ULTRASTRUCTURE OF THE PRESSURE ZONE OF HUMAN PERIODONTAL LIGAMENT INCIDENT TO ORTHODONTIC TOOTH MOVEMENT

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INTRODUCTION

It is a recognized fact that if a force is placed on a tooth for a sufficient period of time, the tooth will move. The amount of force necessary and length of time needed depend upon which tooth is being moved and the type of movement. Generally it is easier to move an incisor than a molar or canine and it is usually felt that tipping tooth movement requires less time and less force than bodily movement or intrusion. One widely accepted explanation for this is that ease and rate of movement is related to root surface area. The less root surface area that is moving through bone, the easier the resultant movement.

The exact physiologic and biochemical mechanisms of tooth movement are still unknown, but some details have been described by scientific investigators. As the tooth moves, the bone around it is remodeled to accommodate the new position. Essentially, resorption occurs on the side toward the direction of movement (the pressure side) and apposition occurs on the tension side.

This remodeling process requires a patent vascular supply to remove the organic and inorganic breakdown products of bone and to maintain nourishment for the fragile osteoclast cells. The periodontal ligament (PDL) in its normal state has an adequate blood supply to permit the resorption process to occur. Investigations have shown, however, that if forces are placed on the tooth so that capillary blood pressure is exceeded, the vessels collapse and the blood supply in this region of the periodontal ligament is cut off. This occurs rapidly in a narrow band on the pressure side of the tooth, even with forces as light as 70 grams.

Within two days after force application, an actual cell free zone appears which may have areas of hyalinization. The cell free zone has been investigated but the fate of the fibers and the exact character of this area is not known.

Since the devitalized periodontal ligament cannot supply the area with necessary elements to carry on bone resorption, the tooth to this point, can only move within the confines of the periodontal ligament and to the extent that the alveolar bone is bent. It is

several days before undermining resorption erodes the bone ahead of the tooth in an attempt to restore circulation in the periodontal ligament. This time period has been referred to as a lag phase of tooth movement.

One question that arises from this is whether or not the bone remodeling continues unchecked. Does the tooth continue to move constantly with no further formation of cell free zones and no further lag periods, or does it go through stages where each time the force is reapplied (spring or elastic reactivated) a new cell free zone forms and another lag phase is initiated.

The structure of this altered collagen and reorganization of the material in this area probably play an important role in the recovery of the periodontal ligament after longstanding pressure trauma. The clinical significance must be viewed in light of previous investigations which suggest there is a lag phase of tooth movement associated with cell free zone formation. The effect of repetitive tooth movement on the periodontal ligament is also considered. Whether or not the cell free zone forms a second time upon reapplication of force is an

important question. An attempt is made here to describe the ultrastructure of the cell free zone as it is seen under the Transmission Electron Microscope.

REVIEW OF THE LITERATURE

Interest in the biological basis of tooth movement has been evident since the early days of orthodontic treatment. Reitan[17] relates how Harris (1863), Talbot (1888), and Guildord (1898) explained tooth movement as a result of bone resorption on one side of the root and bone apposition on the other. Also reviewed was Sandstedt's[21] (1904, 1905) classic research on dogs which was the first biologic description of the hyalinization of the periodontal ligament under tooth movement pressure. Since that time there has been general agreement that tooth movement causes resorption of bone on the pressure side and apposition of bone on the tension side of the periodontal ligament-alveolar bone interface.

Almost all early work was done on experimental animals such as dogs or primates. Examples are Gottlieb and Orban (1931-as related by Macapanpan)[12], Reitan and Skillen (1940)[15], Schwarz (1931, 1932)[22, 23], Oppenheim (1911, 1912, 1936, 1942, 1944)[14, 15, 16, 17], Johnson, Appleton and Rittershofer (1926)[9], and Marshal (1933)[13].

Herzeberg (1932)[7] was among the first to use human tissue with his histologic description of a single patient's premolar experimental site. Reitan (1951)[17] has done extensive histological studies on dog and human tissue where tooth movement was of short duration (up to 48 hours). Macapanpan, Weinmann and Brodie (1954)[12] described early changes in rat periodontal ligament following tooth movement of 1 to 72 hours. They found formation of a hyalinized periodontal ligament area after as short a time period as 6 hours of pressure on the tooth.

Kvam (1969)[10] studied the cell free zone of periodontal membrane following tooth movement of from 2 to 9 days in rats. His photomicrographs verified the disintegration of individual collagen fibers but gave no information with regard to finer decomposition of collagen. The fibers extruding from the cementum and the bone were found to have lost less of their usual morphological characteristics within the hyalinized area. This difference probably related to the slower turnover rate of these more mature fibers. It was shown that the duration of the hyalinized area was dependent upon both the resorption

rate of the altered collagen and the rate of undermining bone resorption. Kvam and Reitan (1971)[18] studied humans, rats, monkeys and dogs and found hyalinization to form within 30-40 hours in the human and in as few as 6 hours in rats.

Buck and Church (1972)[2], using human tissue, found compression of the periodontal membrane greatest after 7 days of tooth movement with frontal and undermining resorption following. It was observed that simply the loss of cells in the pressure area did not result in hyalinization of the fibers. True hyalinization and loss of the fibrillar nature of the collagen occurred only rarely and only in areas of extreme compression. After 2 weeks, undermining resorption had altered the alveolar PDL space to allow revitalization and return of cellular elements to the area. From that time on, periodontal ligament reorganization was the dominant finding.

The use of electron microscopy has been essential in ultrastructural research. Ten Cate (1971, 1975)[28, 29] examined the breakdown of connective tissue during tooth eruption in monkeys using the transmission electron microscope. In the forming attachment around

the tooth, areas of breakdown and synthesis were found to occur simultaneously. The fibrocyte was implicated as playing an important role in the mechanism of connective tissue breakdown and synthesis with the suggestion that the fibrocyte is capable of modulation to become synthetic, maintaining or degradative.

Shackleford (1971)[25] used the scanning electron microscope to study dog periodontium. The presence of a fiber plexus was described which fills the distance between bone and cementum and forms anastomoses with the principal fibers that perforate bone and cementum. This plexus and its relationship with the principal fibers was proposed as a possible explanation of the adaptability of the periodontium to the process of tooth movement. Under heavy forces, the principal fibers were able to tear without interrupting the functional connection between tooth and bone.

Rygh (1972)[19] described ultrastructural changes in pressure areas of periodontal membrane of rat molar following tooth movement of 30 minutes to 28 days. Some intracellular changes were noted after 30 minutes of pressure and the changes appeared to be irreversible

with subsequent cell death after 2 hours. The cementoblasts, fibroblasts and osteoblasts all showed the same reaction to the orthodontic forces. Regeneration was the predominant finding after 7 days. Rygh (1972)[20] also described vascular changes in the high pressure region. Stasis of blood flow occurred after 30 minutes of force application. After 24 hours, fragmentation of erythrocytes occurred. Blood vessel walls began disintegration after 1-7 days. After 7 days, regenerative processes predominated.

In conclusion, it can be said that tooth movement pressures not only cause alteration of the alveolar bone interface, but affect the soft tissue PDL as well. The research tool of electron microscopy has been of particular value in the documentation of these changes. It may well be that the limiting factor in tooth movement is not the skeletal tissue remodeling but rather the remodeling of the connective tissue proper. Much fruitful work remains in this most germane area of basic research for application to the clinical sciences.

MATERIALS AND METHODS

Experimental tooth movement was carried out in 4 male and 3 female humans ranging in age from 12 to 24 years of age. Subjects were chosen who required removal of upper first premolars as part of their orthodontic therapy. A lingual appliance with finger springs which created buccal tipping of the maxillary first premolars was activated with a force of 70 $\pm$ 7 grams and stabilized in the mouth by cemented molar bands. The finger springs were fabricated from .018 inch standard stainless steel orthodontic wire with the design of a double helix and a 5 mm-7 mm lever arm (fig. 1). Calibration was done by a dead weight loading device with the required load yielding a deflection of 3-4 mm in the spring.

Patients were randomly divided into 4 groups and the time periods selected were 2 days, 4 days, 10 days and the fourth category was a sequence of 10 days of force followed by 11 days of no force and then a repetition of the first 10 days of force. Only 1 patient fell into the 4-day category with all other time points including 2 patients.

Maxillary premolars were removed surgically to include several mm^2 of intact buccal alveolar bone in a manner similar to that described by Buck and Church (1972)[2].

Healing was uneventful following surgical closure of the mucoperiosteal flap, and at examination 30 days postoperatively, patients who had undergone this procedure were indistinguishable clinically from patients in whom routine extractions had been performed. Clinical results were similar to those observed by Buck and Church (1972)[2].

The two surgical sections taken from each patient were fixed immediately; one tooth-alveolar bone tissue section being placed in Karnowsky's solution (glutaraldehyde plus cacodylate buffer, pH 7.2) at $42^{o}C$ and the other being fixed in recently prepared Zenker's solution at room temperature.

The glutaraldehyde fixed specimens were later sectioned with a diamond disc at low speed under a cold glutaraldehyde bath to prevent destruction of the ultrastructural characteristics from excess heat. The tissues were decalcified in a buffered 4% glutaraldehyde - 0.1

Molar EDTA solution at pH 7.3. They were then grossly sectioned into transverse 1 mm wide samples, washed free of EDTA in Millonig's phosphate buffer, post fixed in 1% buffered osmium tetroxide, treated with 3% buffered formalin and dehydrated in four progressive acetone solutions, from 30% to 100%. After treating in 50% acetone-50% Spurr resin then 100% Spurr resin the specimens were embedded in complete Spurr resin and heat cured until solid.

For orientation purposes, 1-2 μ sections were cut with glass knives on a Porter-Blum ultramicrotome. The sections were stained in metachromatic 1% toluidine blue in borax and scanned with a light microscope. Black and white photomicrographs were taken on a Zeiss photo-microscope.

The blocks were then trimmed to include the area of highest compression of periodontal ligament plus adjacent tooth and alveolar bone. Thin sections, 700-800 Å in thickness were cut with a diamond knife on the same ultramicrotome. Specimens were placed on #200 copper mesh grids coated with formvar carbon substrate. They were then stained with 1% uranyl acetate in 15% acetone, washed, and stained

with Reynold's lead citrate for 8 minutes. Sections were then examined in electron microscopes (Phillips EM 300 and Phillips EM 200) operated at 60 KV.

FINDINGS

LIGHT MICROSCOPE OBSERVATIONS

For orientation and comparison purposes, 1-2 μ thick sections were studied under a light microscope. Due to artifacts and trauma during surgery, not all the Spurr resin embedded specimens yielded sections which were suitable for further evaluation. However, at least one good sample from each experimental time period was obtained.

Examination of the pressure zone of the 2-day specimen at 128 X magnification revealed an extensive fibroblast-free area, extending from the alveolar bone to the tooth and following the entire mesial-distal length of the periodontal ligament interface (1½ mm on this block section). This section, which was taken from an area about 2 mm apical to the alveolar crest, showed a slight variation in the PDL thickness, with a range of 160-180 μ. A large number of Sharpey's fibers could be seen extending into the cementum, especially on the periphery of the high pressure area. (fig. 2) In the area of highest pressure, there were fewer distinct fibers and these were more lightly

stained. The PDL itself consisted mostly of a mass of curled fibrillar structures with no apparent directional grouping except along the cemental PDL junction where most fibers entering the cementum were at approximate right angles to the tooth. (fig. 3) Some areas appeared somewhat more amorphous than others, possibly due to the following reasons: 1) slight pressure differentials in various areas of the PDL; 2) earlier loss of patent blood supply to some areas; or 3) the inability to obtain enough depth of field for usual fiber orientation in such thin sections.

Some partly collapsed blood vascular channels could be seen within the PDL. Scalloping resorption could be seen along several areas of the alveolar bone-PDL interface. This remodeling was extensive along the highest pressure region although it was fairly shallow, the deepest area being about 25 μ. No intact osteoclasts were visible in or near the scalloped sites, and no cell debris or nuclear remnants were discernible at this magnification. No resorption of cementum or tooth surface could be seen in this section.

The specimen from the 4-day experimental time period was also an

horizontal section taken approximately 2 mm apical to the alveolar crest. It showed more pronounced compression of PDL in the highest pressure zone. The range was 220 μ at the lateral extremities of the section to 88 μ in the central pressure area. (fig. 4) The PDL exhibited the non-uniform fibrillar structure similar to that in the 2-day specimen except here there was considerably more amorphous area. Some areas appeared more hyalinized than fibrillar, especially toward the middle of the PDL.

The number of fibers entering the cementum perpendicular to the tooth was seen to be much smaller than in the 2-day specimen. Those that were present were thick and diffusely stained.

The alveolar bone showed extensive remodeling, with many resting or reversal lines and scalloped depressions as deep as 130 μ. The largest Howship's lacunae were in the peripheral areas lateral to the main pressure point, but the concentration of resting lines was much higher in the area of high compression. In the PDL, usually situated close to scalloped alveolar bone,were large, (some 160 μ in diameter) reasonably well delineated masses with shadowy, fibrillar, fractured

internal structures. One such structure had blood vascular channels running through it which gave rise to the possibility of its being a glomerular body. (fig. 5).

Two 10-day specimens were available. The first appeared to have considerably less fibrillar structures in the PDL than previously discussed sections. Even in the lateral extremities, there was an amorphous, almost glassy appearance. (fig. 6) Many large round empty vacuoles were present throughout the PDL. (fig. 7) Whether these structures were preparation artifacts or existed in vivo was not known. The possibility that these vacuoles contained lipids was a reasonable assumption on the basis of previous work by Buck and Church[2].

Resting or reversal lines as well as resorption scalloping were evident in the alveolar bone along the entire mesial-distal length of the specimen (3 mm). The small number of Sharpey's fibers present were thickened, short and diffuse.

The other 10-day specimen showed extreme compression of the PDL, 60 μ thick in the highest pressure area. Little fibrillar structure

could be seen at 128 X magnification. Several deep Howship's lacunae could be seen with shadows of possibly dead osteoclasts in them. (fig. 8) Sharpey's fibers appeared in the cementum, but were not seen to extend into the PDL. Large round empty vacuoles appeared in some areas. Undermining resorption was the prominent feature in this specimen. Several extensively resorbed alveolar bone areas were present, with one communicating with the PDL. Shadows of cell remnants could be seen in the areas of undermining resorption.

Extensive but even resorption of the alveolar bone was present in the 31-day section throughout about 2 mm of the pressure zone. There was also a 25 μ deep resorption area on the tooth surface which extended into the cementum but not into the dentin in this section. (fig. 9)

The fibrillar structures seen in earlier samples were almost nonexistent in the 31-day sample. Sharpey's fibers were apparent in the high pressure area, and the large round vacuoles seen in previous sections were noted.

FINDINGS

ELECTRON MICROSCOPE OBSERVATIONS

The 2-day specimen had a highly variable appearance over the length of the PDL. Some areas had normal, viable looking cells (fig. 10), while other areas showed several stages of cell degeneration (fig. 11). Adjacent to the alveolar bone (fig. 12) could be seen a cell with a fairly normal looking cell membrane and the cytoplasmic extensions of the cell did not appear to be unusually vacuolated. Periodicity of the adjacent collagen bundles appeared to be about 610 Å. This area of the PDL did not have the appearance of having been adversely affected by the applied pressure. Areas such as this were exceptional, however. Most cells present did not appear healthy. Near the PDL-cementum interface were aggregations of degenerating cells. Some had cell membranes and nuclear membranes mostly intact and others showed moderate and severe disruption of membranes. There was a considerable amount of vacuolization and cellular debris present. Nuclei of some cells contained nothing more than small amounts of debris,

consisting mostly of empty space. (fig. 10) Collagen fibers in this time period had a fairly normal appearance at 8,500 X magnification. Under higher power (47,500 X) (fig. 13), periodicity was evident in almost all fibers, the average major period being close to the normal 640 Å. At extremely high magnifications (275,000) (fig. 14), the distance between major bands averaged about 640 Å. Major banding near the PDL-alveolar bone interface appeared shorter than normal, about 620 Å. (fig. 15)

A cross-sectional cut was made through a nerve bundle in fig. 16, showing both myelinated and non-myelinated fibers in good condition. Fat droplets were visible in other sections. Fig. 17 showed a high magnification (112,500 X) view of a fat droplet near a relatively intact cell membrane.

Considerable spacing was evident in many areas even at this early time period. (fig. 16, 18, 19) The area of greatest spacing was found near the nerve bundle, which was probably part of a neuro-vascular channel, and near a mast cell in fig. 18.

A conspicuous finding in the 4-day specimen was many areas of

densely packed collagen fibers with some scattered pyknotic fibroblast nuclei. (fig. 20) All the cells that were present were in advanced stages of degeneration. (fig. 21, 22) Large vacuoles were present in most cells, and nuclear and cell membranes were extensively disrupted. (fig. 22) Lipid droplets in their characteristic spherical shape were seen near some of the cells. (fig. 20, 21, 22) Some small veins were seen which were packed with erythrocytes and white cells. (fig. 23, 24) The red blood cells were compressed into unusual angular shapes and showed evidence of venous stasis, but were not highly degenerated. The cells of the venous wall appeared to be in good condition.

Several pictures were taken of a Sharpey's fiber entering bone. (fig. 25, 26, 27, 28) The average collagen band width in these photomicrographs was 630 Å, which was close to the normal of 640 Å. In other places, however, such as Fig. 29, 30, high magnifications of a densely packed area, the periodicity was about 600 Å. The minor cross-bandings which divided the major periods into smaller increments, were, in some cases, quite prominent, and made an accurate determination

of band width difficult. But the periods generally appeared to range from 600-610 Å.

Spacing was quite evident in many areas, especially around the degenerating blood vascular channels. (fig. 24) The cell free zone at 10 days showed a broad area across the PDL with no intact cells. Near the borders between PDL-bone and PDL-cementum, were cells which showed less complete breakdown, but were nonetheless, in some phase of degeneration. Some fibroblasts had relatively intact outer membranes, but were vacuolated in the main cell body and, more frequently, in the cytoplasmic extensions of the cell. (fig. 31) Other cells had perforated outer membranes and nuclear membranes. Still others showed frank necrosis. (fig. 32, 33, 34) The latter stages of degeneration produced a ghost-like structure in the cell. Most obvious was a nuclear outline filled with a spider web-like stroma and some larger nuclear remnants. (fig. 34) Where the cells had been completely lysed, spacing was evident, accompanied by surrounding cell debris.

Vascular channels were usually engorged with degenerating

erythrocytes. Disruption of endothelium and loss of membrane continuity was obvious. Some erythrocytes were lying outside the vascular channel in direct contact with the collagen fibers. The more highly degenerated red blood cells had lost their dense homogeneous appearance and had taken on a ragged, honeycombed look. (fig. 33)

The collagen fibers were easily distinguishable in most areas of the cell free zone, with the periodicity evident in longitudinally cut fibers. (fig. 31, 34) Most of the collagen appeared to be in good condition. In all cases, the average period of cross-banding was less than the normal 640 Å. Most measurements indicated periods in the 560-580 Å range. (fig. 35, 36, 37, 38)

Lipid droplets were evident in some areas. (fig. 31) They appeared to be intracellular, possibly closely associated with the lysosomes of ingesting cells.

Many areas of spacing were visible throughout the PDL. Some regions showed punctate areas of spacing which seemed to follow channels, possibly the remains of cellular cytoplasmic extensions. In several

heavily spaced areas (fig. 32, 39, 40) were seen very active, viable cells with heavy concentrations of rough endoplasmic reticulum. One of these active cells (fig. 39) appeared to have many small strands of unorganized collagen surrounding it in the extracellular fluid.

The 31-day specimen showed necrotic cell remnants in very close proximity to fresh viable cells. A blood vascular channel could be seen in Fig. 41 which had a fairly normal looking endothelial wall. The red blood cells within the vessel were rounded and not tightly packed together, probably indicating that the vessel was viable at the time of biopsy. Cross-banding was evident in most of the collagen and the periodicity range was approximately 530-550 Å.

Near some largely spaced areas could be seen healthy viable cells with active rough endoplasmic reticulum, (fig. 42, 43), and pinocytotic vessicles along cell borders. There was some indication of the presence of strands of unorganized collagen.

DISCUSSION

This study was mainly descriptive in nature and few definitive conclusions could be made from the work done here. Much more work on both animal and human subjects could be done on this subject.

Several technical problems were encountered in the experimental procedures used, from patient selection to production of the electron photomicrographs. The sample size was fairly small, necessarily so from the standpoint of availability of patients and the nature of the project. A continuing study in future years would be useful and appropriate for a project of this complexity. The surgical procedure necessary for obtaining intact tissue samples was difficult and required nearly ideal conditions for good results. The histological work-up and decalcification in EDTA were very lengthy procedures. The slow rate of decalcification was necessary to prevent damage to the tissues at the ultrastructural level. Although a diamond knife was used for cutting thin sections, obvious, heavy chatter marks were produced in some specimens which made scanning with the electron

microscope very difficult. Two electron microscopes and several operators were used in this study. Surprising differences were seen between quality of photographs from different machines and developing and printing procedures. In several pictures from one microscope, a grainy spotted surface covering the field indicated the presence of a fog of particles in the microscope which had settled on the specimen and obscured the pictures. Focusing was also a problem at times, especially when particles or chatter lines on the specimen reduced their clarity.

The choice of 2-day, 4-day, 10-day and 31-day samples was made to get the widest range possible from the small sample size. Many other time periods, from as short as a few minutes up to the 10-day period could have been studied. Control specimens would have been helpful, since photomicrographs from existing ultrastructural studies were difficult to usefully compare with present work. Differences in tissue thickness, staining procedures, decalcification methods, electron microscopes, photography and printing procedures all introduced variables which made it essential that controls for each experiment

be worked up along with the experimental samples.

The 31-day sample was used to observe the tissue changes that took place after application of force for 10 days, relief of force for 11 days and re-application of force for 10 days. If a larger sample size had been available, a more useful time period range could have been used, including a sample each 24-48 hours during the relief of pressure period and also several sequential samples during the re-application period.

One interesting discovery was that many of the cell free zones, which appeared cell free under the light microscope, did not give the same results with the electron microscope. Under EM, many supposedly cell free areas had remnants of cells and nuclei and cytoplasmic extensions of dead or dying cells scattered throughout the PDL. Some actual cell free zones were found with the EM, but these were usually small and isolated.

