HUMAN MAXILLARY AND MANDIBULAR BONE STUDIED BY CONTACT MICRORADIOGRAPHY AND HISTOLOGIC STAINING

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Presented to
Department of Orthodontics
University of Oregon Dental School

In partial fulfillment of requirement for a
certificate in Orthodontics

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INTRODUCTION, PURPOSE AND REVIEW OF LITERATURE

Contact microradiography utilizes soft X-Rays to provide an image of a thin section of tissue on an X-Ray film. The specimen should be organic material of low atomic number so that soft X-Rays of about 5 A wave-length can produce an image with high contrast on the film. The image on the film shows the relative penetrability of the structures in the specimen to X-Rays. Since soft X-Rays are easily absorbed , the window of the X-Ray tube must be very thin and have a low absorption coefficient. In this study a Phillips C.M.R.5 unit was used which utilizes a window of Beryllium.[1] This method provides a good picture of the microdensity of bone because hydroxy-apatite is relatively radiopaque.

In this method the photographic material determines the resolving power. Advances in film manufacture make magnification to about 500 diameters possible with resolution below 1 micron. Engstrom,[5] utilizing voltages of over 500v and Lippmann photographic plates, obtained a resolution of about .5 microns.

The microradiography techniques do not damage the ground sections and they may be further examined by another histologic technique after the X-Ray picture is taken. Thus it is possible to compare the microradiograph, which shows relative densities, with the same section, decalcified and stained. Cellular detail, however, is destroyed by preparation of the ground section.

The purpose of this study was to develop a method whereby the relative density and morphology of the bone of the dental area could be studied by means of microradiographic techniques and a comparison made to the same section, decalcified and stained with hematoxylin and eosin. Information

gained on the density of the bone around the tooth would be useful in determining whether the initial lag in tooth movement is due to the bone density of the lamina dura as well as other factors. There is a disagreement in the literature concerning the density of alveolar bone.[4,13,14] Information gained here would also be useful in demonstrating the relative calcification of cementing (resting) and reversal lines, a subject about which little is known.

The first attempt to reach the microscopic level with X-Rays was made only two years after their discovery in 1895. Heycock and Neville[6] attempted this by photographically enlarging the original negative. Contact microradiography has been used since 1913 to study almost all tissues of the body.[7] The dental literature shows many studies of enamel and dentin by this method. Considerable microradiography has been done on bone. Bhussry and Parikh[2] studied developing bone of the rat; Scott, Nylen, and Pugh[18] used the Phillips C.M.R. 5 machine on 50 micron sections of human compact bone to show the relative density. Their photographs show that there is considerable variation between the densities of the different osteones of compact bone. Radiopaque lines show in their pictures as concentric rings around canals and as lines separating osteones. Miller, Istock, and Lyon[15] studied humal fibula and also reported various degrees of density between osteones. They also observed that lines of increased density that appear toward the preiphery of the osteones may be associated with cementing lines. They did not show stained sections of the same areas. Miller and Lossee[16] who used soft X-Ray on 30 micron sections of human femoral bone stated that haversian systems differed between themselves and from the surrounding bone in density. Their pictures also show a complex system of haversian and Volkmans canals.

Molenaar[8] utilized bone sections embedded in methyl-methacrylate and described a technique for lapping such sections to a thickness as low as 10 microns. Bohr and Rollerup[9] examined samples of bone showing osteomalacia with microradiography followed with chemical analysis to correlate density with chemical content.

Cementing lines have been described in decalcified stained sections and in ground sections as the lines which separate two periods of bone development.[3,12] These lines show as basophylic (blue) lines in hematoxylin and eosin stained sections and as white or light lines in ground sections. Orban[17] makes a distinction between cementing or reversal lines in which the Howship's lacunae turn their convexity toward the old bone as darkly stained lines, and the lines left by a more or less continuous process of apposition which he calls resting lines. This determination is based on formation and resorption of bone and the patterns of the lines but not on staining reaction. Weinmann and Sicher[19] also make this comparison adding that the inactive surface of bone shows a peculiar staining reaction - becoming increasingly more basophylic with H & E - showing as a dark blue line, "aplastic line or limiting membrane". When new bone is laid down this line is incorporated as a resting or reversal line. No mention is made by these authors as to the relative calcification of these lines.

MATERIALS AND METHODS

Blocks of bone were dissected from the jaws of cadavers which had been maintained in formalin after gross dissection. The blocks were removed with a high speed air drive dental handpiece and a bone burr. 45 blocks about 4 mm square were removed from cortical and trabecular areas adjacent to teeth. These specimens of bone were defatted by 5 changes of acetone of 24 hours each change.

