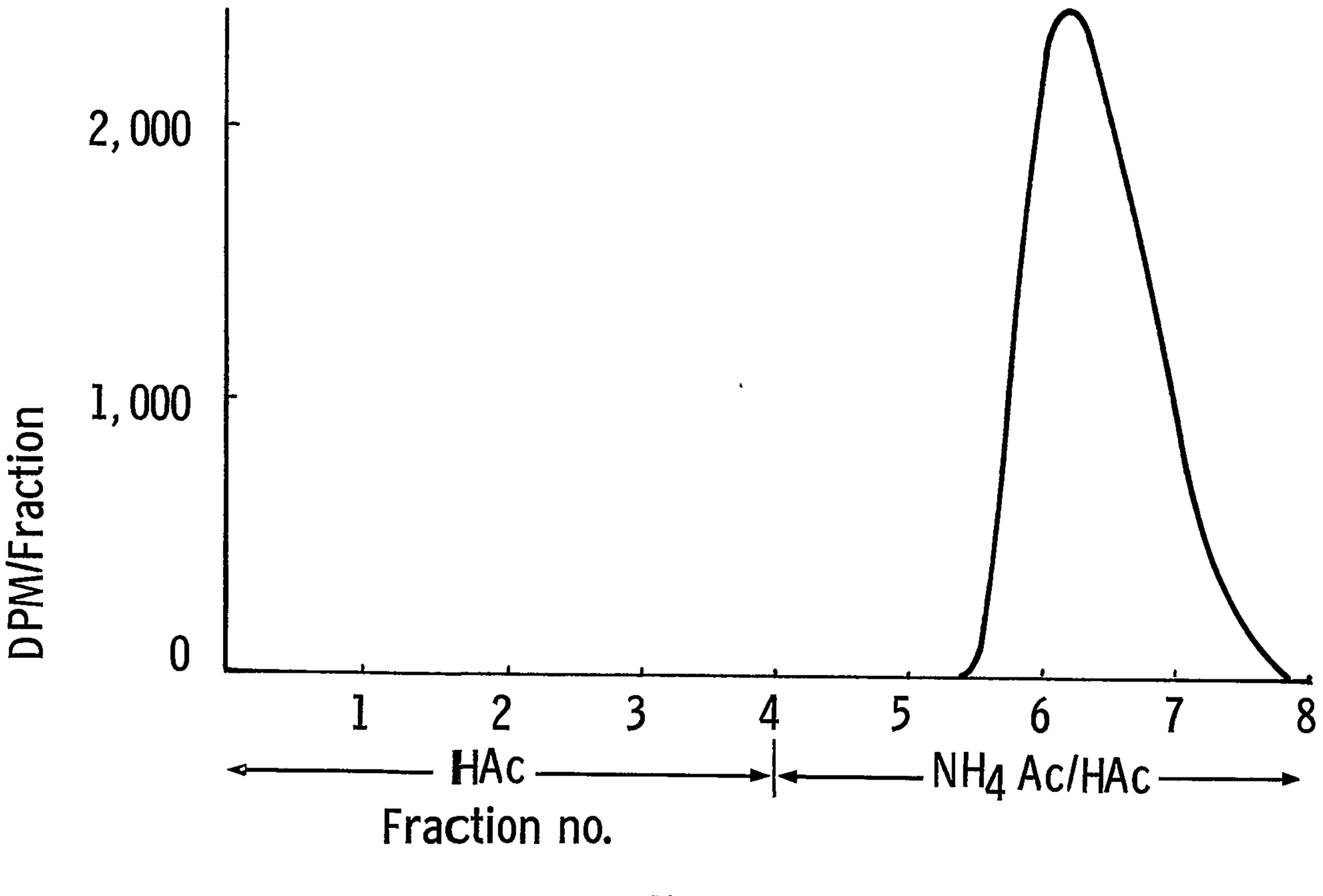