The changes observed in the 2-day sample indicated the rapidity at which the PDL was able to change under pressure. Some places had changed little, with healthy appearing cells, while cells in

other areas were in advanced stages of degradation by this time. This seemed to corroborate previous researchers who found cell free zones after 48 hours[2, 17, 18]. Areas of cell death and degradation were found in the 4-day, 10-day and 31-day specimens as well.

The most important consideration here was the pattern of degradation and reorganization that occurred over a period of time. When the pressure was applied to the tooth, the blood vascular channels were closed off and the cells in the PDL began to die. The lysosomes within the cell released lytic enzymes and the cells began to degenerate. The cytoplasm became vacuolated to a larger and larger degree and eventually, the cell membrances disrupted. The cellular and nuclear debris, and probably some surrounding collagen continued to dissolve, creating larger and larger spaces in the PDL. Examples were seen of punctate areas of spacing that appeared to follow the remnants of cellular cytoplasmic extensions. Other more concentrated areas of spacing occurred in the areas of previous cell bodies and around blood vascular channels. At some point, when enough spacing was present, new cells invaded the area, became very active and produced

new collagen.

In several specific examples from the photomicrographs, it appeared that the areas nearest to the blood vascular channels were lysed more rapidly and more completely than other areas. In Fig. 16, from a 2-day specimen, the area around a neurovascular channel showed a high degree of spacing even though pressure had been on the tooth for only 48 hours. One possible explanation was that neutrophils, which have enormous amounts of lytic enzymes and are the first to go to a wounded or damaged area, immediately after pressure application invaded the tissue surrounding the vessel by moving through the endothelial wall. These cells had more immediate access to the regions surrounding the blood vascular channels and these were the areas that were expected to lyse and show spacing first. Also in Fig. 16, the axons showed some degeneration but the myelin sheath had resisted degeneration characteristically well. Many cell remnants in the immediate area were probably from the endothelial lining of the nearby vascular channel.

Another example was Fig. 18 which showed a great deal of spacing.

Also evident on that picture was a mast cell wrapped around a collagen bundle. The heparin contained in the mast cell, which worked as an anticoagulant, tended to liquefy the area and allow much easier movement of repairative cells in the damaged site.

Areas of spacing and degeneration were evident at all of the time periods studied. Therefore, it was obvious that under constant pressure, the repairative process could not regenerate the damaged areas, at least not within a 10-day period. By the 10th day, however, there was clear evidence of some attempt at reorganization. There were numerous large, degenerated areas with considerable spacing, and in very near proximity could be seen new viable active cells. In some cases, the endoplasmic reticulum of these cells were heavily loaded with ribosomes, indicating active protein synthesis. Pinocytotic vessicles were seen on the membranes of one cell, and surrounding these cells in some areas could be seen small strands of seemingly disorganized collagen. There was a distinct possibility that this was evidence of new collagen (or tropocollagen) being produced, moved through the cell wall in a disorganized state, and becoming

organized in the extracellular fluid.

Pressure was applied, relieved and applied again in the 31-day specimens in an attempt to monitor the degree of breakdown of cells and fibers upon reapplication of force as compared to an initial application. The state of degradation and reorganization in the 31-day sample appeared to be very similar to that seen in the 10-day specimen. Degenerating cells were seen scattered throughout the PDL and many healthy viable cells were also seen, some closely juxtaposed to badly destroyed cell remnants. Possibly the biggest difference between the 10-day and 31-day specimens was the apparent presence of more healthy blood vascular channels in the 31-day specimen. Although no definitive conclusions were drawn from that finding, it was speculated that the viability of the blood vascular system was either not damaged as much upon reapplication of force as the first time, or the vessels were regenerated more quickly after the second application. More samples between 10 days and 31 days would have been useful here. It would have been helpful to follow the reorganization of the PDL for a longer period of time with no relief of pressure to see how relevant

the relief of pressure was to the healing process. Also a sample taken just prior to reapplication of pressure would have helped in an analysis of comparison of damage caused by the two force applications.

The question of where the new cells come from was not satisfactorily answered in this study. Buck and Church[2] indicated undermining resorption renewed the PDL in their studies within 14 days after pressure was applied. In the light microscope findings of this study, reference was made to obvious evidence of undermining resorption in the 10-day specimen. Judging from the severity of vascular stasis seen in all the samples, the most reasonable assumption was that undermining resorption did indeed play a major role in the reorganization of the PDL. Another possibility was the ingress of viable cells from the lateral and apical directions through the PDL itself where the pressure was not so great, and therefore stasis of the blood flow would not be expected to occur. In both instances, the spacing that follows degeneration of cells was felt to be extremely important and probably necessary for movement of new cells any appreciable distance

into the wounded portion of the PDL.

One unusual and unexpected finding concerned the periodicity of the collagen fibers in the various time periods. The major banding of normal collagen has been known to occur at 640 Å intervals. Measurements taken on the photomicrographs of the 2-day specimen indicated the periods were fairly normal, with a measured range of 620-650 Å. In the 4-day sample, the banding range was 600-630 Å with most measured period in the 600-610 Å group. By the 10th day the observed spacing was even further from the normal 640 Å. The range at 10 days was 560-580 Å. In the 31-day sample, the range was even lower, 530-550 Å.

In doing these measurements, random collagen strands were chosen, usually being the strands with the best striations visible on the photographs. No explanation was given for this discrepancy of cross-banding. The possibility of its being purely artifactual has been considered, however, the chance of it occurring by chance alone was considered remote. Additional samples would have again been helpful in determining if this phenomenon reversed itself during the relief of pressure phase of the study.

SUMMARY AND CONCLUSIONS

A descriptive study was done where a 70 gram force was placed upon a human tooth and the resultant changes in the PDL were studied with the use of a light microscope and an electron microscope.

Seven samples of tooth and intact adjacent buccal alveolar bone were obtained from various time periods; 2 days, 4 days, 10 days, and a fourth category treated by applying force for 10 days, relieving the force for 11 days and reapplying the force for 10 additional days.

Cell death and degradation was found to occur after 48 hours with considerable spacing evident. Cell degeneration continued and the amount of spacing increased up to the 10-day time period. At 10 days, new cells were seen invading the area. Evidence was seen of reorganization of the collagen. The fourth category tested was histologically very similar to the 10-day specimens.

The spacing that formed in the areas of degenerated cells seemed to be very important to allow ingress of new cells into the damaged areas.

Evidence was seen of a change in the banding pattern of the collagen

after pressure application. The major cross-bandings were found to be closer together, with shorter inter-band intervals in the samples that had undergone constant pressure for long periods of time.

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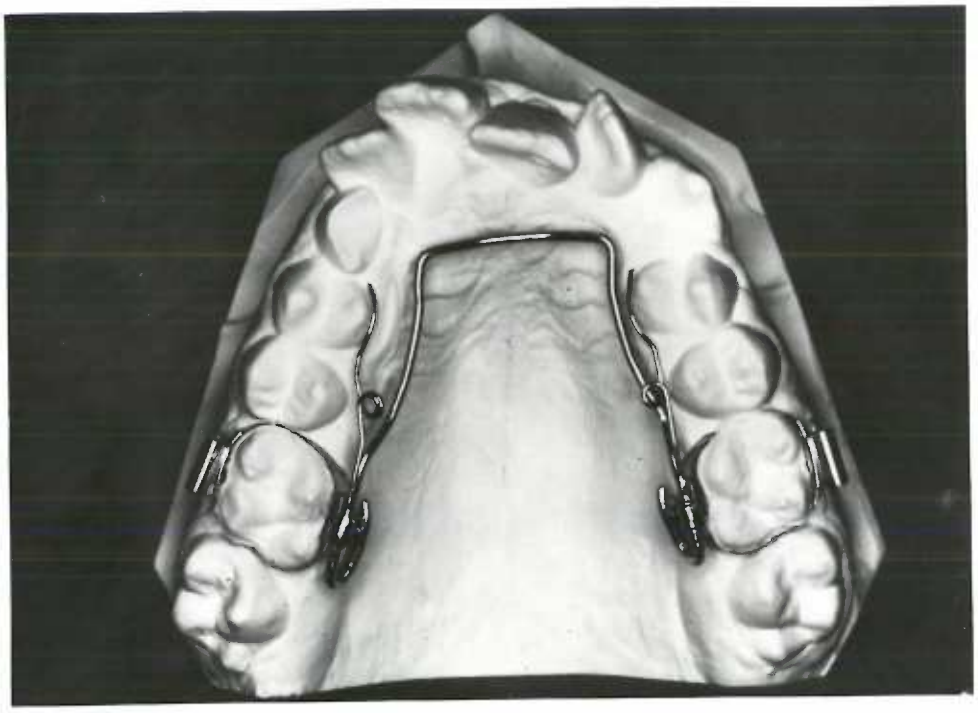

Fig 1. Appliance used in the experiment - shown on a plaster model.

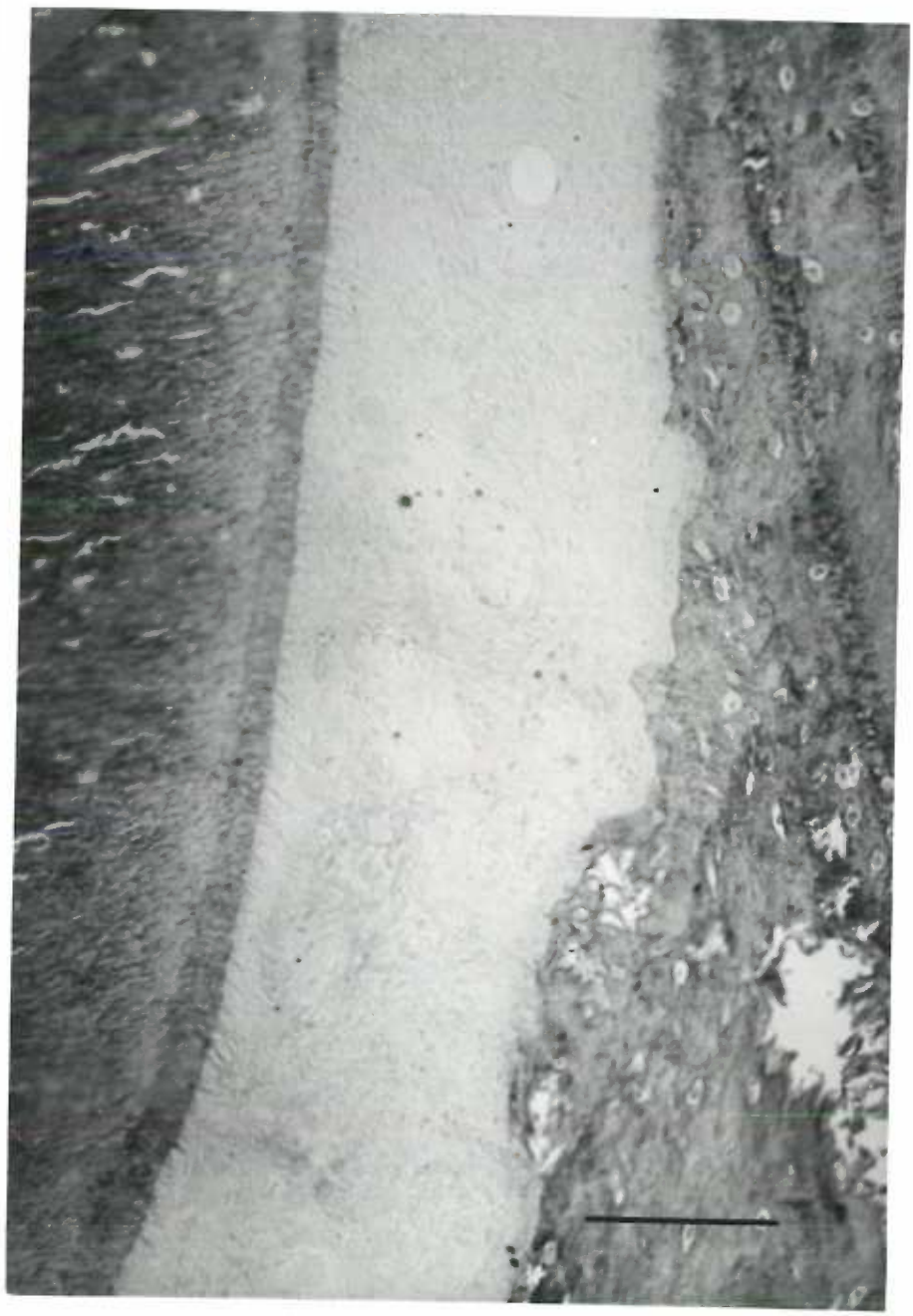

Fig 2. Pressure zone of PDL; 2 day; 256X. Scale 100μ.

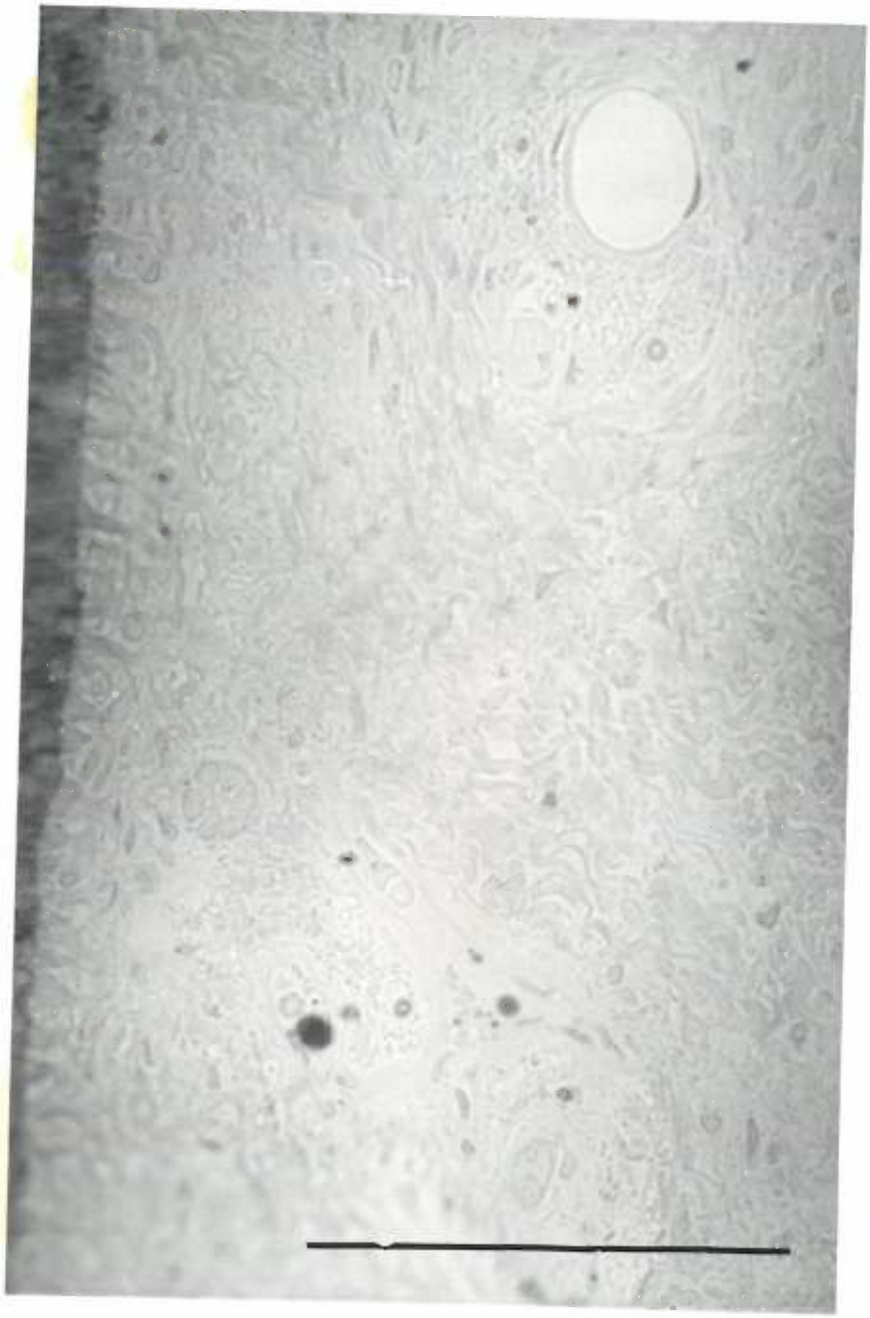

Fig 3. Pressure zone of PDL; 2 day; 640X. Scale 100μ.

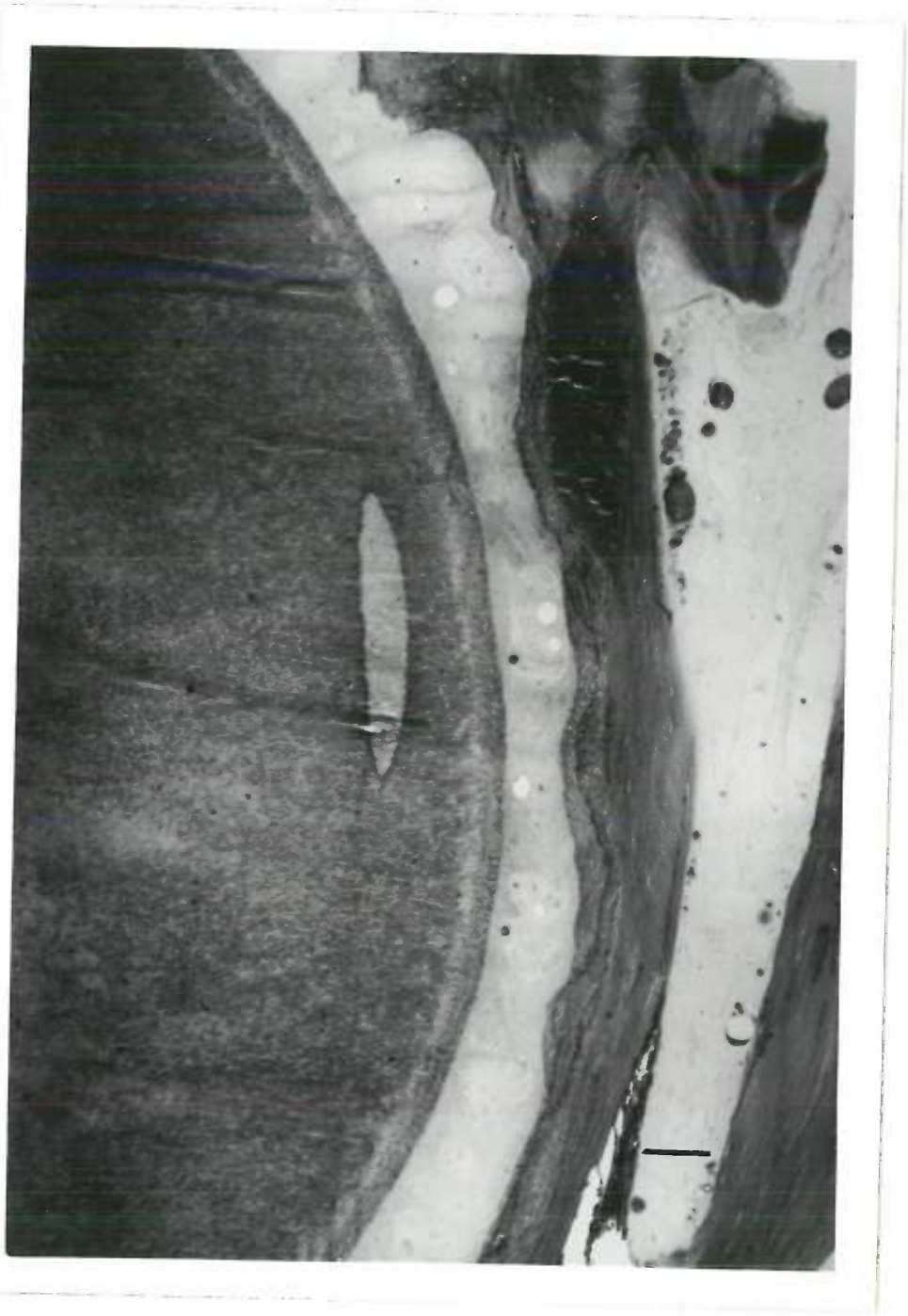

Fig 4. Pressure zone of PDL; 4 day; 100X; showing variations in thickness of PDL. Scale 100μ.

Fig 5. Pressure zone of PDL; 4 day; 256X; remodeling including reversal lines, resting lines, Howship's lacunae. Scale 100μ.