Ten of these specimens were then embedded in Wards Bioplastic as directed by the manufacturer and heat cured. These embedded blocks were then sectioned to 250 microns with a Motters rotary blade sectioner and then lapped down to about 90 microns with a Motters lapping machine. The Wards Bioplastic gave very poor penetration into the spaces in the trabecular bone and below 90 microns the pieces of cancellous bone tended to fall out of the section. The cortical pieces of bone also tended to fall out if extensively handled.

The remainder of the sections were embedded in acrylic after the method of Jee.[9] The sections were given 5 changes of acetone followed by 10 changes of anyhdrous diethyl ether. The blocks were then dried in a vacuum flask followed by 2 changes of washed uncatalyzed methacrylate without inhibitor. The specimens were then placed in small vials with the area to be parallel to the sectioned surface placed down. They were then covered with partially polymerized methacrylate to a depth of one inch. The vials are placed in the vacuum flask for 30 minutes, then cured under 50 Lbs. pressure at 44°C for 24 hours, followed by 24 hours at 60 C.

The glass vials were then broken off of the cured plastic and the diameter of the acrylicc trimmed with a dental model trimmer so that the diameter of the plastic cylinder was less than the diameter of the target

area of the Phillips Contact Microradiography 5. Brass rings the size of the vice on the sectioner were lined with vaseline, placed on a flat surface and filled with quick cure acrylic. The plastic cylinders containing the bone blocks were centered in the quick cure acrylic. This allowed the acrylic cylinders, which were otherwise too small, to be held firmly in the vice of the sectioner. The cylinders were then sectioned to 250 microns as before, and lapped to 100 microns.

Selected sections were then lapped to about 50 microns for use as specimens to be microradiographed directly. Ten of the specimens were glued to plastic coverslips in an attempt to lap them thinner than 50 microns. Below 50 the unsupported section would tend to warp and come apart. Elmers "glueall" and duco cement were tried as adhesive agents but both did not hold uner the wet conditions in the lapper. A thin layer of catalyzed acrylic cured between the specimen and coverslip did not hold either. A commercial plastic cement "Quick Seal" (Doughboy Industries, New Richmond, Wisconsin) usually used for repair of soft plastic articles without a patch, held very well. The sections were clamped to the coverslips with two flat pieces of wood while the cement set in order to get an even layer. With a coverslip as a backing the sections could be easily lapped to 25μ and one was lapped to 8μwithout loss. It was later found that the soft X-Rays would not penetrate the thick coverslip, so after lapping the section to the desired thickness it was turned over and the coverslip was lapped to a thickness of about 65μ which allowed the X-Rays to pass through.

The measurements of thickness were made with a Starrett micrometer.

The standard error of the measurement was 1.68μ . (Table 1)

TABLE 1

Section Number	Measure No. 1	Measure No. 2	d	d^2
1	66μ	66μ	0μ	0
2	222	218	4	16
3	35	34	1	1
4	348	348	0	0
5	362	358	4	16
6	298	300	2	4
7	93	92	1	1
8	350	350	0	0
9	417	414	3	9
10	435	436	1	1
11	370	368	2	4
12	274	270	4	16
				68

Standard error of the measure $\sqrt{\frac{\Sigma d^2}{2N}}$ $\sqrt{\frac{68}{24}}$ or 1.68μ

The second set of measurements was made without reference to the first, with a time lapse between so that the first measurements were not remembered. The specimens and the micrometer were wiped clean before each measurement.

After lapping the sections were smoothed with 3200 mesh grit pumice to remove the lapping lines. Not all of this pumice could be washed free without disturbing the specimen. This produced some small radiopaque artefacts in the microradiographs.

The Phillips C.M.R.-5 microradiography unit was used to take the microradiographs. (Figure 1) Kodak spectroscopic safety film, type 649-O, 16mm, was punched with a special punch to produce discs of film the size of the target area of the unit. The film is placed so that the emulsion faces the X-Ray tube and the section is placed in contact with it. This is covered with a brass ring with a hole in it the size of the exposure area. These are held in place with a rubber ring. (Figure 2)

The exposure were made at 5 kv. and 2 ma. with no vacuum evacuation. This approaches the maximum output of this machine which is 10 watts. The tube was cooled with an air stream as directed in the Phillips manual. The exposure time varied from 25 seconds to 5 minutes, depending upon the thickness of the section (and the backing, if used).

The film was then developed for 6 minutes at 68° F in Kodak D-19 developer, washed for 20 seconds in water, and fixed in Kodak rapid fixer, Solution A, to which hardener (Kodak soln B) had been added. The film was then dried and observed under a light microscope.

The specimens were then decalcified in a solution of formic acid and sodium formate overnight and then stained with hematoxylin and eosin without removal of the supporting plastic. The sections were then compared under the light microscope with the previously made microradiographs.