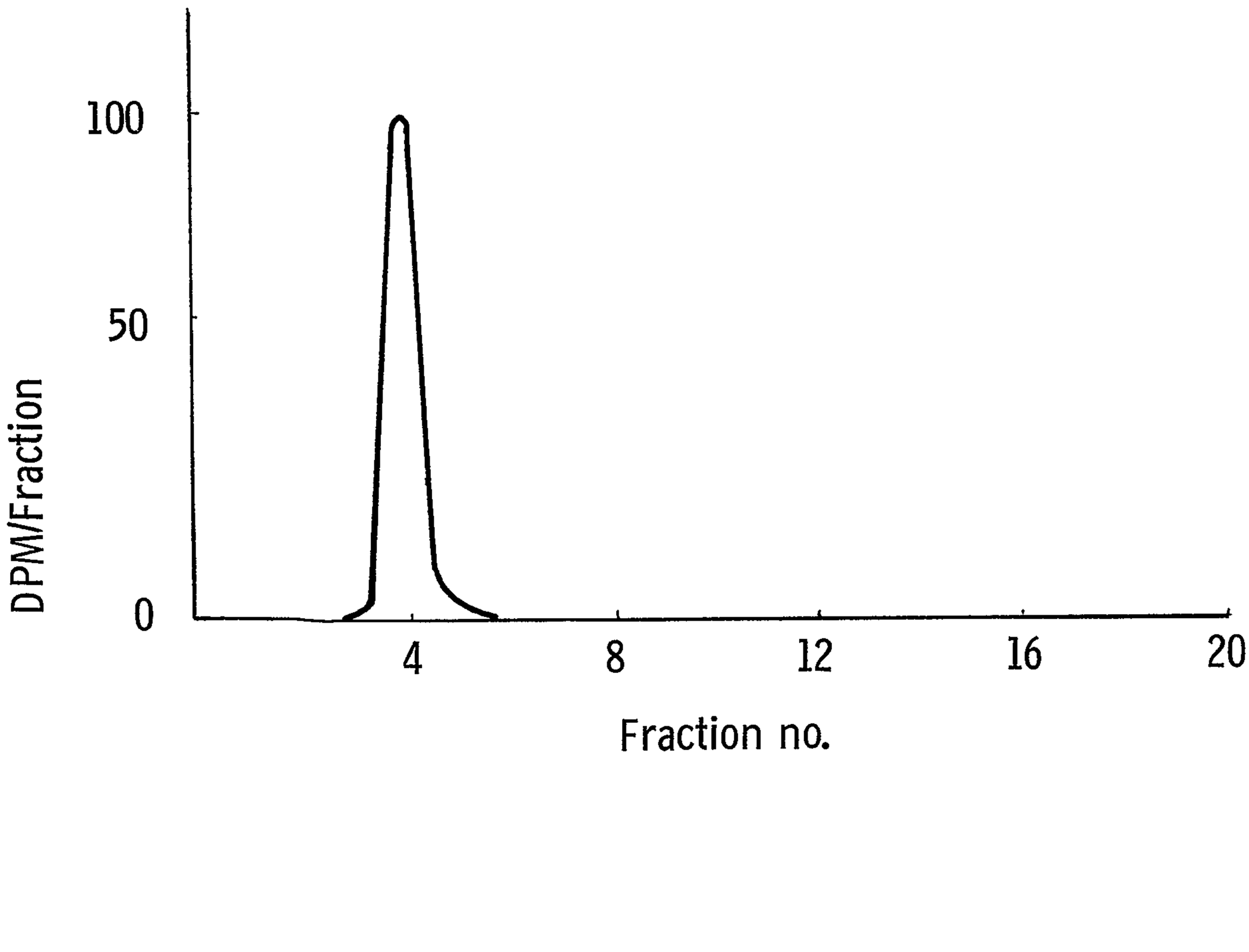