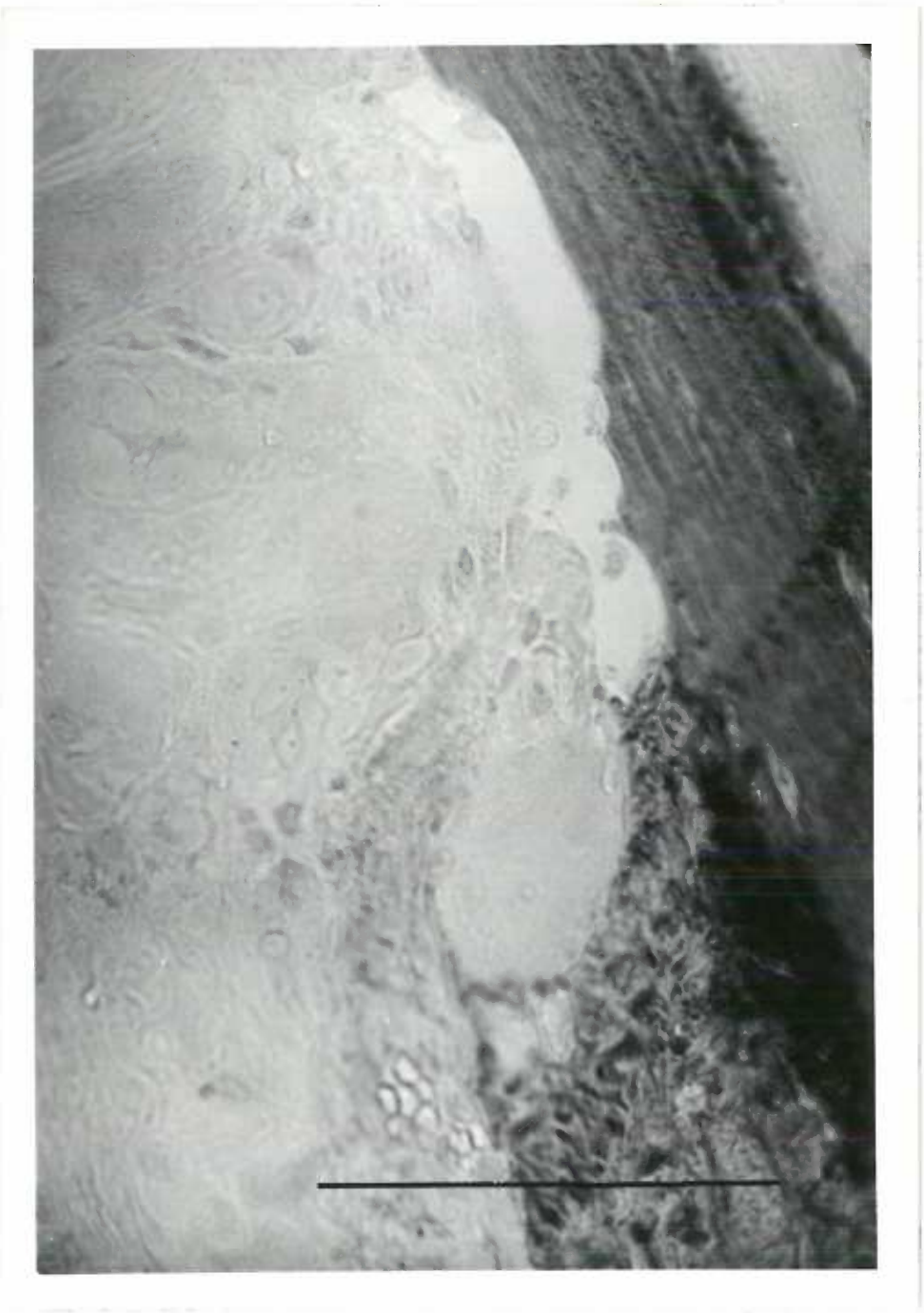

Fig 6. Pressure zone of PDL; 10 day; 640X; amorphous appearance. Scale 100μ.

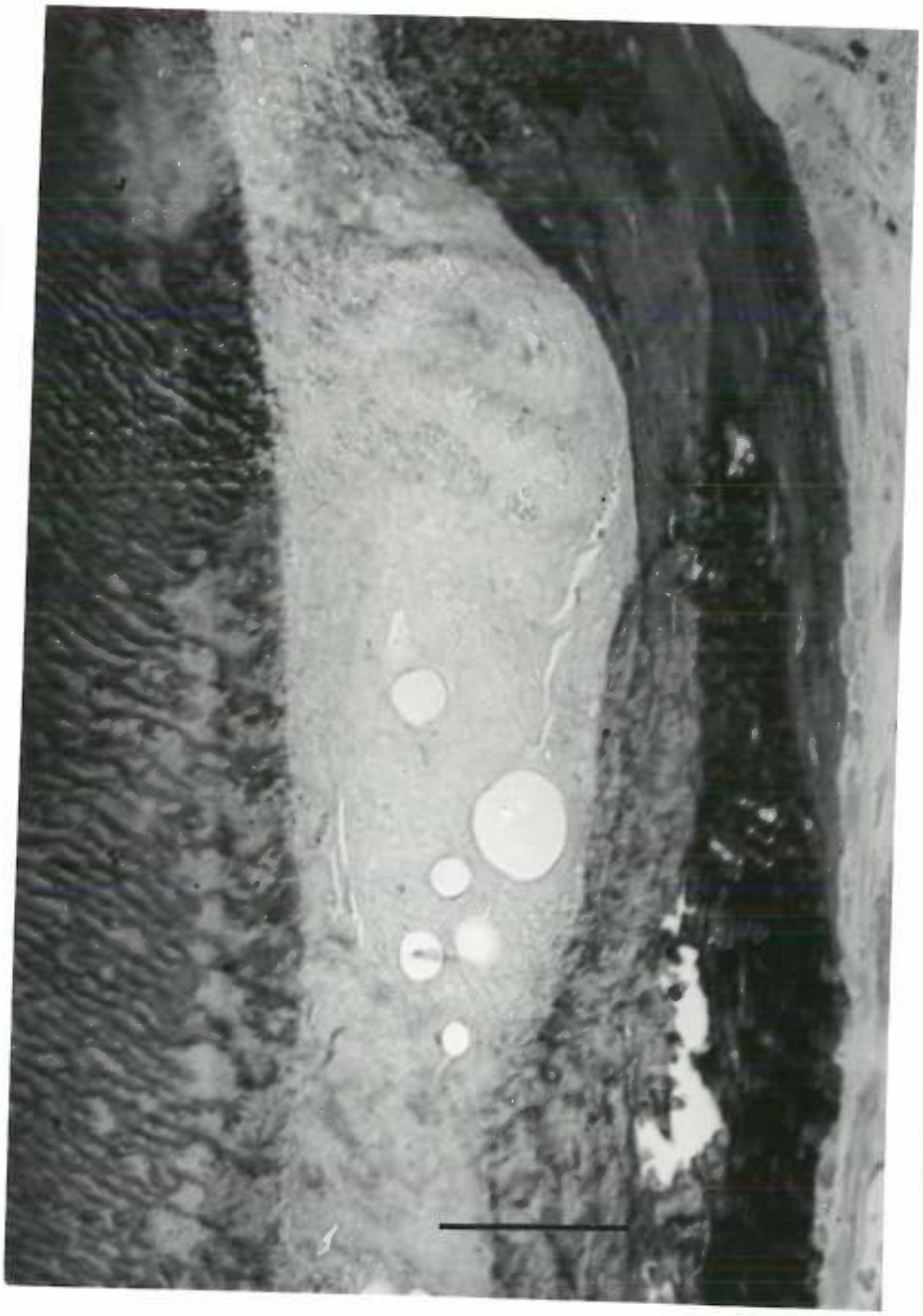

Fig 7. Pressure zone of PDL; 10 day; 256X; vacuoles. Scale 100μ.

Fig 8. Pressure zone of PDL; 10 day; 256X; part of PDL highly compressed; Howship's lacunae present. Scale 100μ.

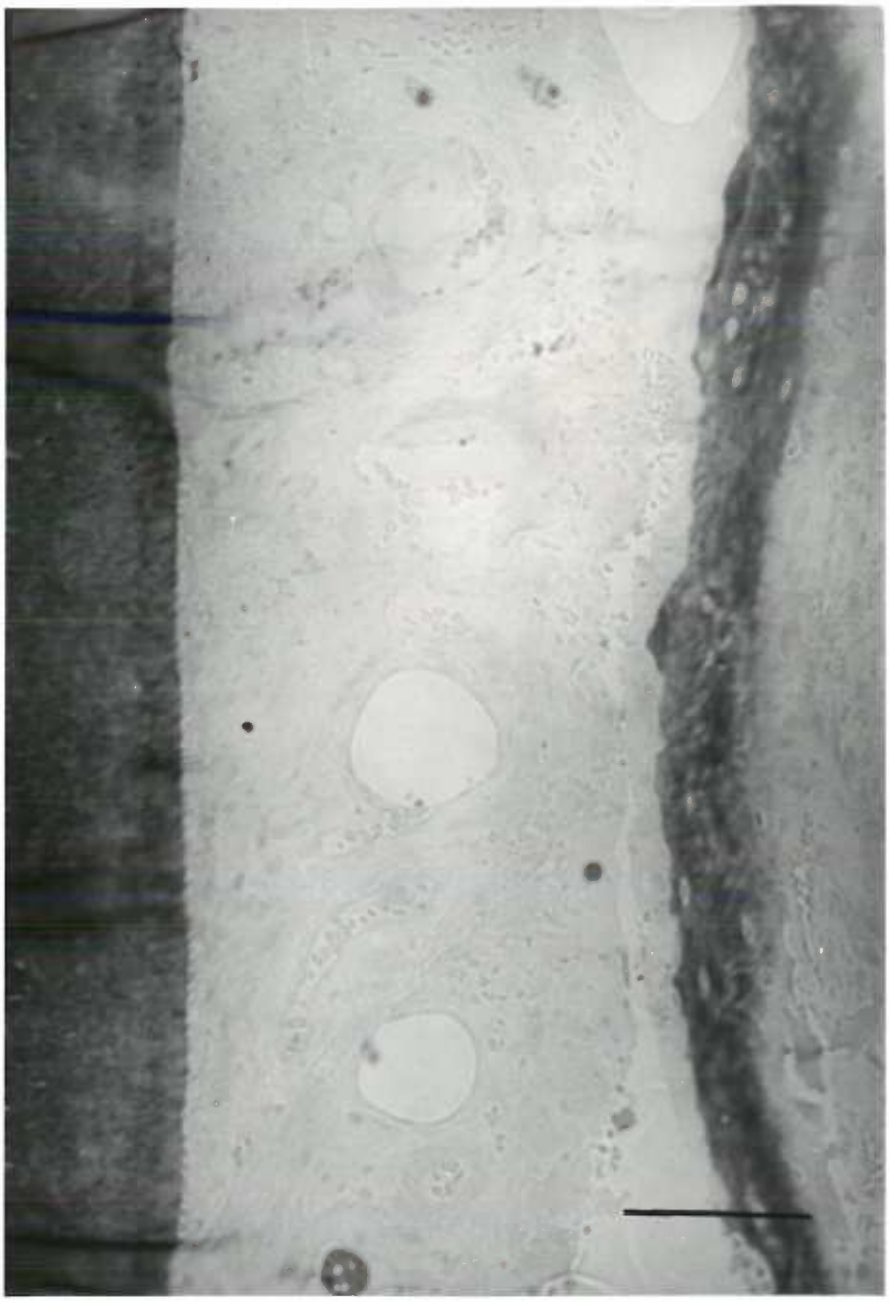

Fig 9. Pressure zone of PDL; 31 day; 256X; even resorption of alveolar bone. Scale 100μ.

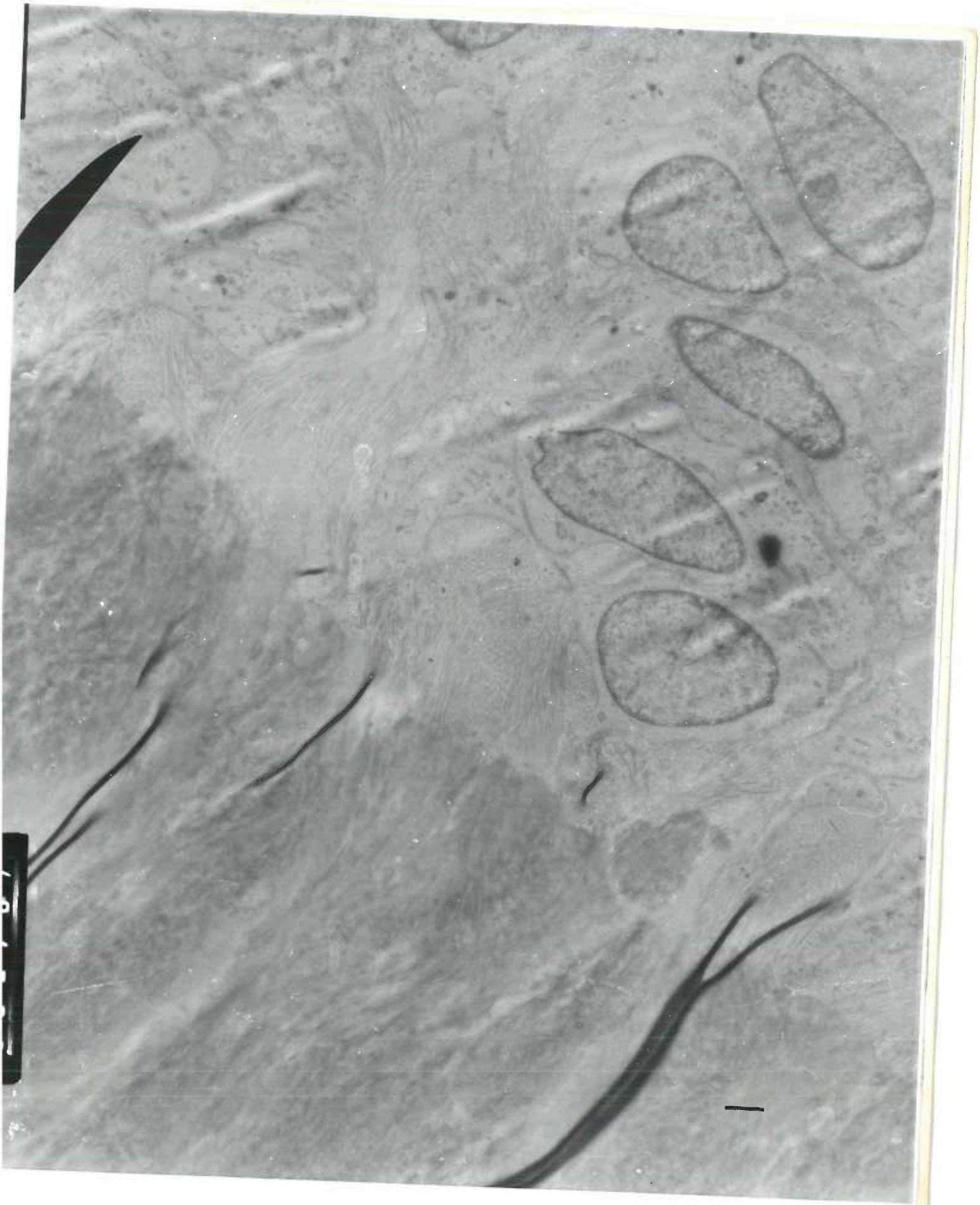

Fig 10. 2 day; 8,500X; fairly normal looking cells and also some degenerated cells. Scale 1μ.

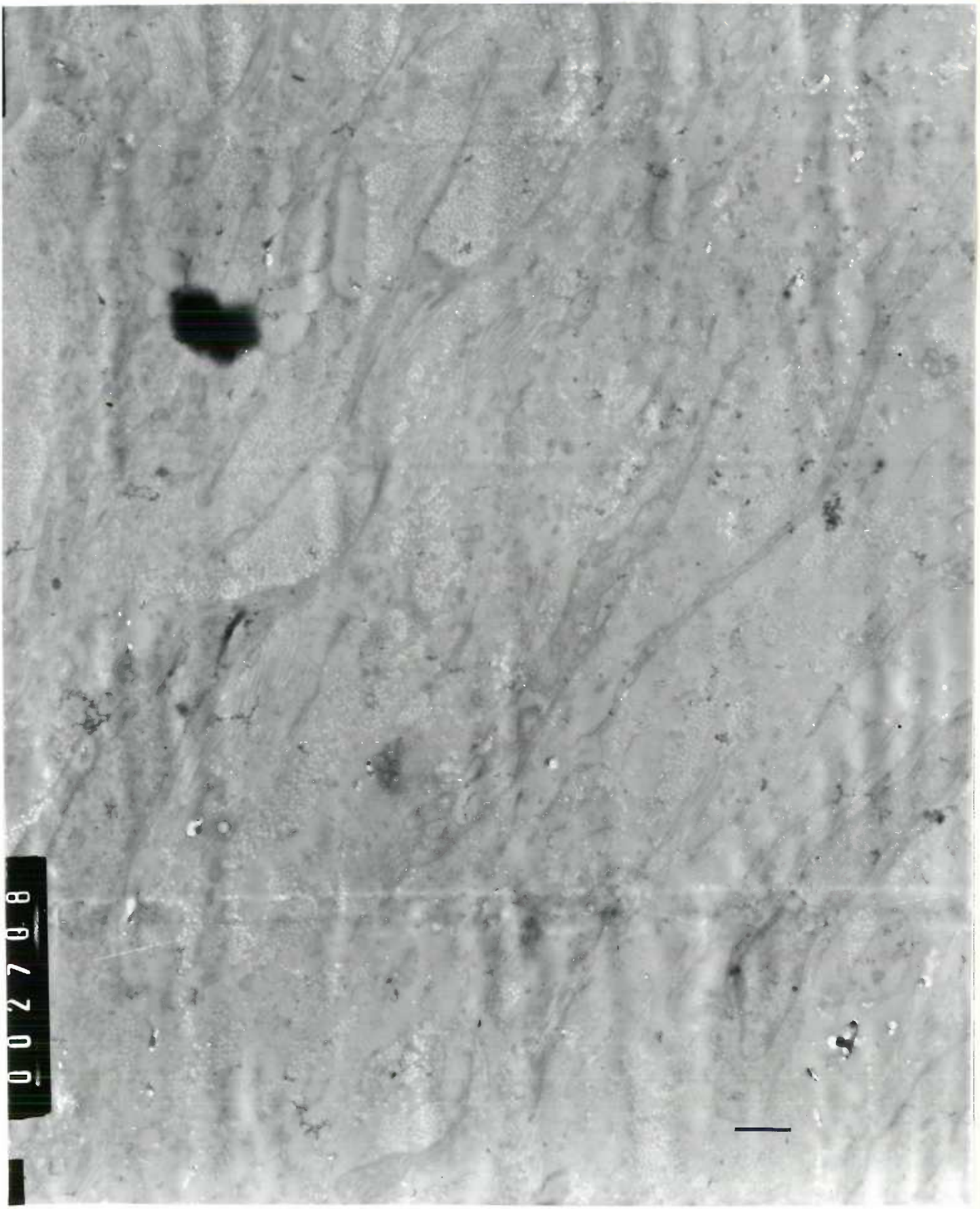

Fig 11. 2 day; 11,250X; cells in various stages of degeneration. Scale 1μ.

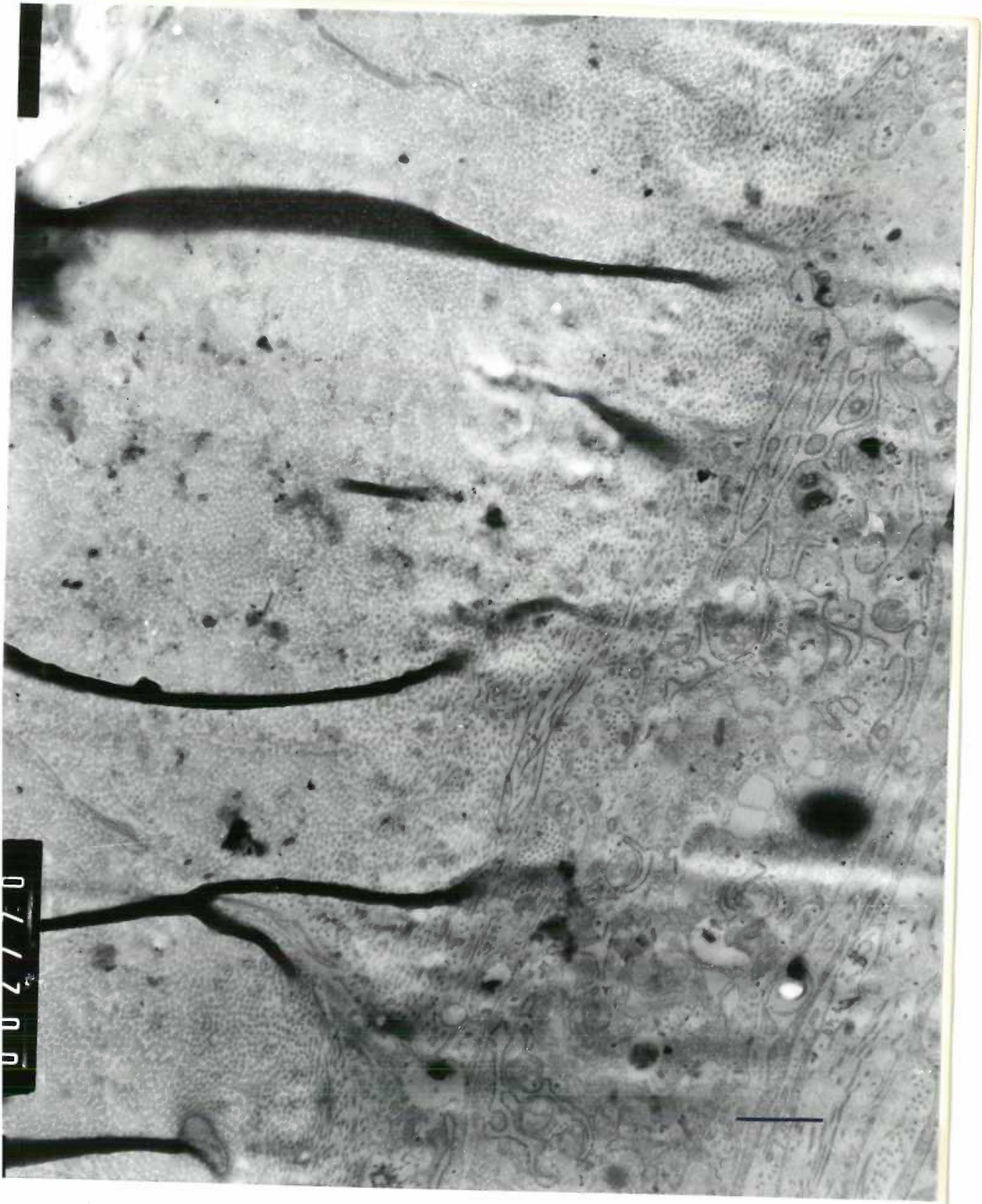

Fig 12. 2 day; 17,750X; fairly normal looking cell. Scale 1μ.