FINDINGS

The microradiographs of human jaw bones demonstrated classic morphological patterns as described in the literature. Well organized osteones of varying densities were found in the cortical specimens. Areas of laminated bone without haversian systems were also seen. Trabecules of cancellous bone were also well demonstrated.

The spaces of bone as classified by Frost[10] are apparent in these specimens. They are: osteocyte lacunae, canaliculi, haversian canals, Volkmans canals, primary longitudinal canals or blood vascular channels not associated with osteones, and marrow spaces.

Specimens after X-Ray and subsequent histologic preparation show characteristic morphologic patterns as described in the literature. The cellular detail is, however, destroyed in the preparation of the ground section. Numerous basophylic lines which are characteristically described by histologists as cementing lines are evident.

A unique finding concerns the calcification of the cementing lines. The cementing lines that were observed as basophylic on the H and E sections appeared as radiopaque lines on the microradiograph of the same specimen. (Fig. 3, 4, 6)

An empirical finding was the impression that most of the cancellous bone specimens appeared more radiopaque per unit mass of bone that did the cortical specimens. This would imply that for unit mass of bone that cancellous bone is more, rather than less, mineralized than cortical bone in the sections studied. The cortical bone seemed to show considerable variations of radiopacity, between osteones, and between osteones and surrounding bone, but the trabecules of cancellous bone all seemed quite radiopague.

The captions of figures 3 through 10 describe the location of the

specimen and unique histologic features. The approximate magnification of figure 9 is 200X, and of the other figures is 50X. It should be remembered that each figure shows an X-Ray and an H and E section of approximately the same field of the same specimen.

DISCUSSION

Sections used for histologic or microradiographic study should be thin to avoid superimposition of the various structures. Thin sections of undecalcified bone, about 10 microns thick, are possible with special microtomes. These microtomes, however, produce compression artefacts. Ground sections can be made but it is difficult to make them as thin as microtome sections because of the handling involved. It is necessary to imbed the bone in some material or hold the parts together at the thickness desired. In this study methacrylate was used which works very well for making the ground sections. The H and E staining and the decalcification were done without removing the plastic, a technique which allows good staining but produces artefacts under the light microscope. Removal of the plastic before placing the coverslip with some solvent such as acetone would probably reduce this.

The thickness of the sections is easily controlled with a micrometer and the accuracy is quite good. The measurement becomes less accurate if the section is of uneven thickness, or if wedging is allowed to occur. The lapping machine produces very flat sections with very little wedging and is in this way preferable to hand grinding which usually produces some wedging. Microradiographs of thicker sections (50μ to 100μ) show more contrast between areas of varying density but they also produce too much superimposition of structures. Thicknesses of 25μ to 50μ show good contrast with little superimposition. Some canals that are present in part show as radiolucencies on the microradiographs but do not show on the H and E sections due to the thickness. The one section presented here that was lapped to 8μ (Figure 10) shows very little except lacunae on the X-Ray. The H and E section shows very little superimposition of

structures, but this section does not make good material for a density study.

Quantitation of the density by means of microradiography requires that a step wedge or some other method be used. A step wedge has levels of known density which produce an image on the film to which the sample can be compared. This is very difficult on the C.M.R.-5 unit without special adapters to make the field large enough to include the wedge and a specimen. So, though it would be useful to know the relative density of bone next to the periodontal ligament, and the amount removed by stress, this would not be easily studied in the 3mm field of the C.M.R.5.

If one desires to take microradiographs with very soft X-Rays (below 3 KV), the specimen chamber should be attached to a vacuum pump and as much air as possible evacuated to avoid scatter of the X-Rays by the air in the chamber.

The density might be measured with a micro-photo scanner which would measure the amount of light that would pass through a small area of the film. This would give quantitative evidence which would help confirm the emperical impression that osteones differ in density and that cortical bone is less dense than trabeculated bone (not measuring the spaces). Photo scanning of the whole film is not possible due to the large amount of spaces present in the bone.

Cementing lines are described in most histology books but only Weinmann and Sicher[19] provide information as to their possible makeup. Miller, et al,[15] mentions that they might be the radiopaque lines seen in his microradiographs. No comparison of cementing lines as described by histologists in stained sections has been made to microradiographs of the same section to our knowledge. It would appear from our sections that some of the cementing lines are indeed more radiopaque to X-Ray. We

attribute this to a higher degree of mineralization than the surrounding bone.

SUMMARY

1. Specimens of human bone were removed from cadavers and studied by means of microradiography and H and E staining. The same fields on the same sections were then compared.
2. A method for making thin ground sections for use in microradiography and a method for producing microradiographs and H and E stain of the same section is described.
3. Cortical bone shows considerable variation in degree of radiopacity between osteones and between osteones and the surrounding bone. Empirically, the spicules of trabeculated bone appear to be more radiopaque per unit mass of bone than does cortical bone. Trabeculated bone, then, may contain more mineral salts per unit mass of bone than cortical bone.
4. Resting or cementing lines, seen as basophylic lines on H and E stained sections of bone, show as radiopaque lines in microradiographs. From this it is concluded that they are more highly mineralized than the surrounding bone.