2,000
1,000
0
DPM/Fraction
1
2
3
4
5
6
7
8
HAc
NH4 Ac/HAc
Fraction no.

Fig. 23. Graph showing the pattern of radioactivity when peak 2 (Fig. 19) was examined by electrophoresis on 15% polyacrylamide gel.


100
50
0
DPM/Fraction
4
8
12
16
20
Fraction no.

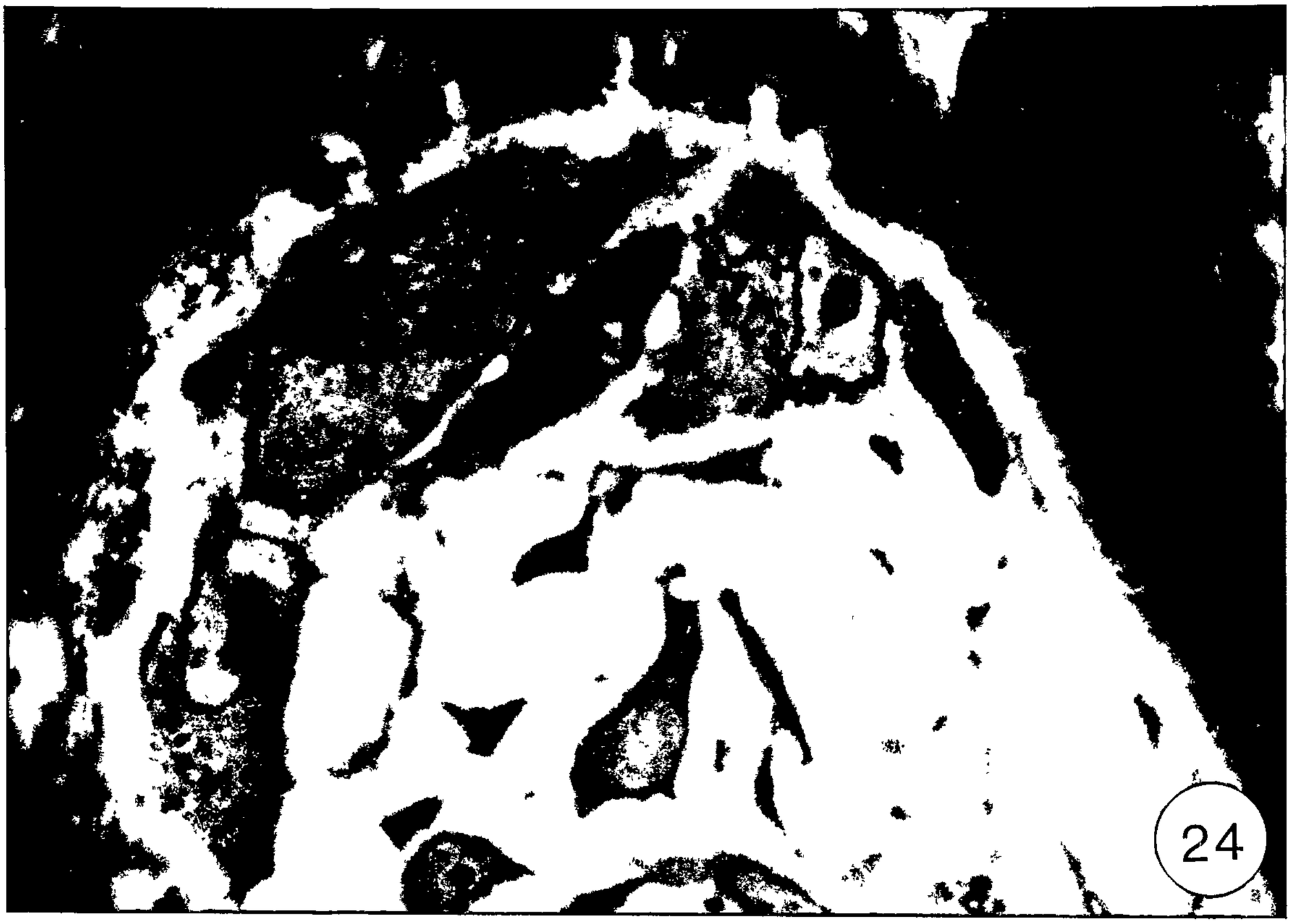
48.K

Fig. 24. Autoradiograph of 0.5 micron section of 2-day old rat parietal bone which had been incubated with ^{14}C-PTH for one hour. The section shows calcified bone matrix with adjacent osteoblasts. Developed silver grains are seen over the peripheries of some of these cells. The cytoplasm of one osteoblast is outlined by some six grains.

x 3,200

Fig. 25. Autoradiograph of a 0.5 micron section from the periosteal surface of rat parietal bone. Silver grains are shown over an osteocyte, over osteoblasts adjacent to the bone matrix, and over osteoprogenitor cells lying further from the matrix.

x 2,400


24


25.