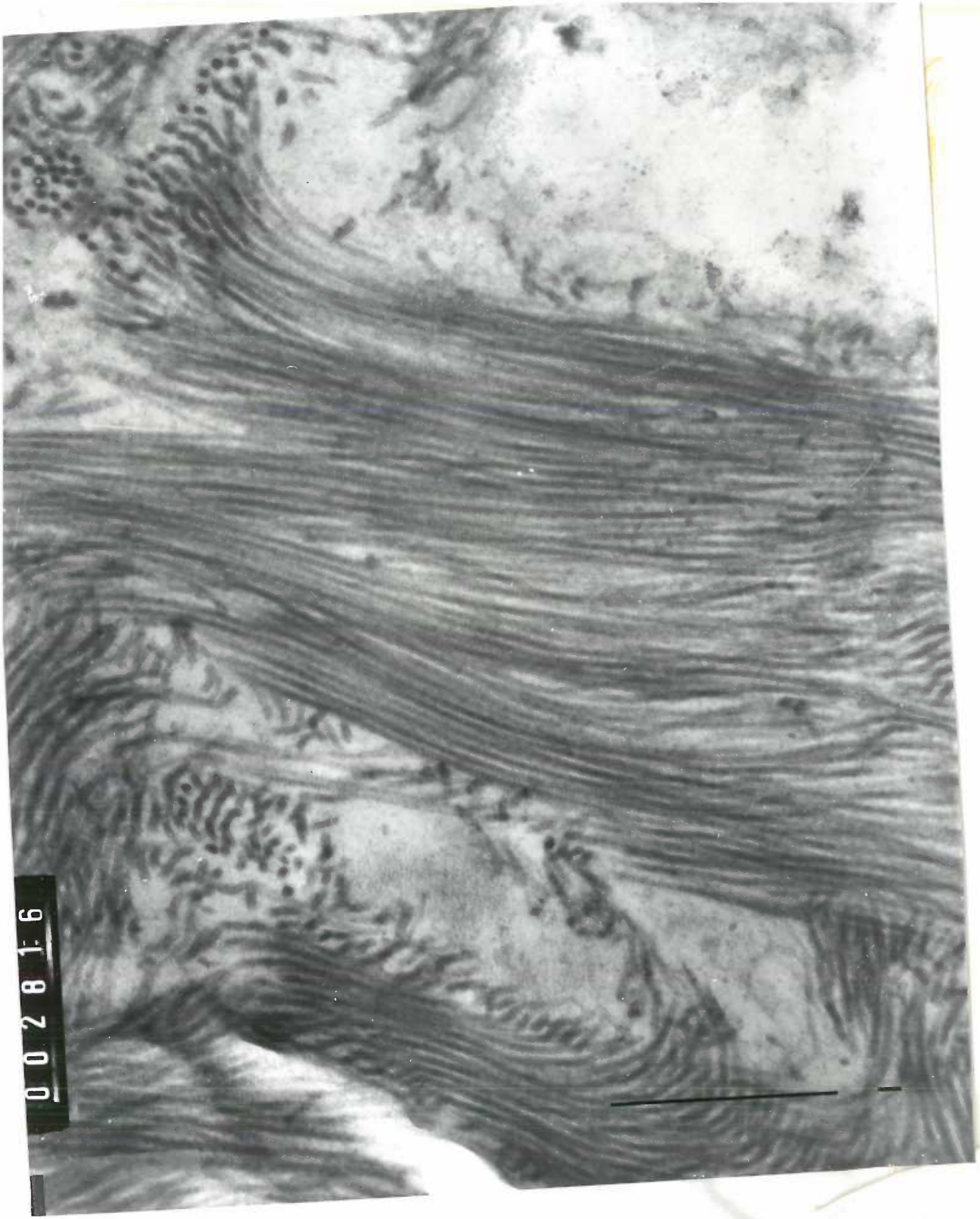

Fig 13. 2 day; 47,500X Collagen bundle. Scale 1μ; 0.1μ.

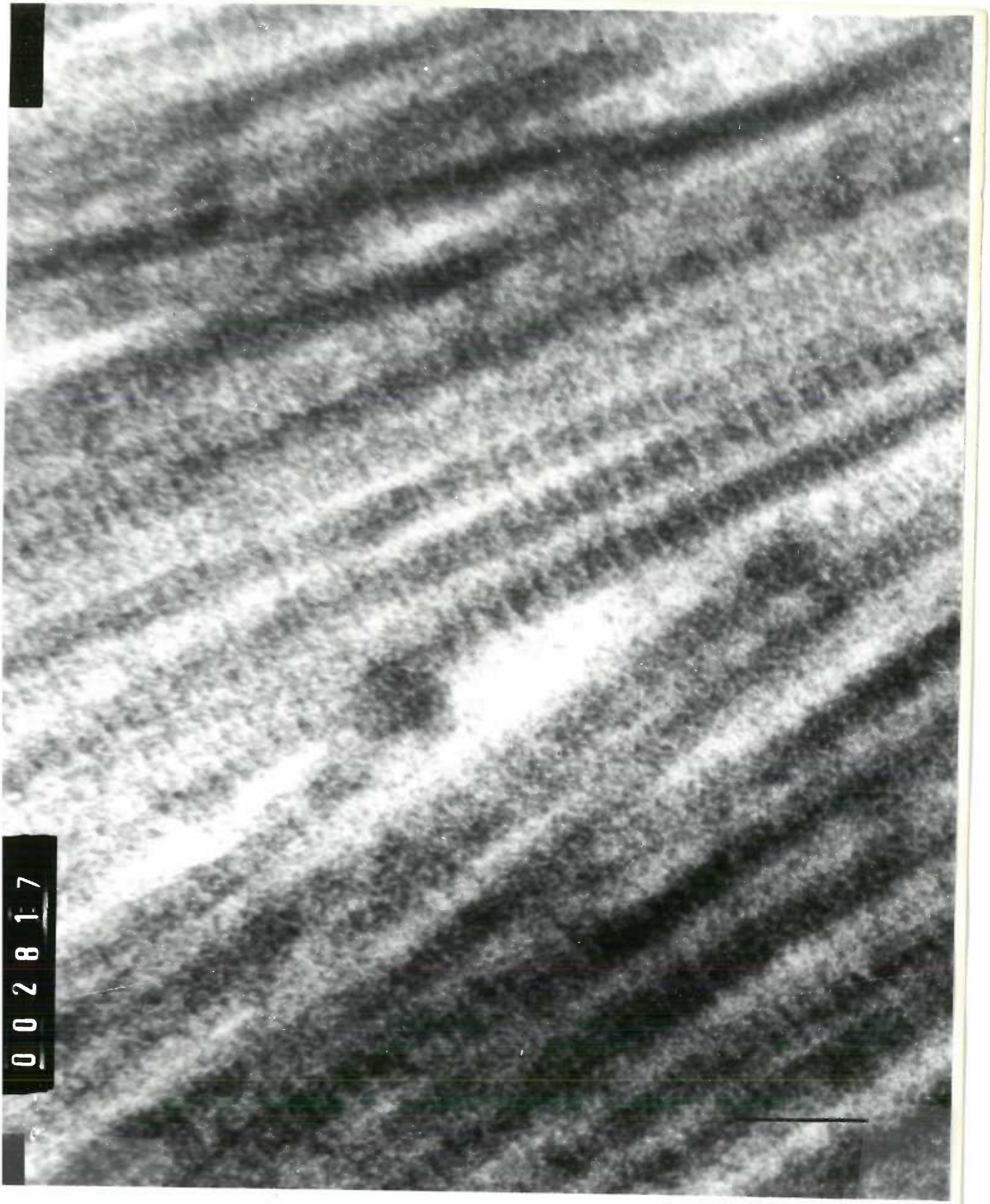

Fig 14. 2 day; 275,000X; As above only higher power. Scale 0.1μ.

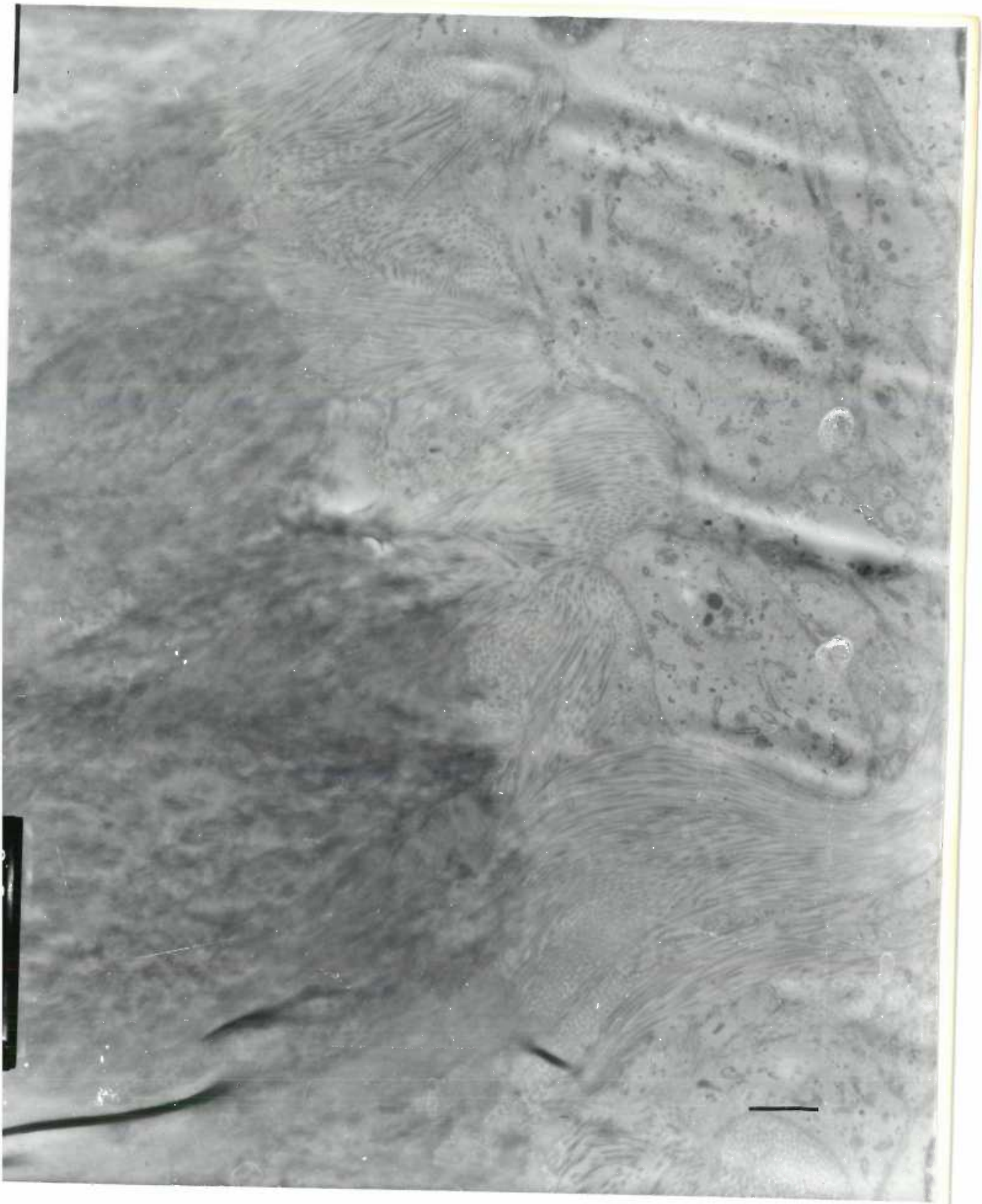

Fig 15. 2 day; 14,250X; PDL-alveolar bone interface. Scale 1μ.

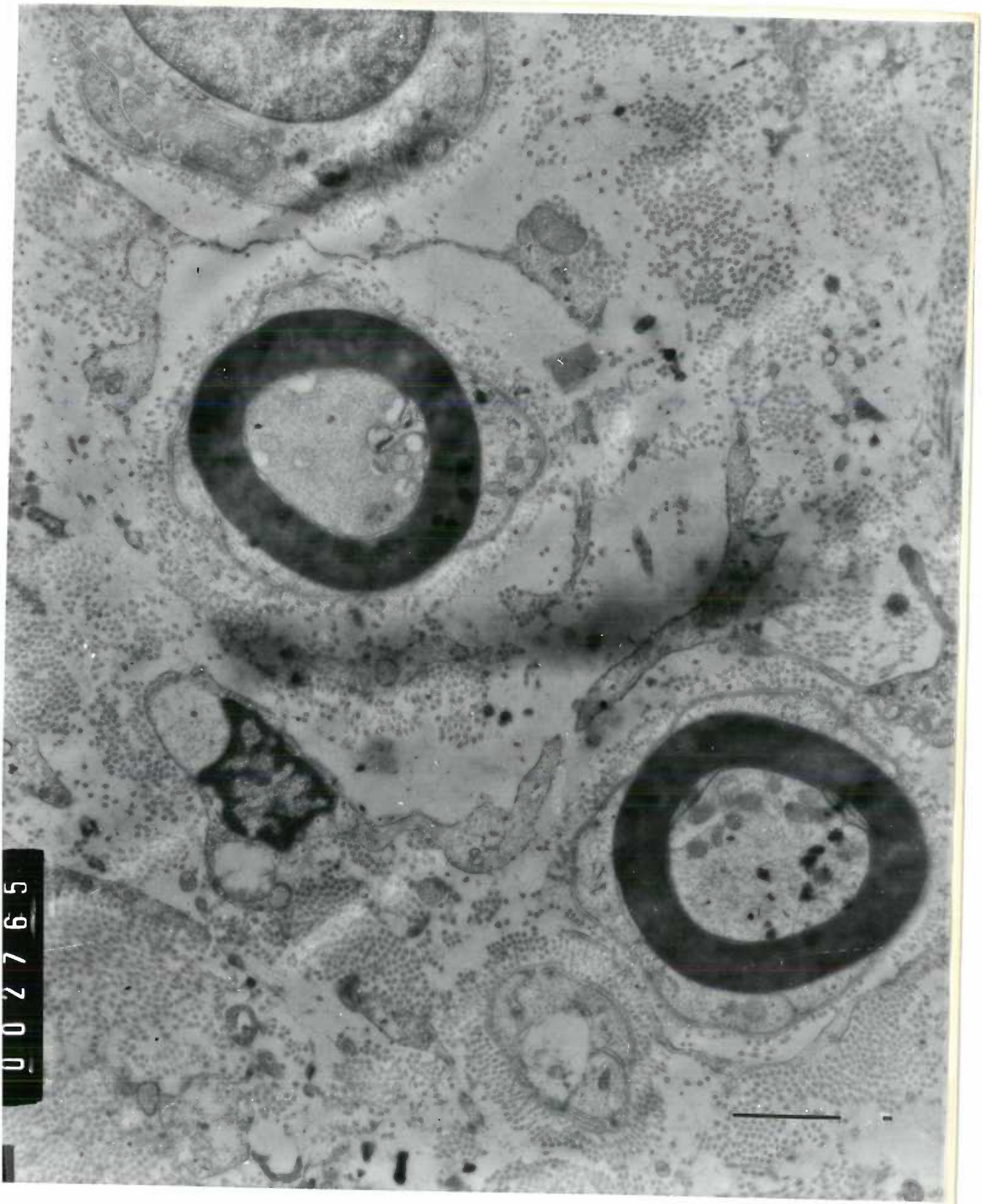

Fig 16. 2 day; 22,750X; cross-section through neurovascular bundle, spacing evident. Scale 1μ; 0.1μ.

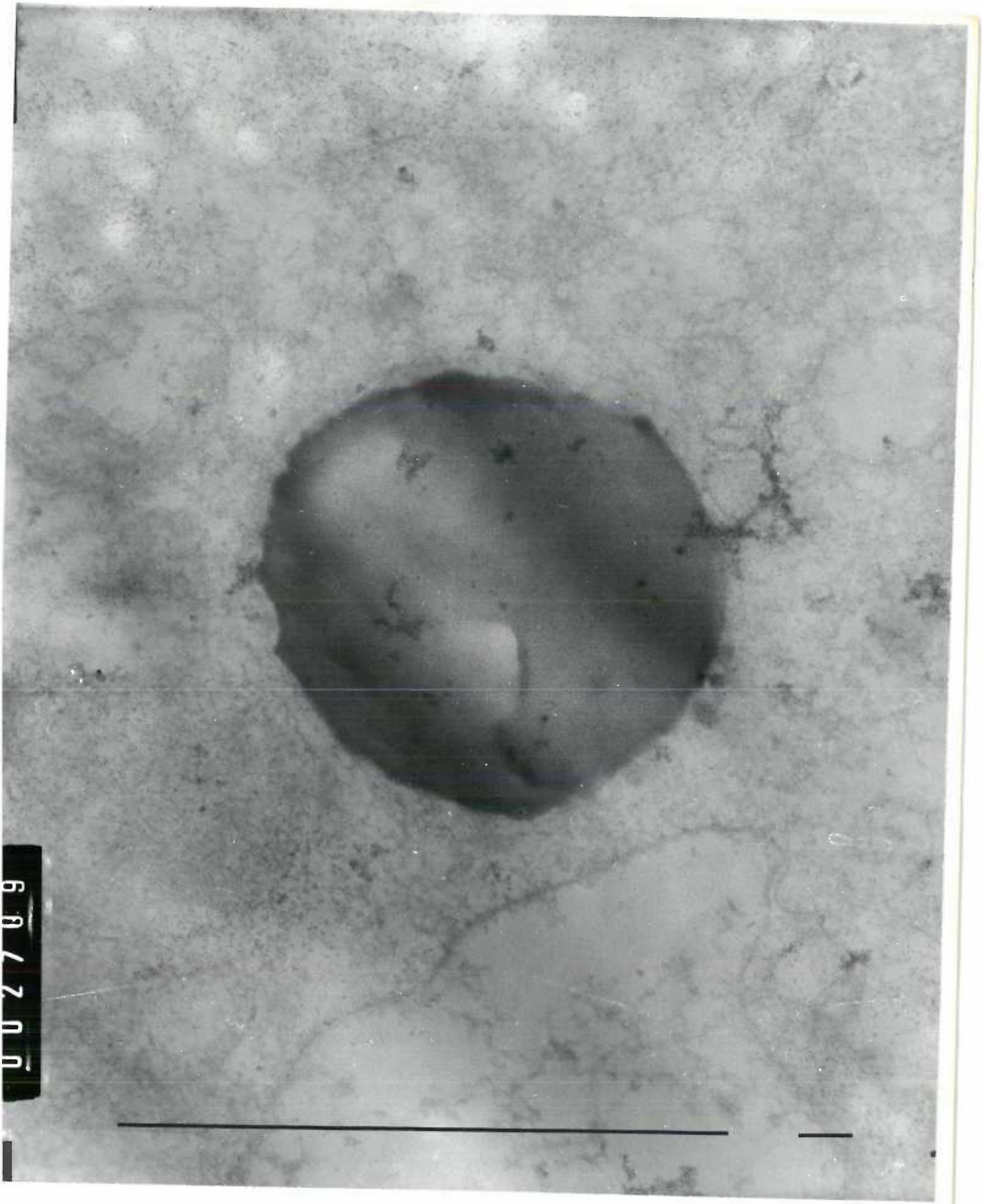

Fig 17. 2 day; 112,500X; fat droplet. Scale 1μ; 0.1μ.

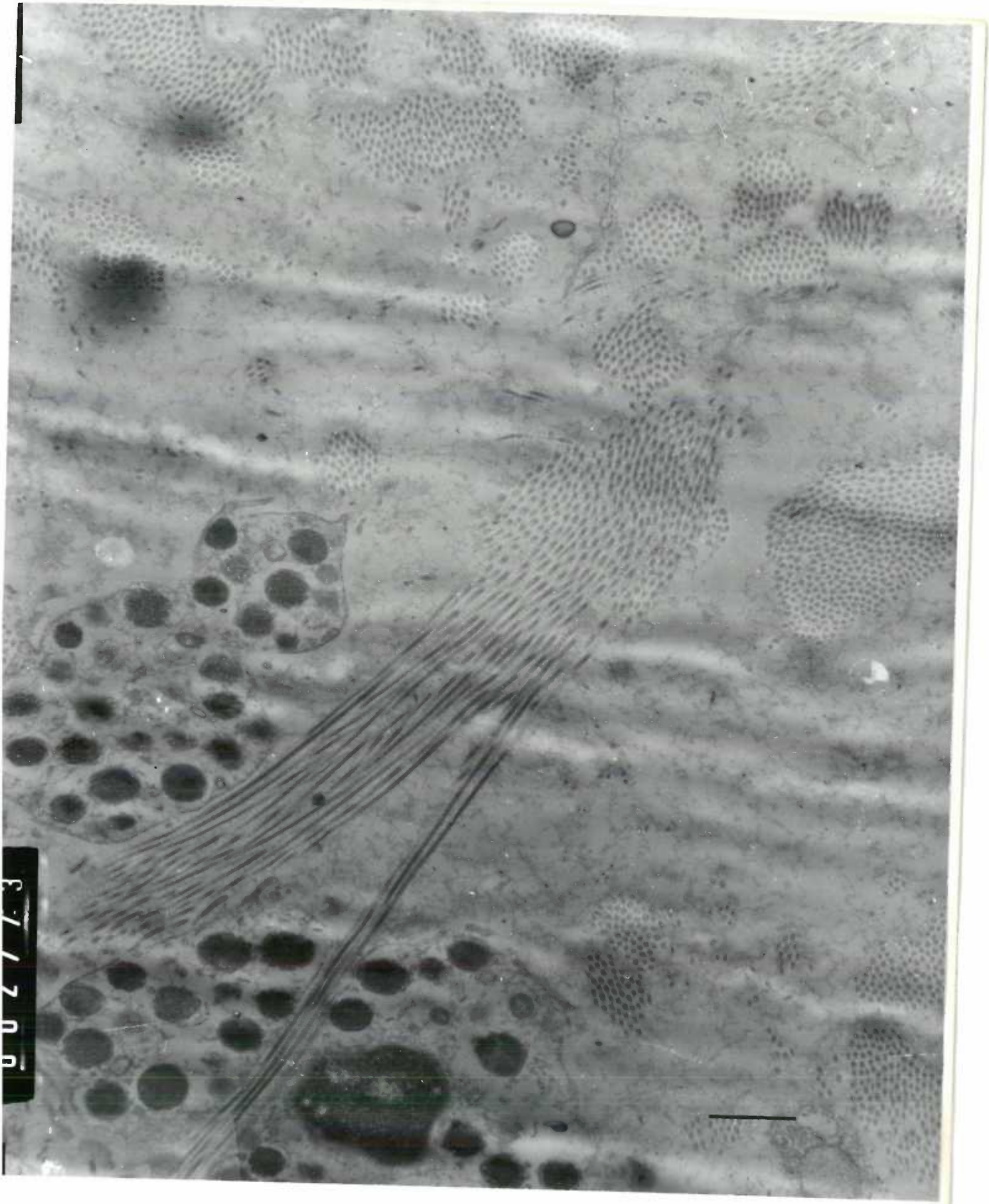

Fig 18. 2 day; 17,750X; collagen bundle surrounded by mast cell, spacing evident. Scale 1μ.

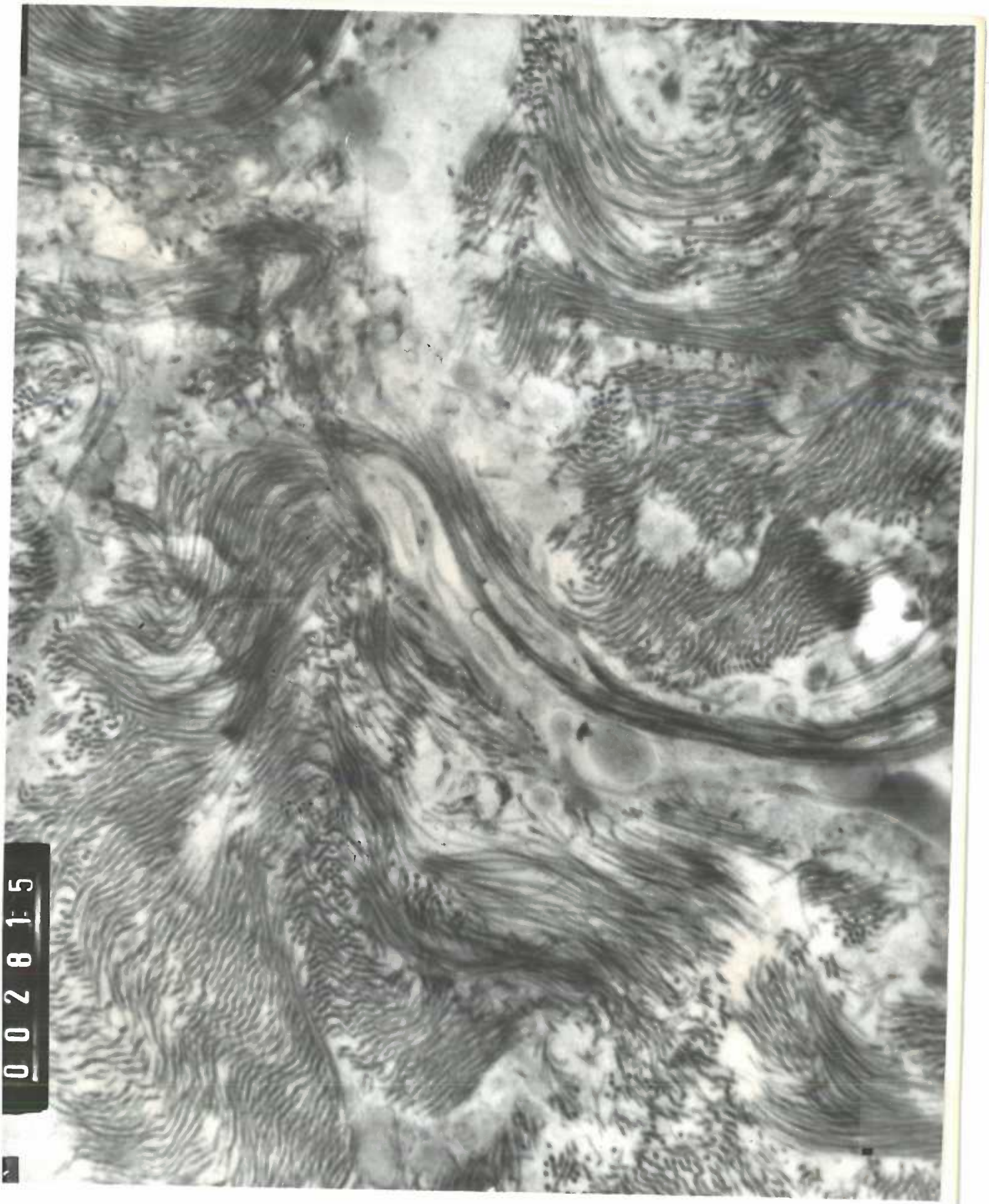

Fig 19. 2 day; 22,750X; spacing evident. Scale 1μ; 0.1μ.

Fig 20. 4 day; 4,750X; pyknotic nuclei, venous stasis. Scale 1μ.

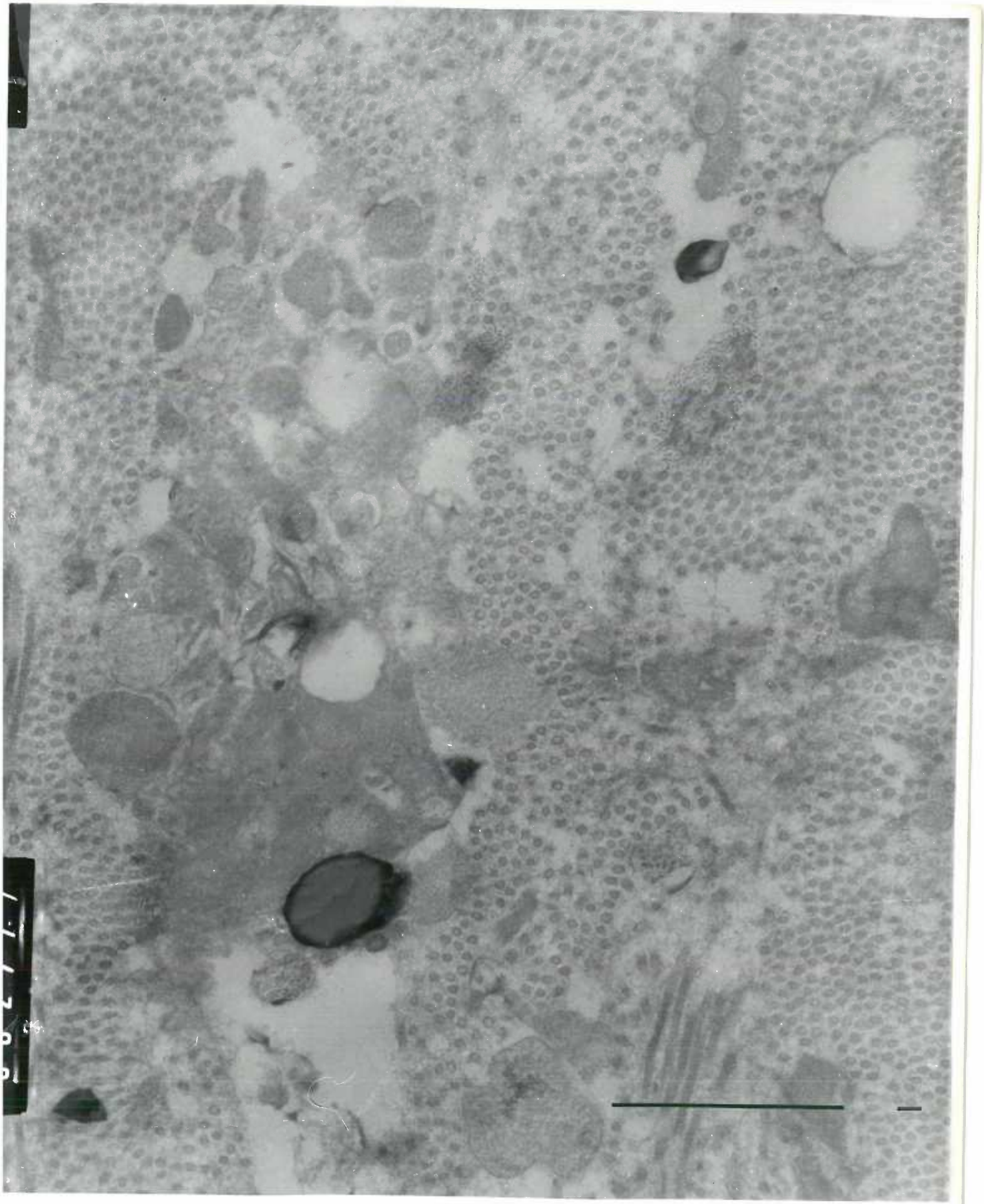

Fig 21. 4 day; 47,500X; highly degenerated cell, spacing evident. Scale 1μ; 0.1μ.

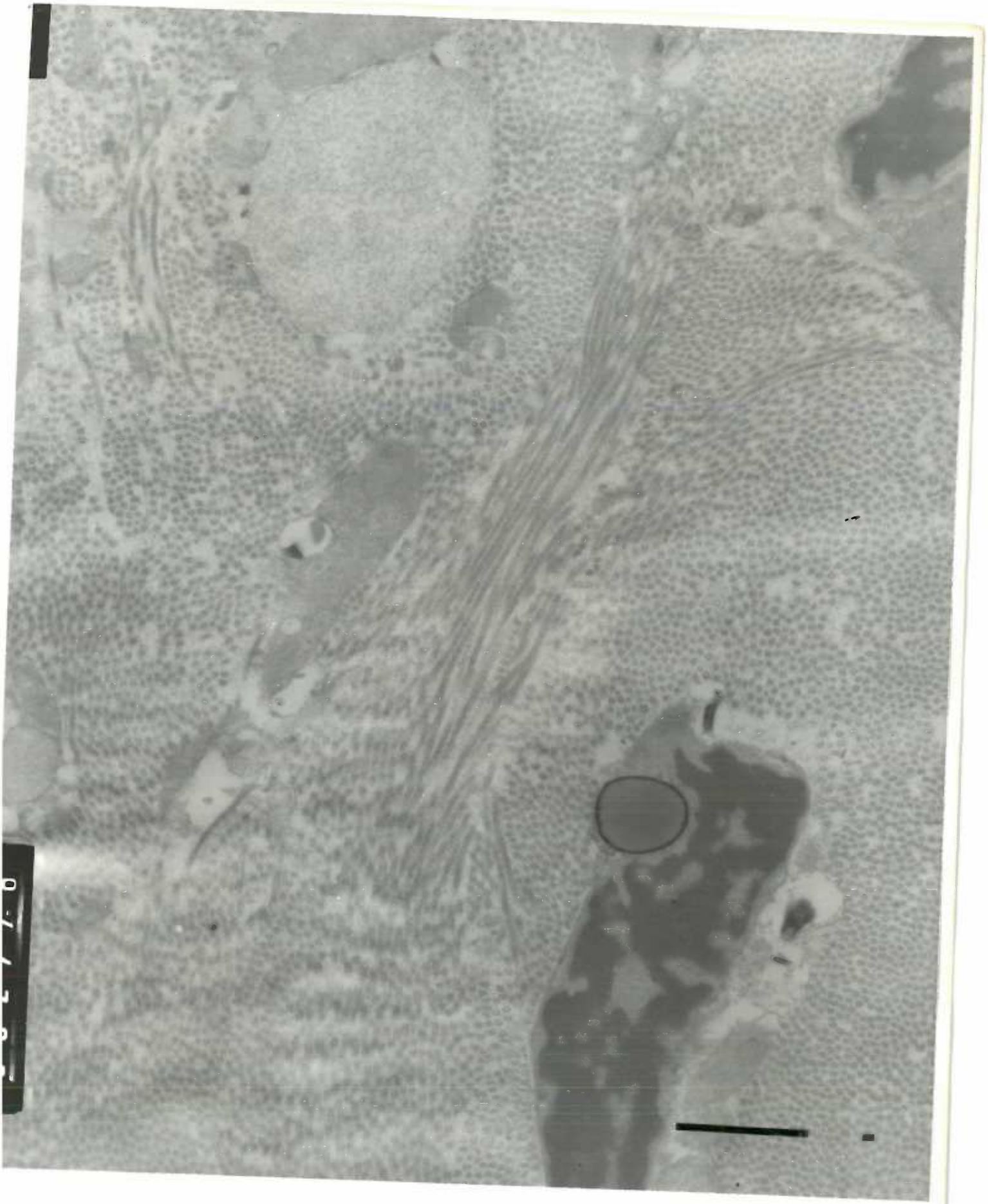

Fig 22. 4 day; 27,500X; degenerated cell; lipid. Scale 1μ; 0.1μ.

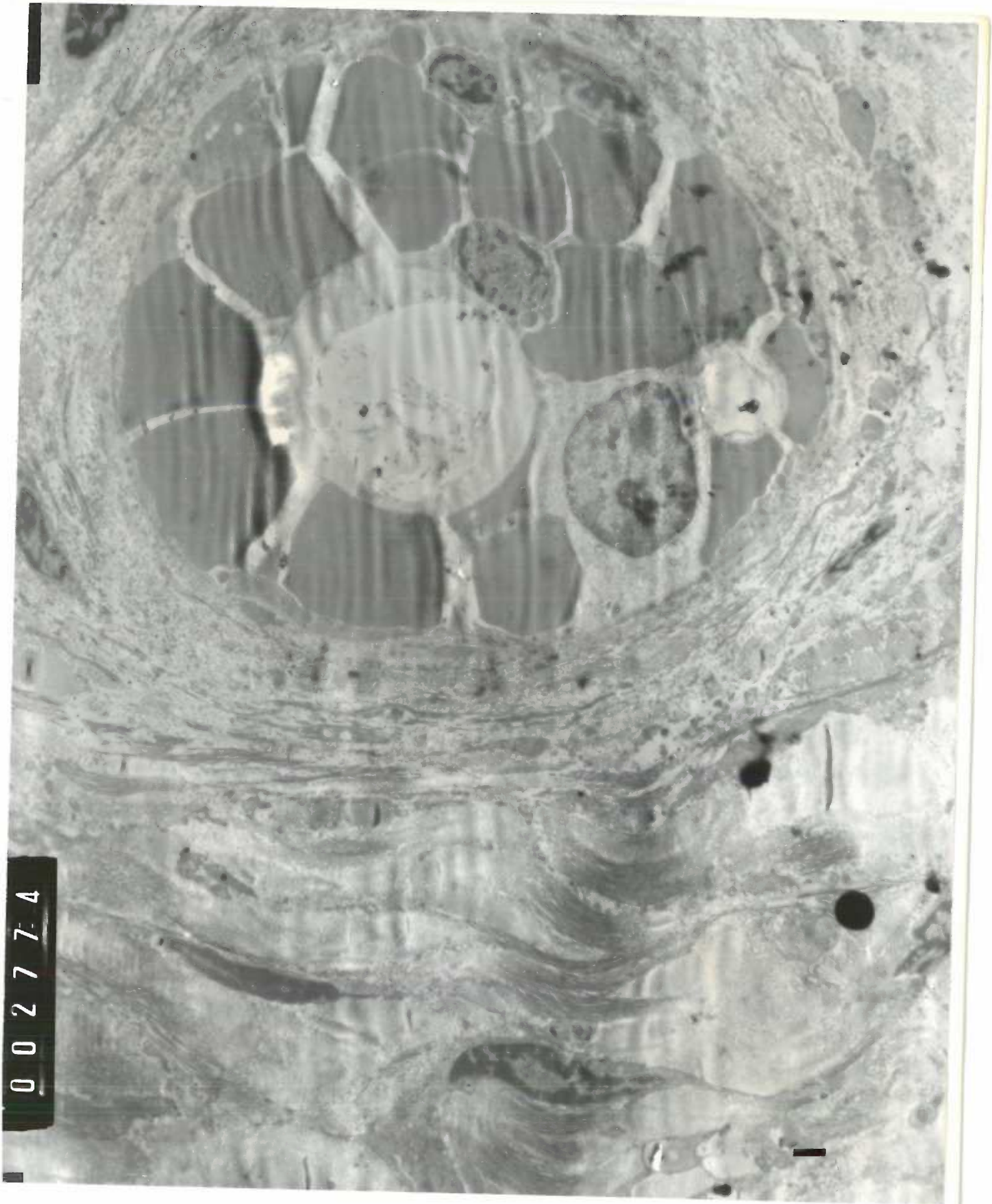

Fig 23. 4 day; 6,250X; venous stasis. Scale 1μ.

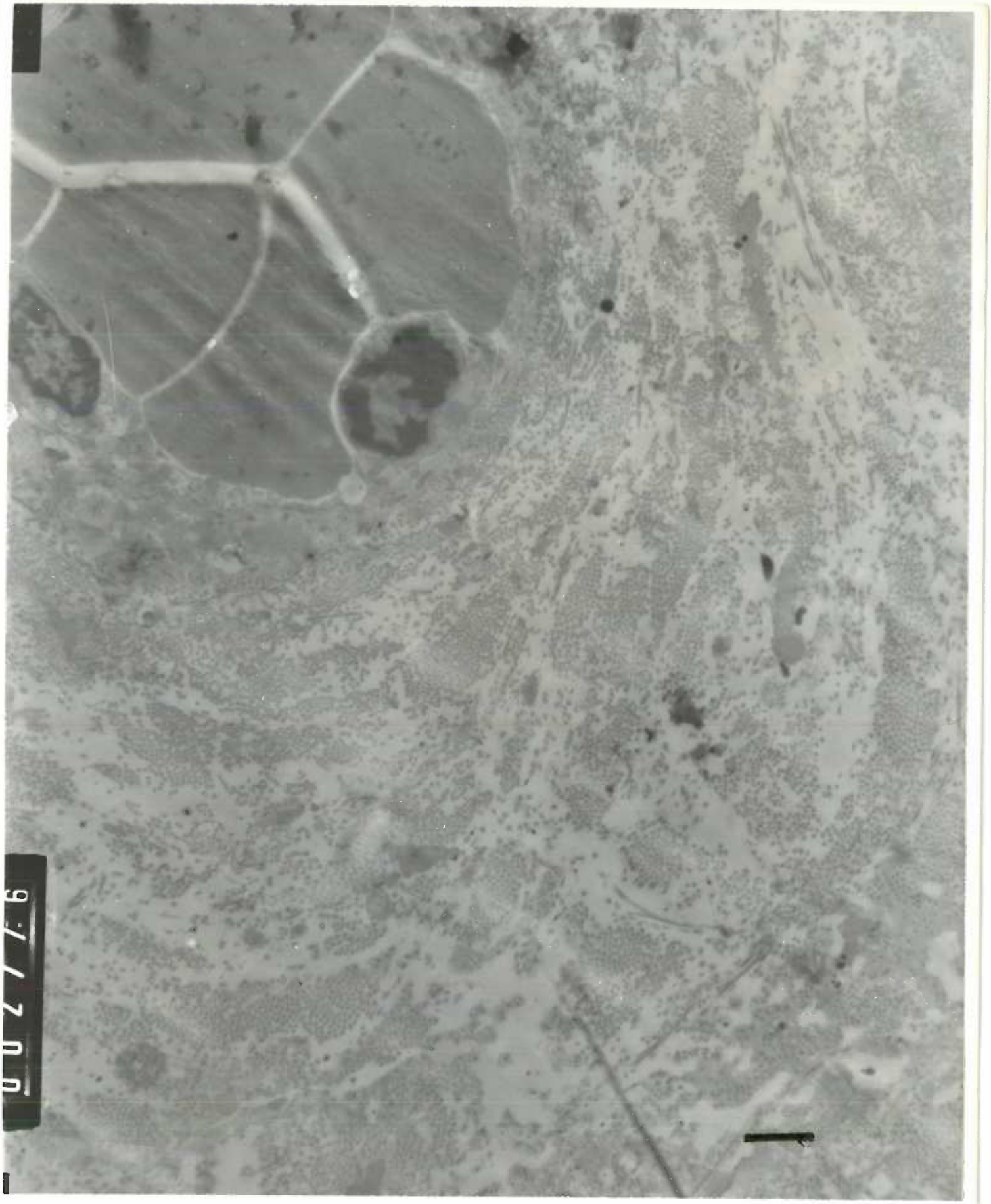

Fig 24. 4 day; 14,250X; venous stasis; spacing evident. Scale 1μ.

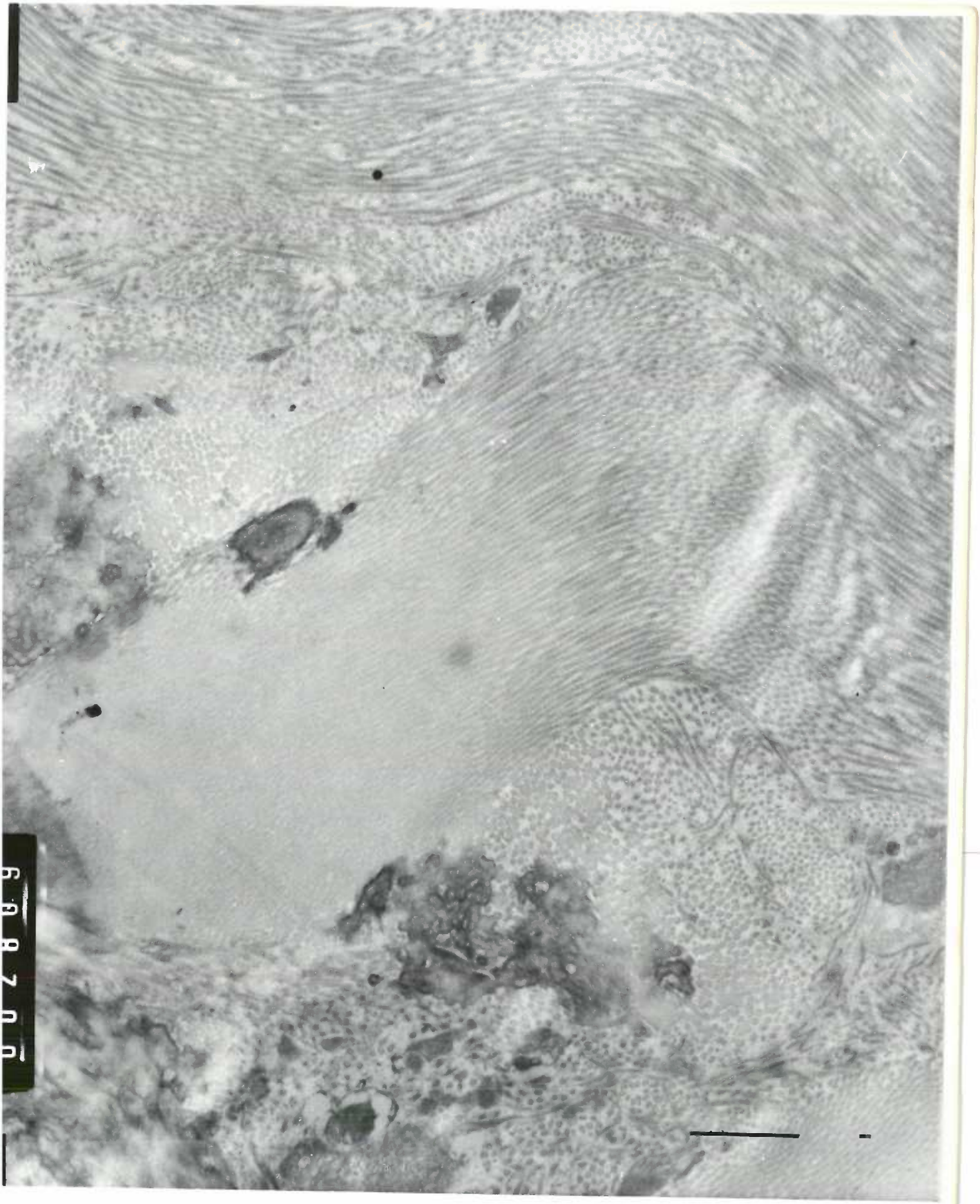

Fig 25. 4 day; 22,750X; Sharpey's fiber entering bone. Scale 1μ; 0.1μ.