Fig. 26. Autoradiograph of a 0.5 micron section of rat parietal bone which had been incubated with ^{14}C-PTH for one hour. Silver grains are shown over osteoprogenitor cells and over osteoblasts. One osteoprogenitor cell shows four developed silver grains even in this thin section.

x 3,600

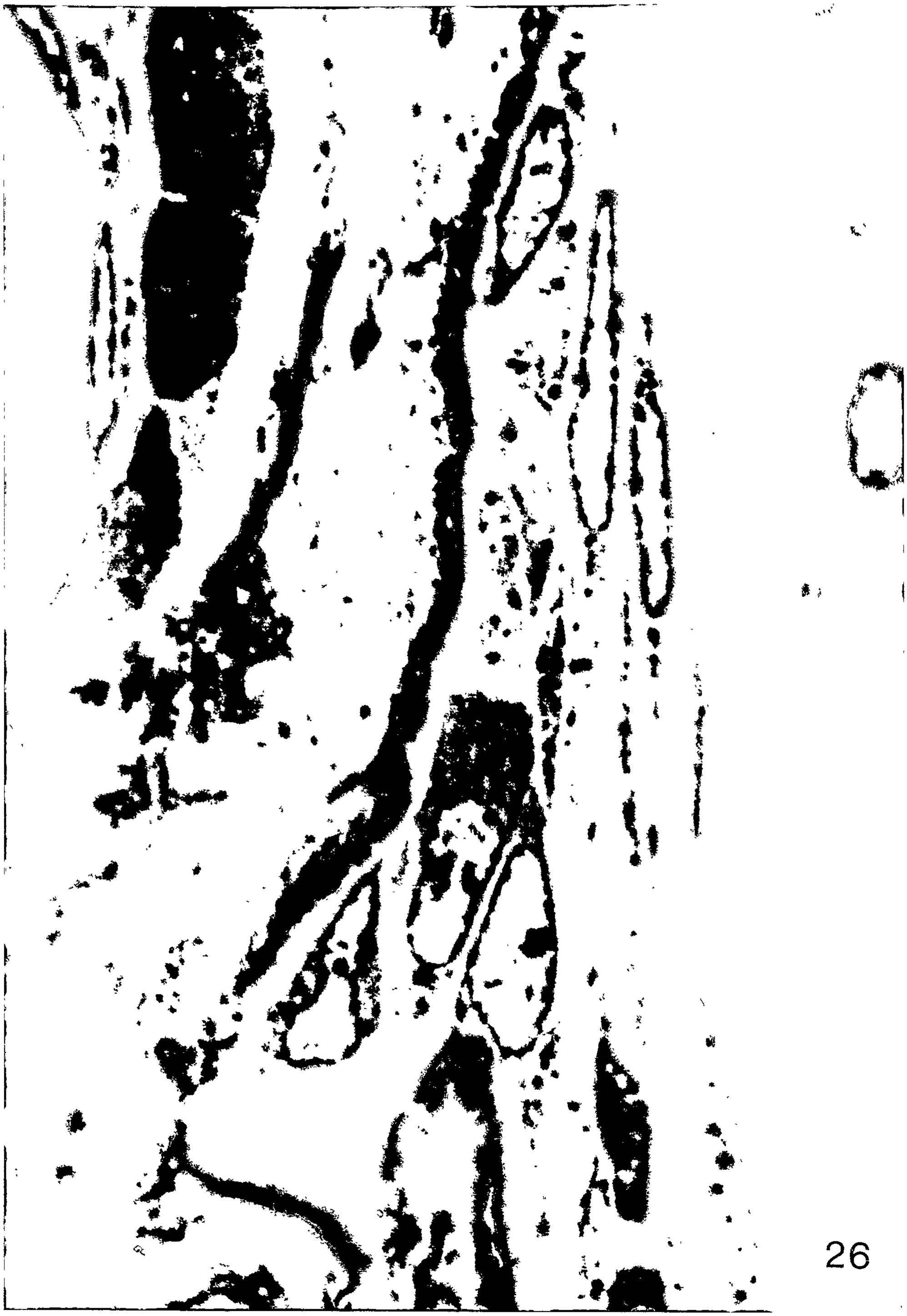


Fig. 27. Autoradiograph of a 0.5 micron section of parietal bone. Silver grains are shown mainly over osteoprogenitor cells. One superficially placed cell, probably a macrophage, shows a couple of grains.

x 2,000

Fig. 28. Autoradiograph of a 0.5 micron section of parietal bone which had been incubated with ^{14}C-PTH for one hour. Silver grains are evident overosteoblasts and over osteoprogenitor cells.

x 2,700

27

28