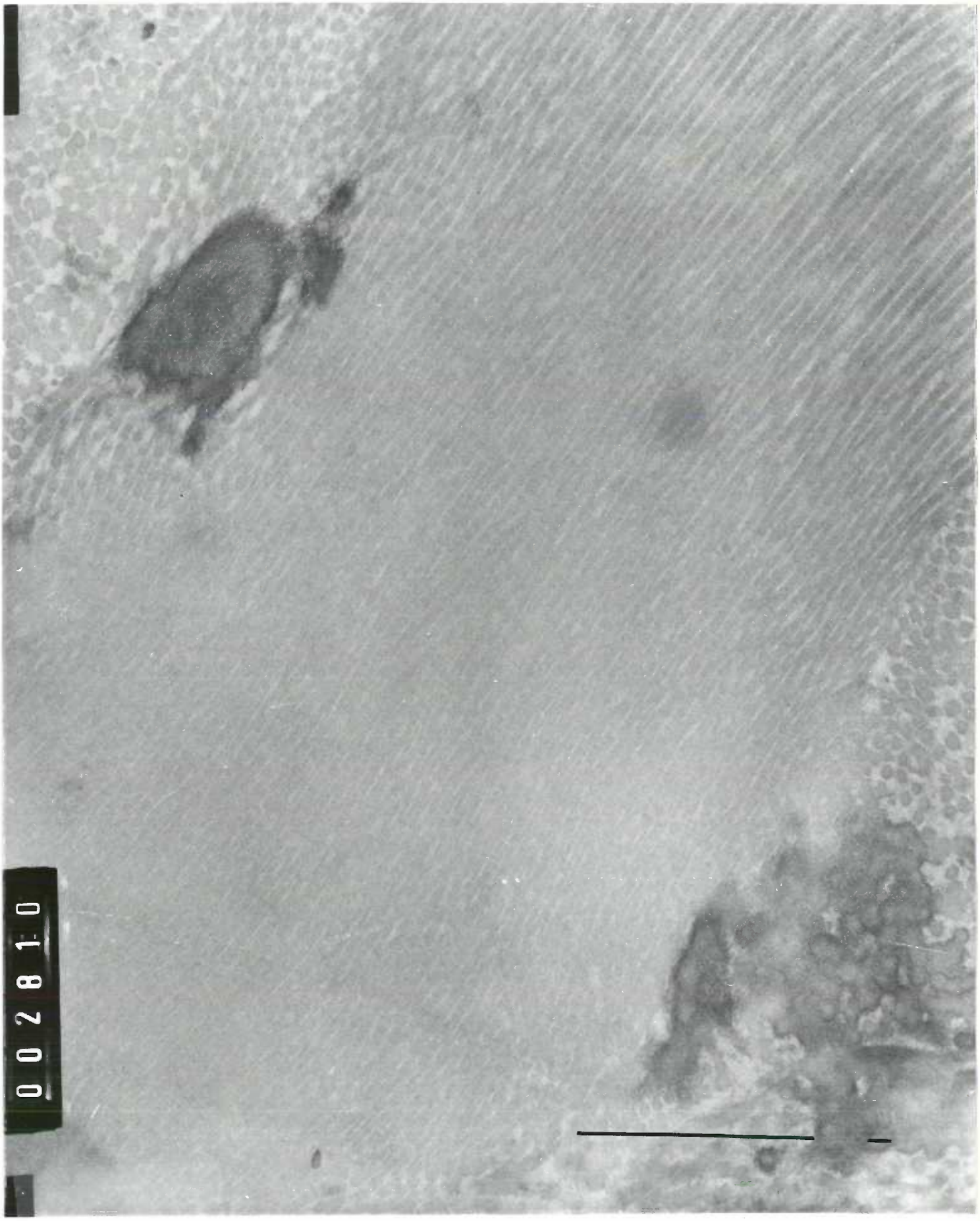

Fig 26. 4 day; 47,500X; Sharpey's fiber entering bone. Scale 1μ; 0.1μ.

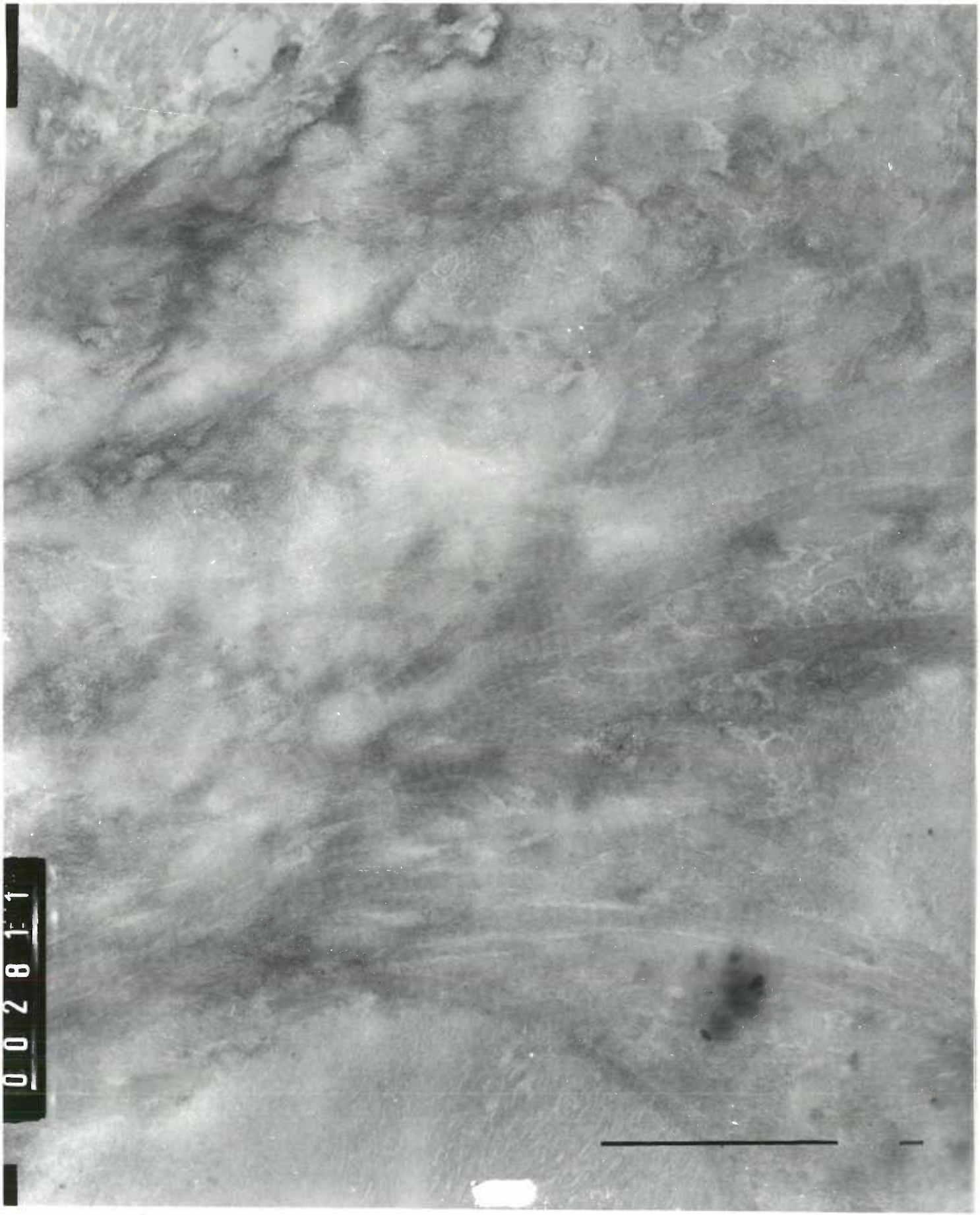

Fig 27. 4 day; 47,500X; Sharpey's fiber entering bone. Scale 1μ; 0.1μ.

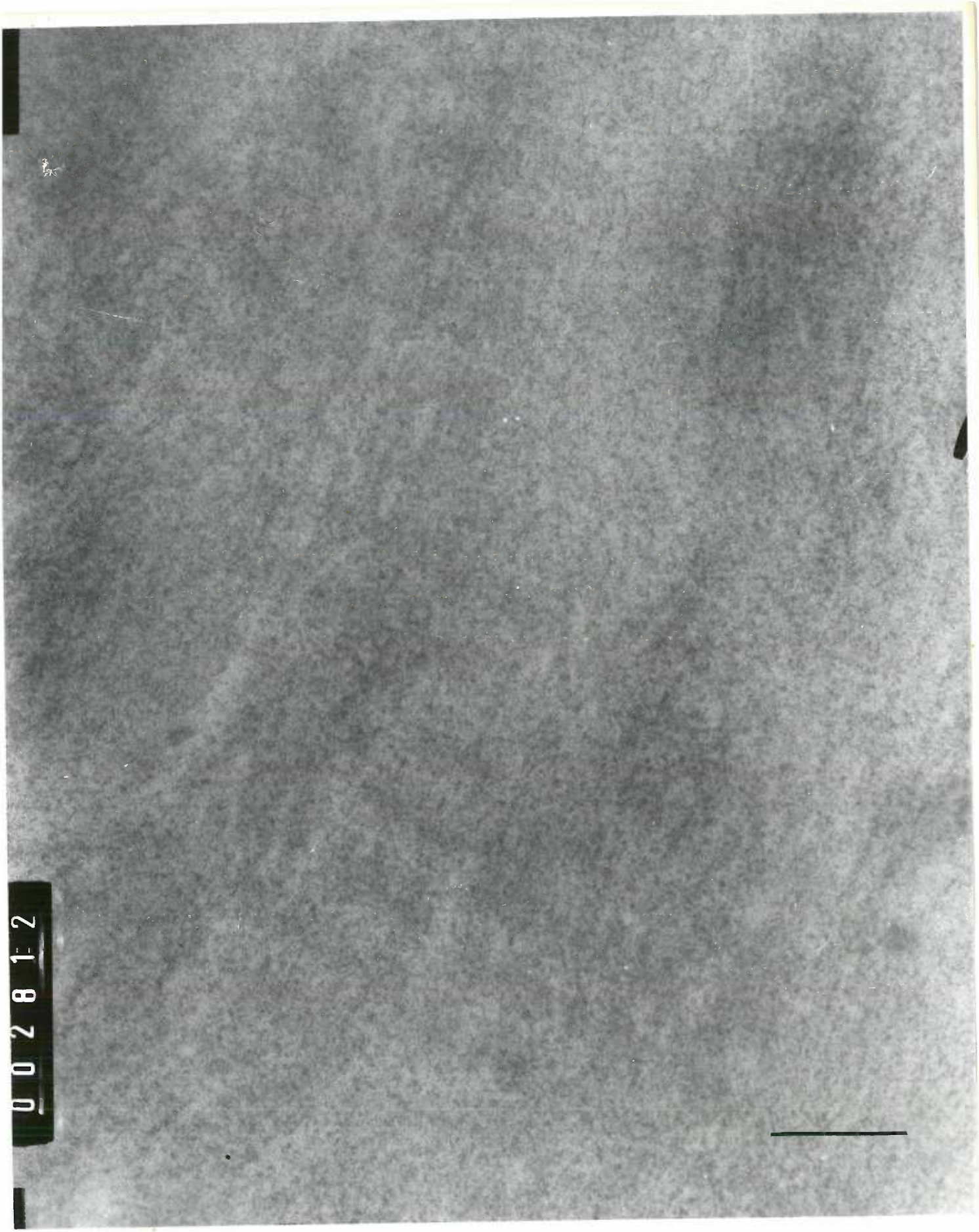

Fig 28. 4 day; 275,000X; Sharpey's fiber entering bone. Scale 0.1μ.

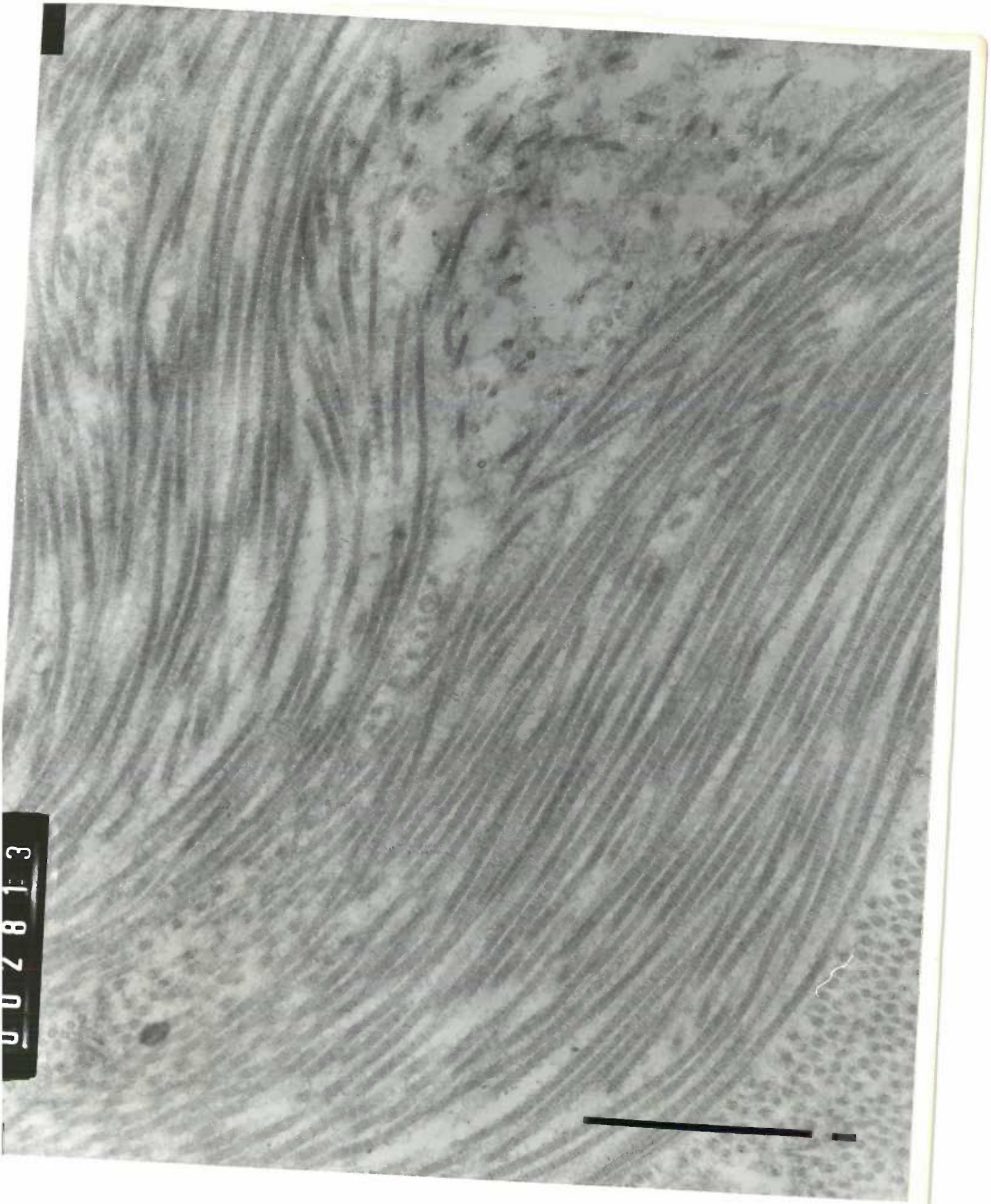

Fig 29. 4 day; 47,500X; dense collagen; periodicity evident. Scale 1μ; 0.1μ.

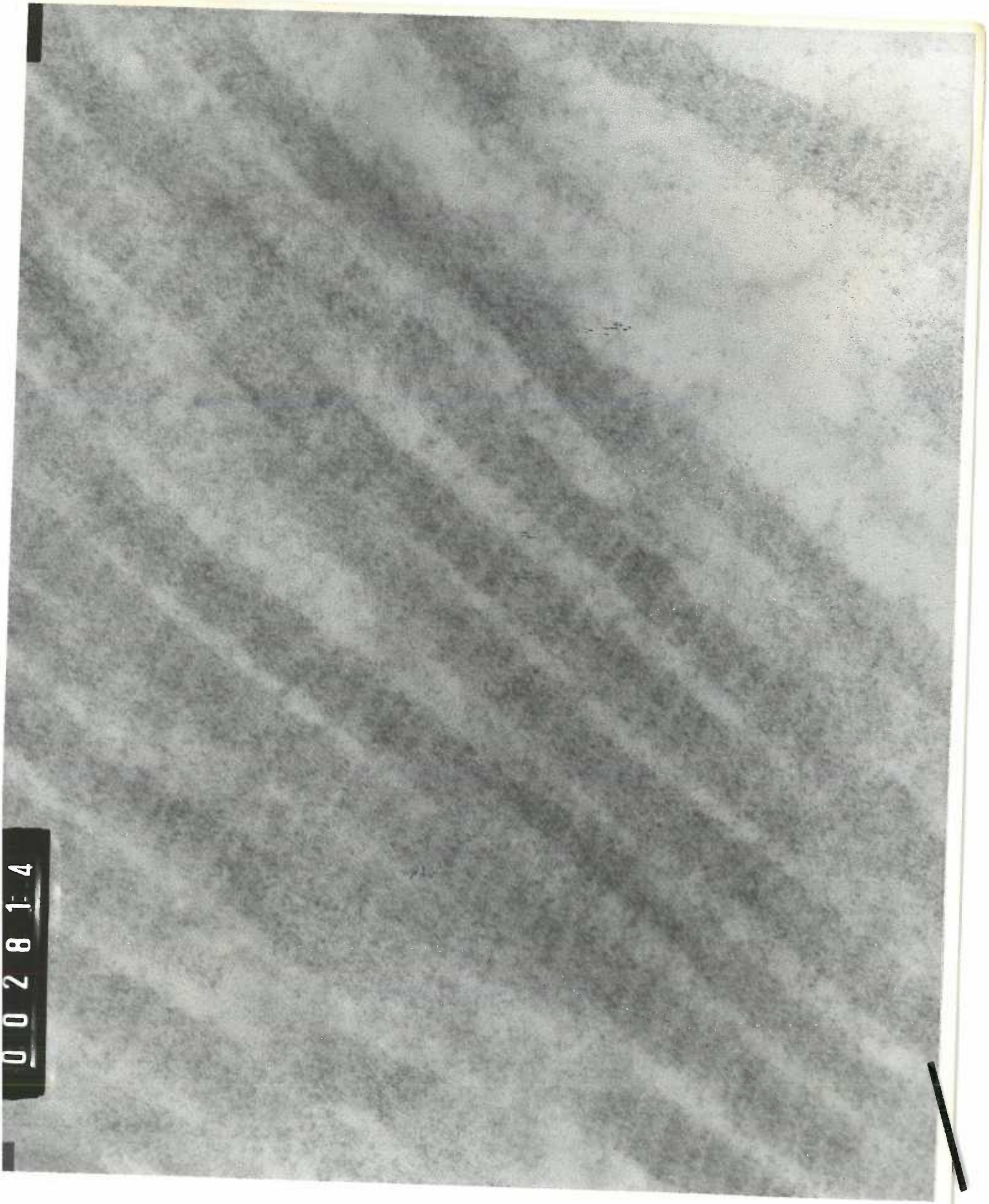

Fig 30. 4 day; 275,000X; high power of dense collagen, periodicity evident. Scale 0.1μ.

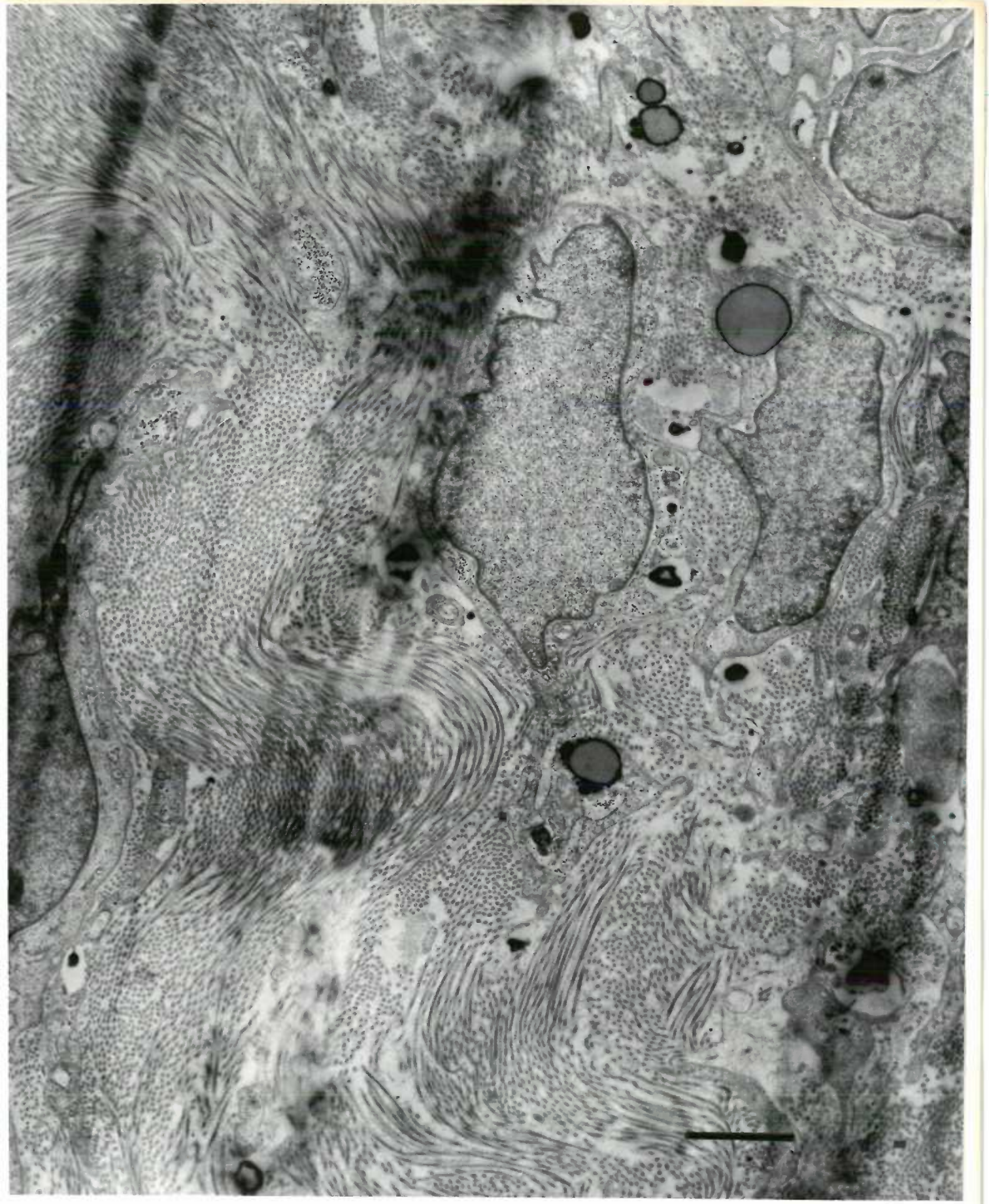

Fig 31. 10 day; 22,000X; fibroblasts with vacuoles and lipid droplets in the cytoplasm. Scale 1μ; 0.1μ.

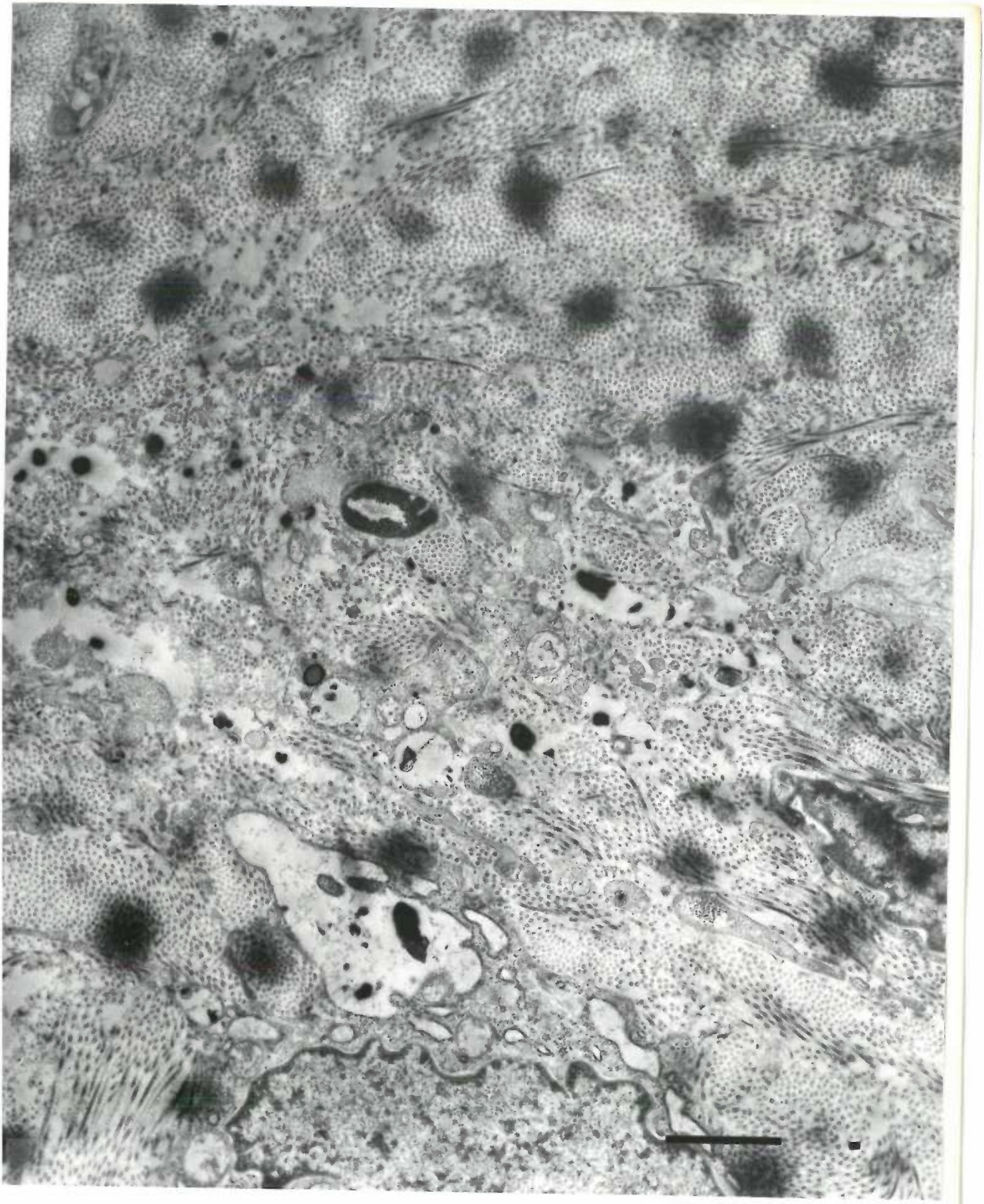

Fig 32. 10 day; 22,000X; cell necrosis, vacuolization, breakdown in cell membrane. Scale 1μ; 0.1μ.

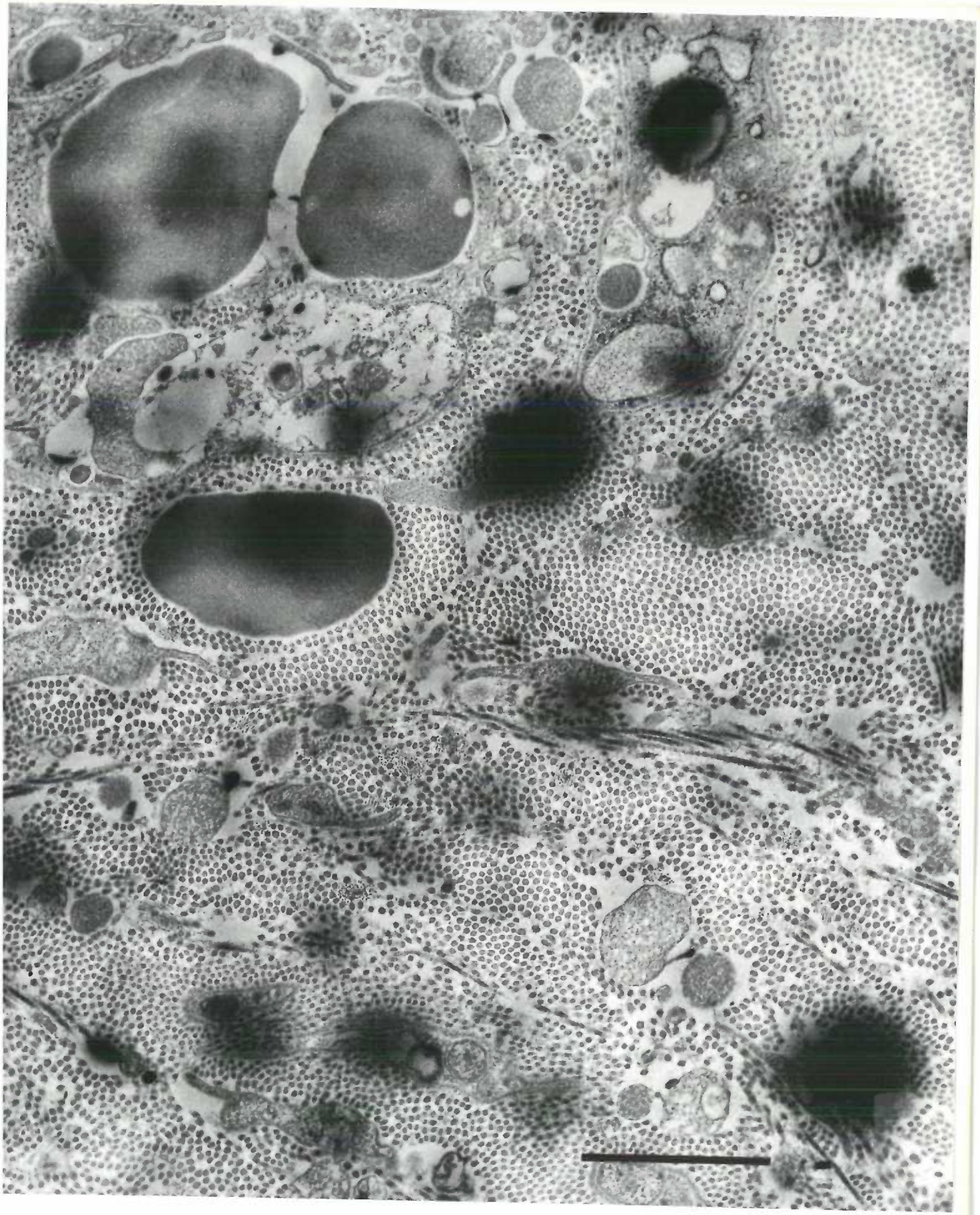

Fig 33. 10 day; 36,600X; cell necrosis, vacuolization, breakdown of cell membrane; red blood cells in advanced stages of degeneration. Scale 1μ; 0.1μ.

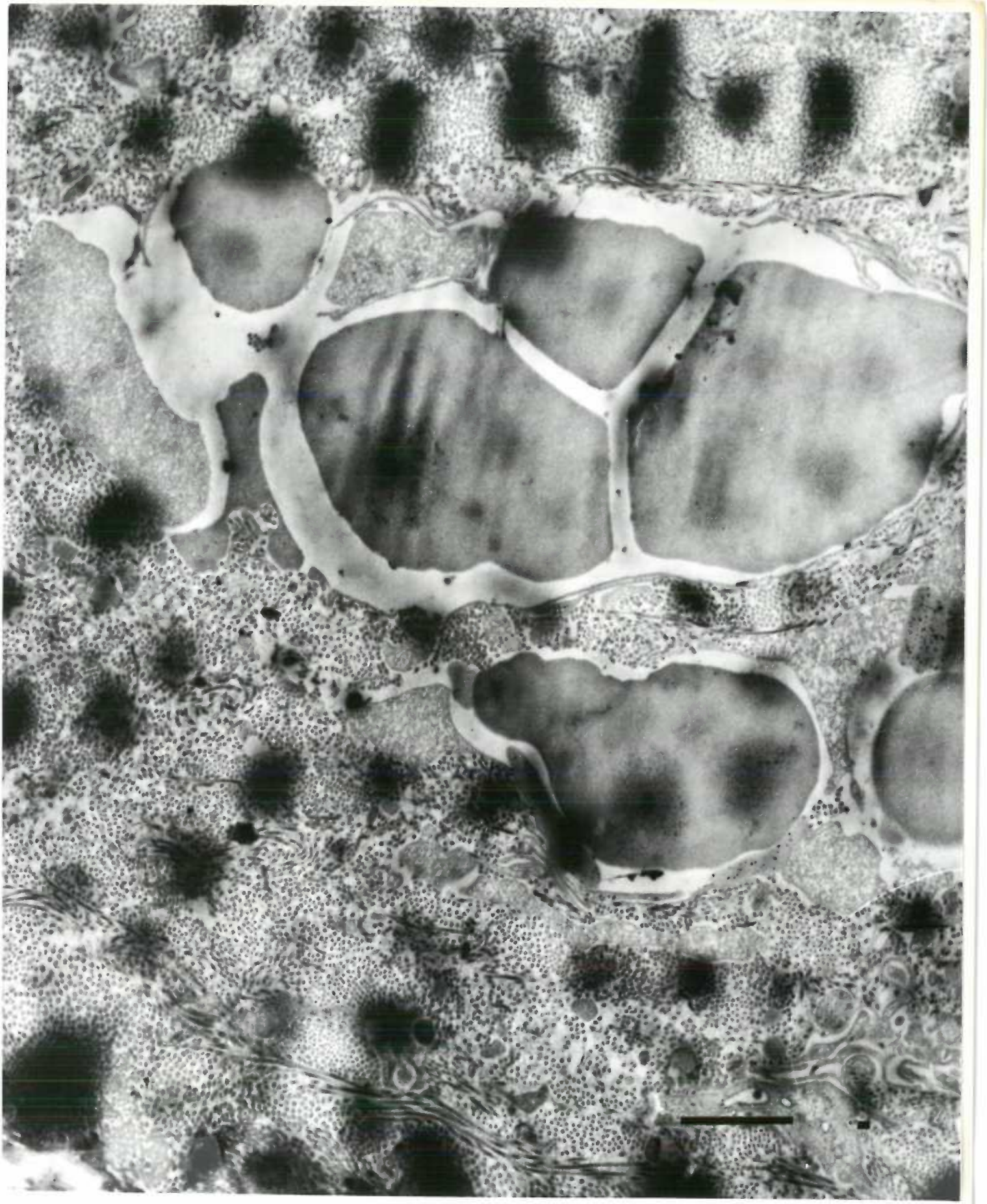

Fig 34. 10 day; 22,000X; venous stasis and advanced endothelial breakdown. Scale 1μ; 0.1μ.

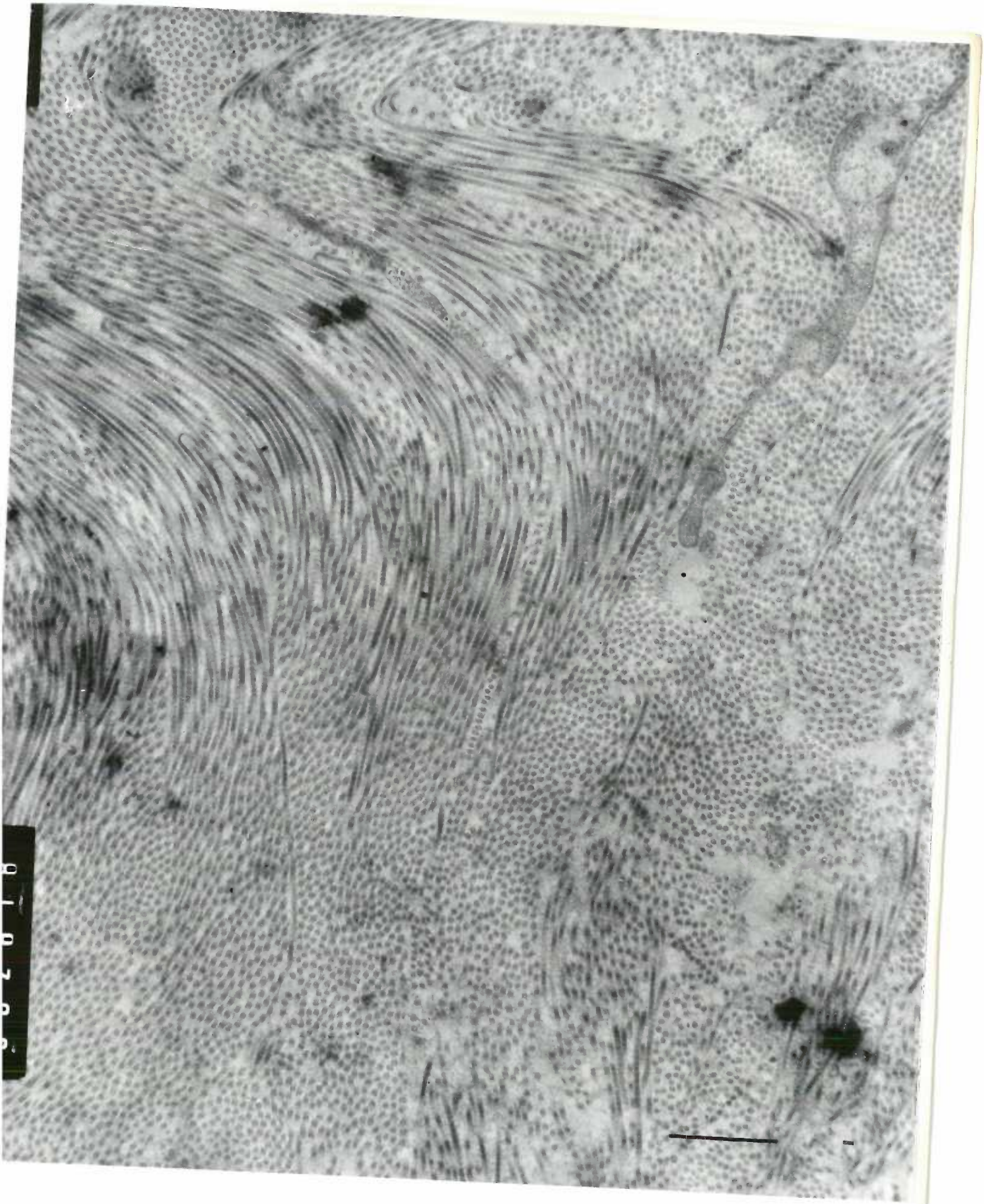

Fig 35. 10 day; 22,750X; Collagen cross-banding about 560Å. Scale 1μ; 0.1μ.

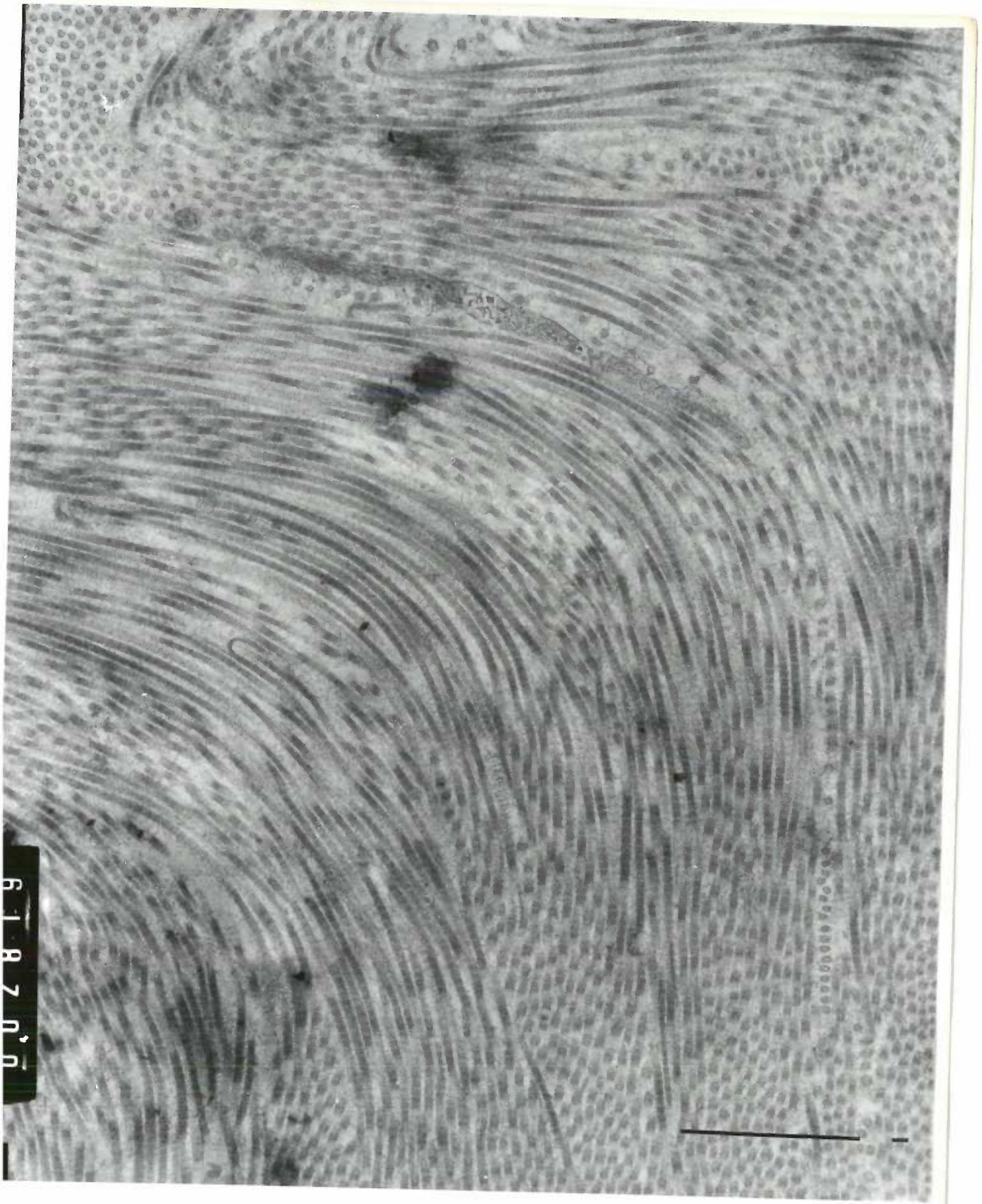

Fig 36. 10 day; 37,500X; collagen cross-banding about 530Å. Scale 1μ; 0.1μ.

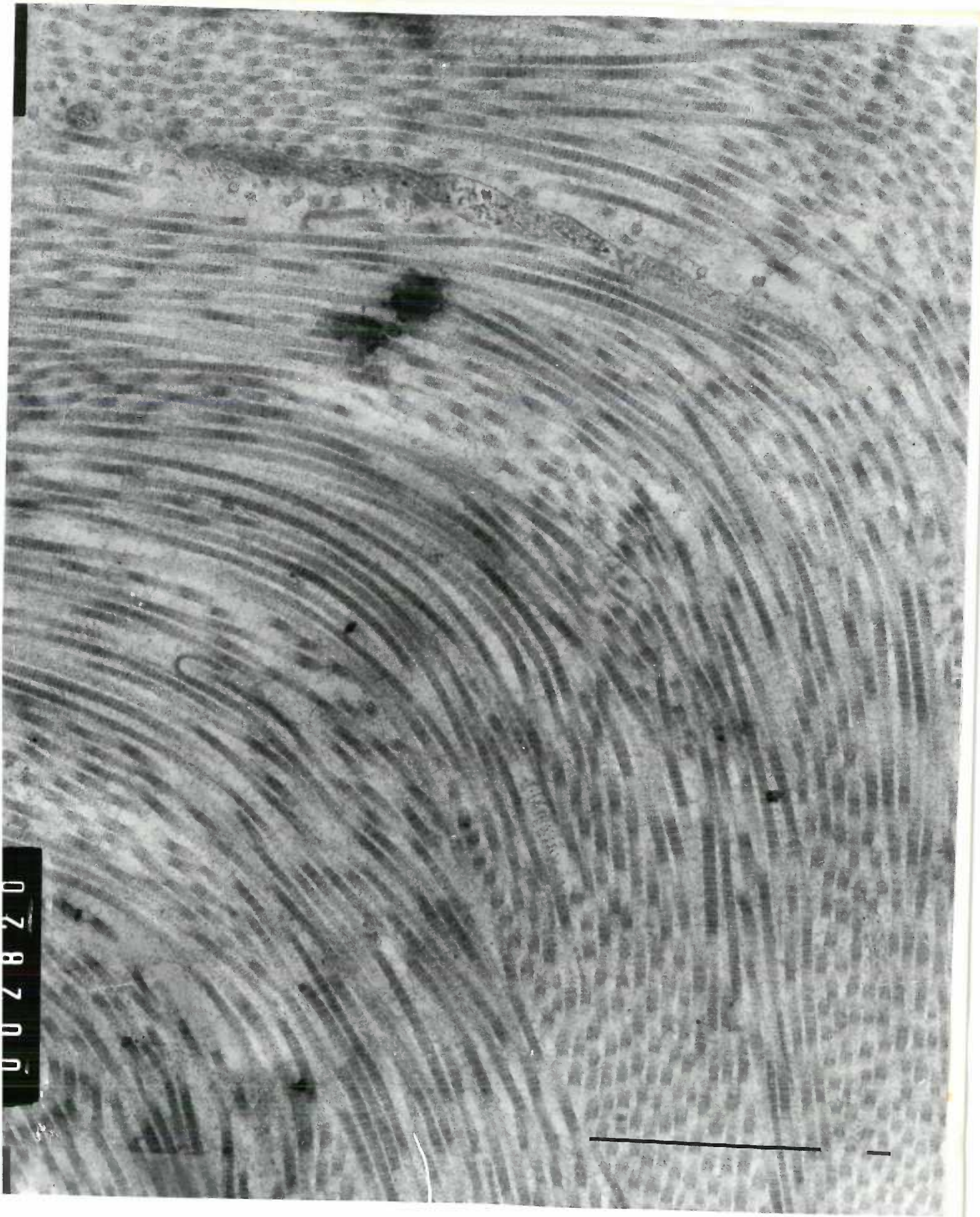

Fig 37. 10 day; 47,500X; close up of above area with collagen cross-banding measuring about 570 Å. Scale 1μ; 0.1μ.

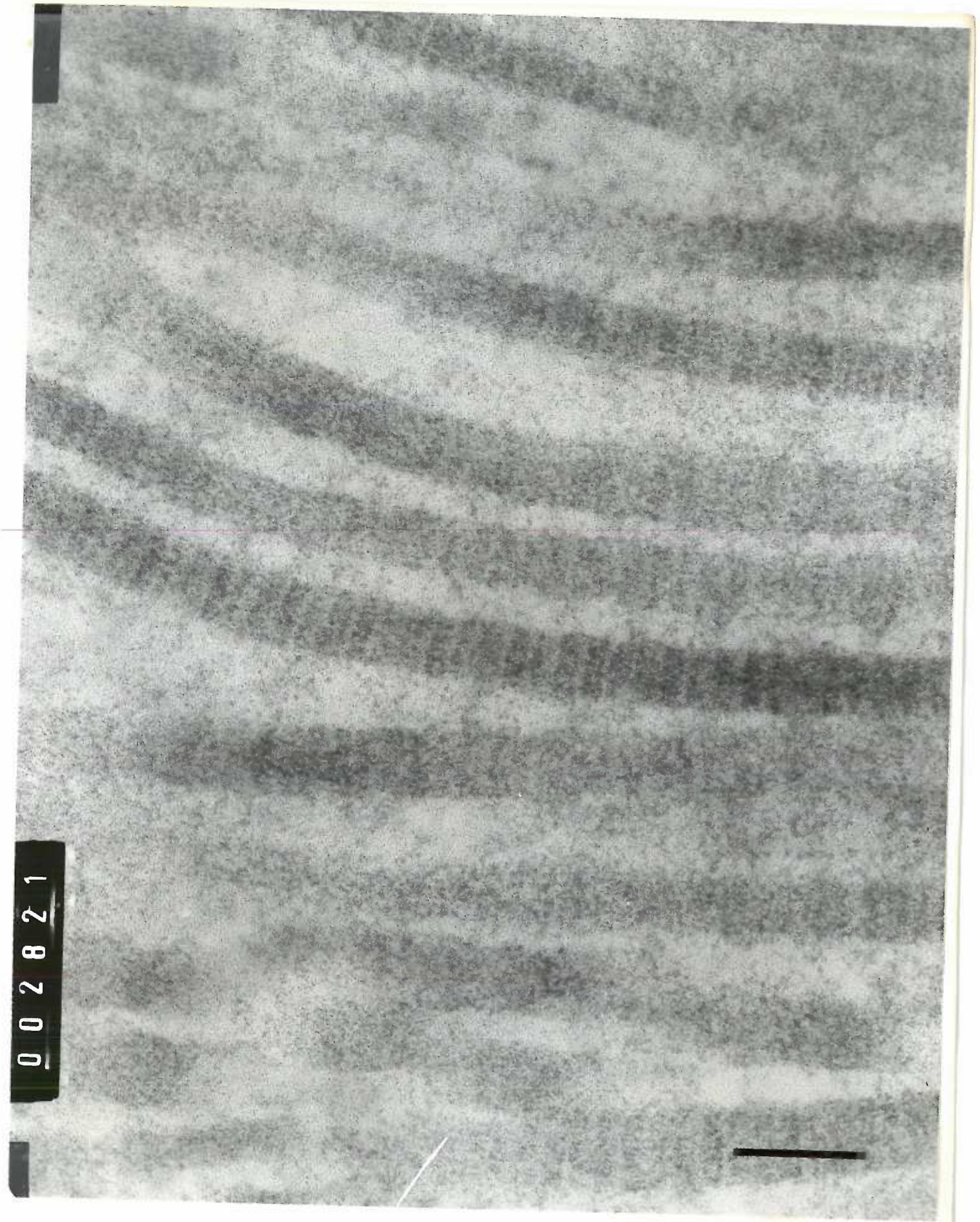

Fig 38. 10 day; 275,000X; collagen cross-banding about 580Å. Scale 0.1μ.

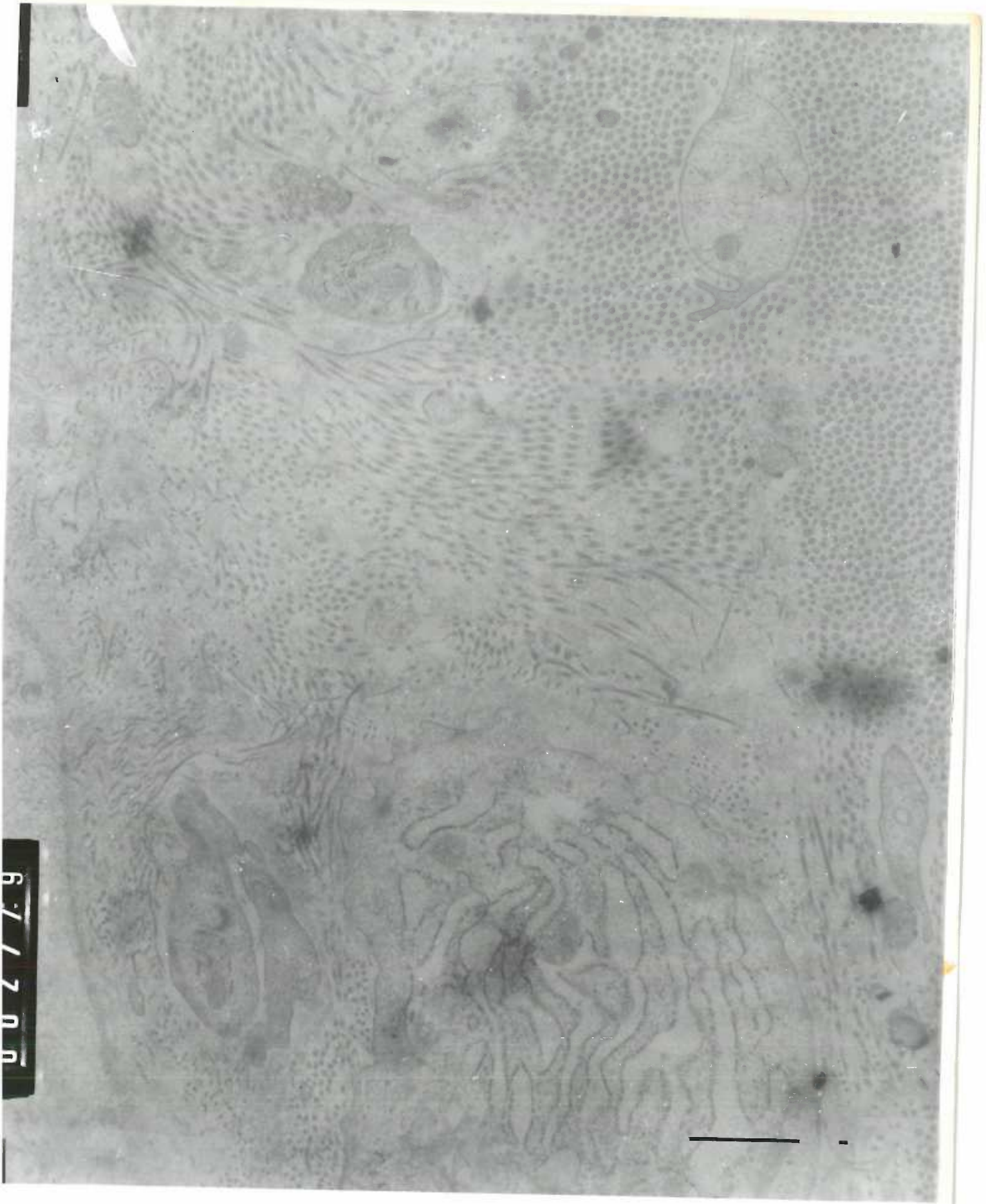

Fig 39. 10 day; 22,750X; active cell, rough endoplasmic reticulum; spacing evident; fibrils of unorganized collagen. Scale 1μ; 0.1μ.

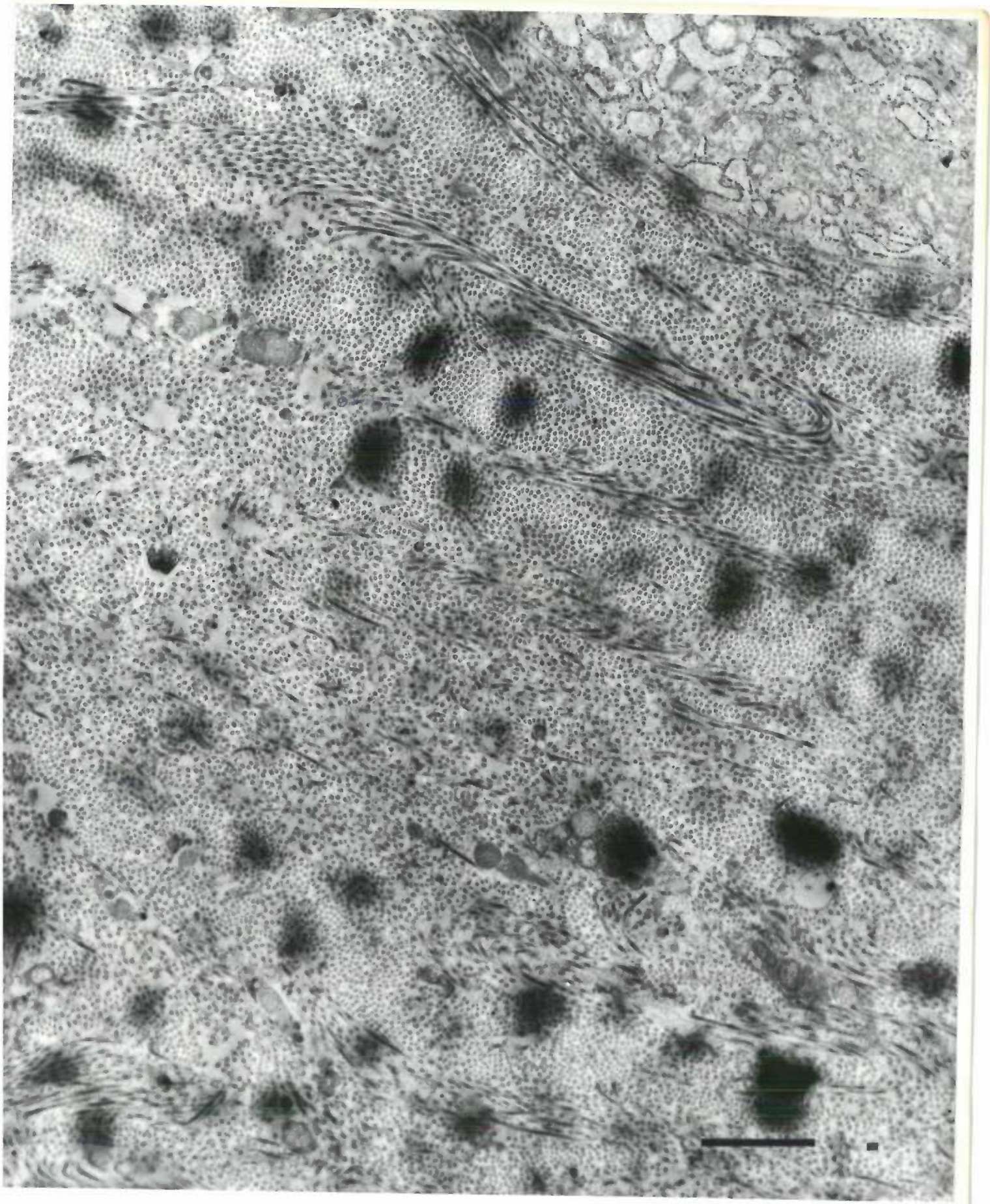

Fig 40. 10 day; 22,000X; punctate areas of spacing, some of which follow channels, apparently areas of previous cytoplasmic extensions. Scale 1μ; 0.1μ.

Fig 41. 31 day; 6,250X; highly degenerated cells in close proximity to viable cells and viable blood vascular channels. Scale 1μ.

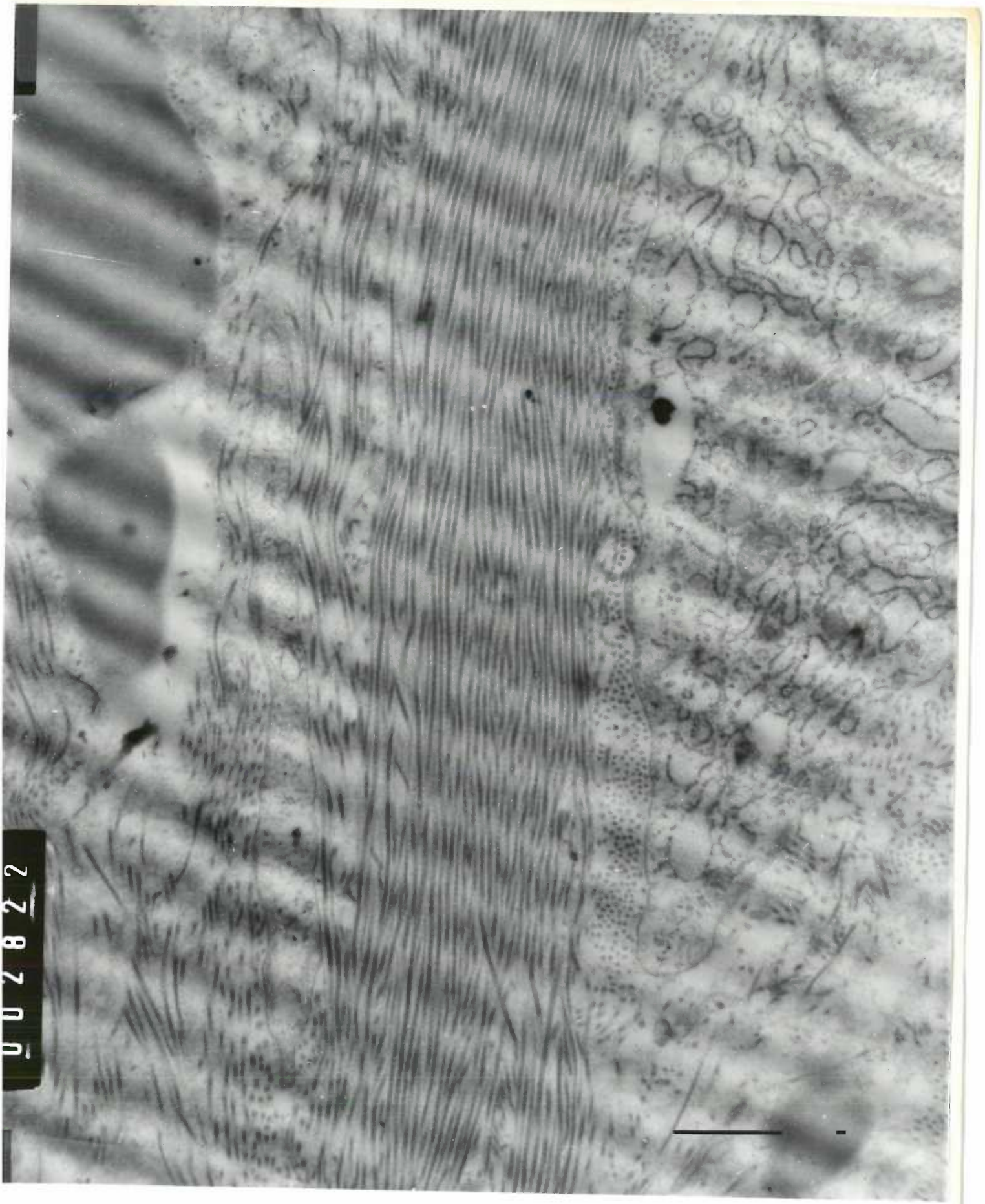

Fig 42. 31 day; 22,750X; active cell, rough endoplasmic reticulum, pinocytotic vessicles, strands of unorganized collagen. Scale 1μ; 0.1μ.

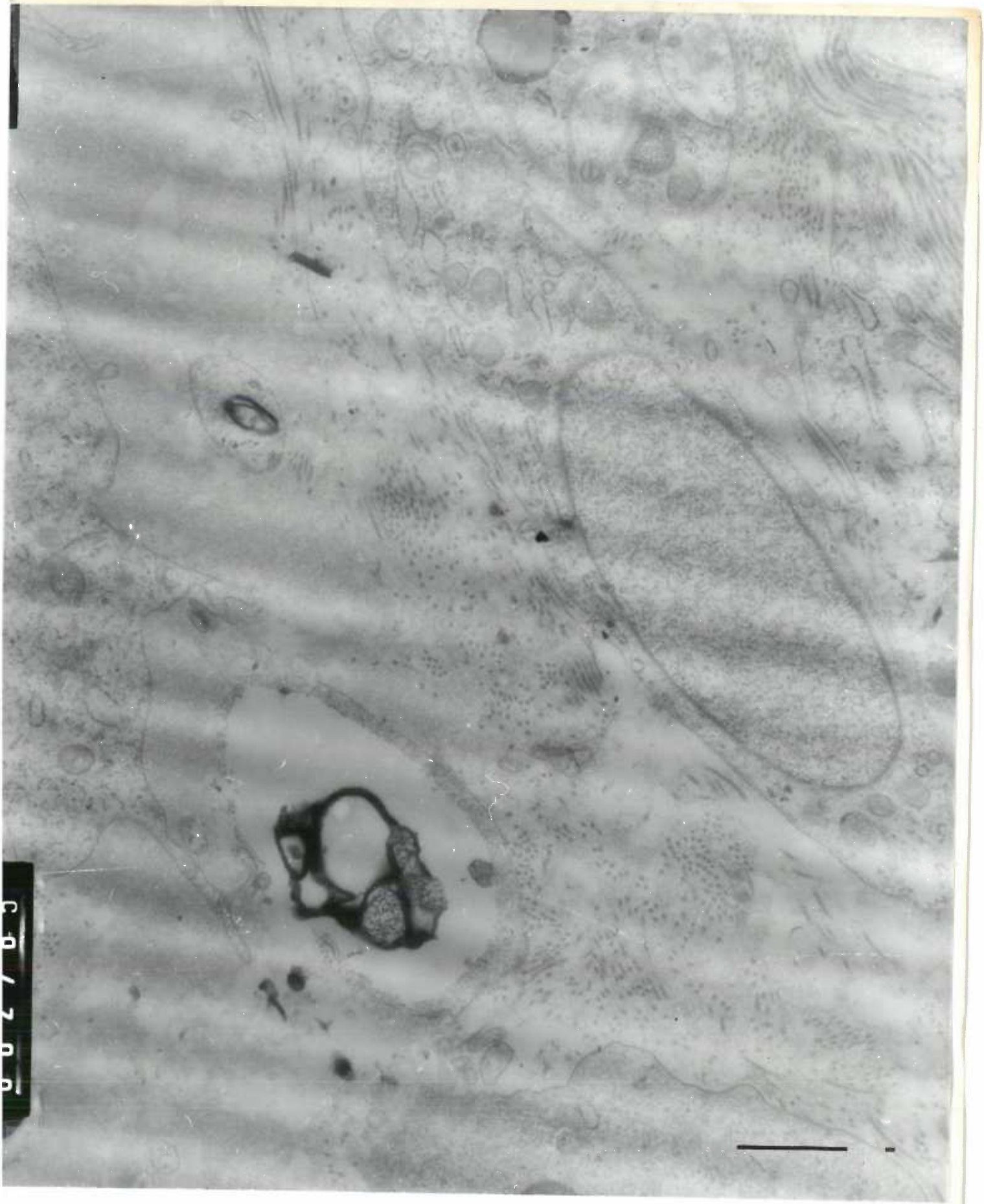

Fig 43. 31 days; 22,750X; highly degenerated cells in close proximity to healthy cells. Scale 1μ; 0.1μ.