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Fig. 1. The Phillips C. M. R. 5 unit showing the control panel. The extension to the right contains the camera and the X-ray tube.

Fig. 2. The camera of the Phillips C. M. R. 5 unit showing the target area, the brass ring which covers the film, and the rubber ring which holds the brass ring in place. The hole in the brass ring is the size of the X-ray field.

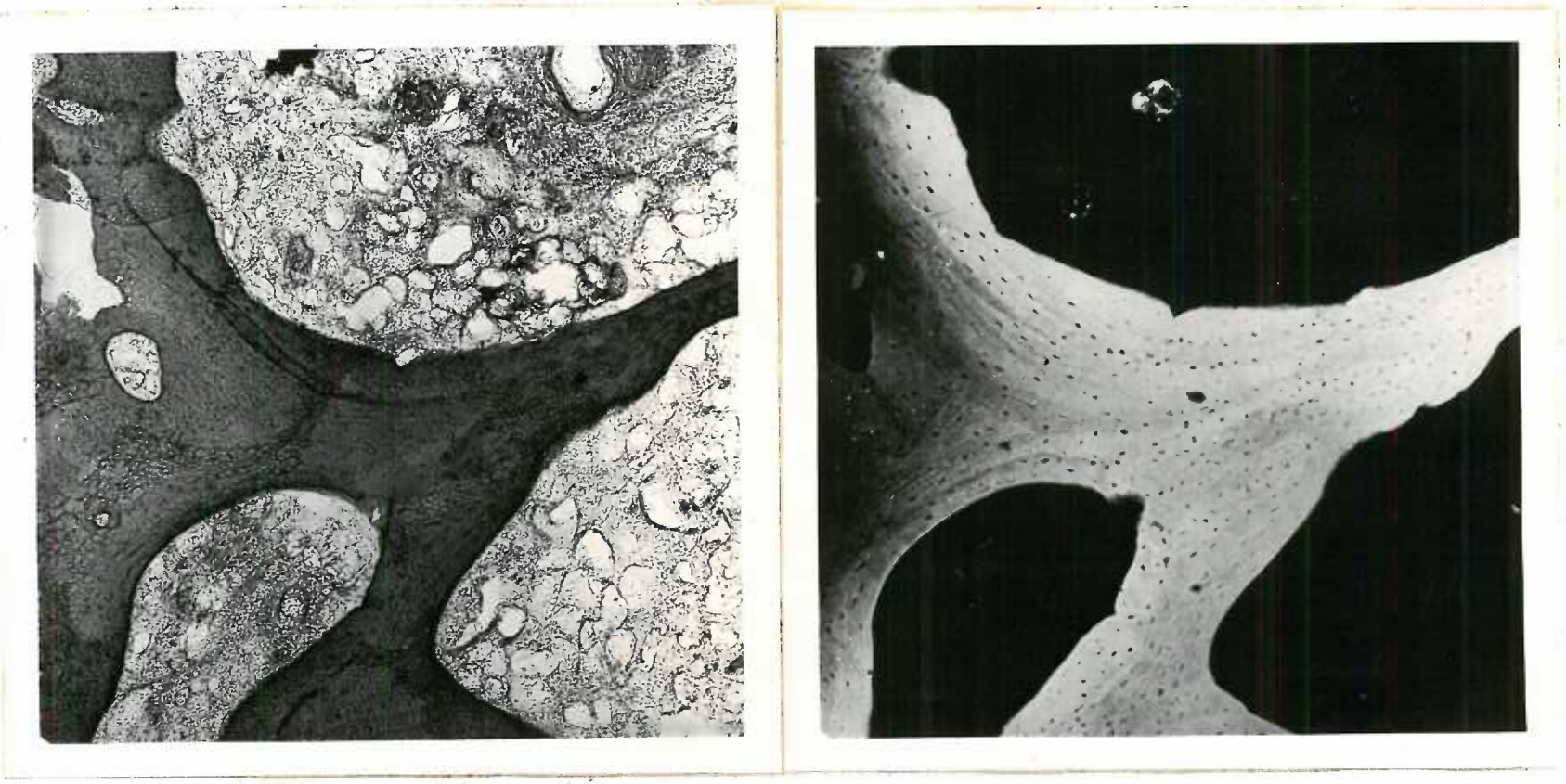

Fig. 3. H and E stained section on the left shows a darkly stained line in the middle and upper left middle of the field. This same line shows as a white line in the microradiograph on the right, indicating that it is more densely calcified. 45 μ section with no backing, 25 seconds exposure.

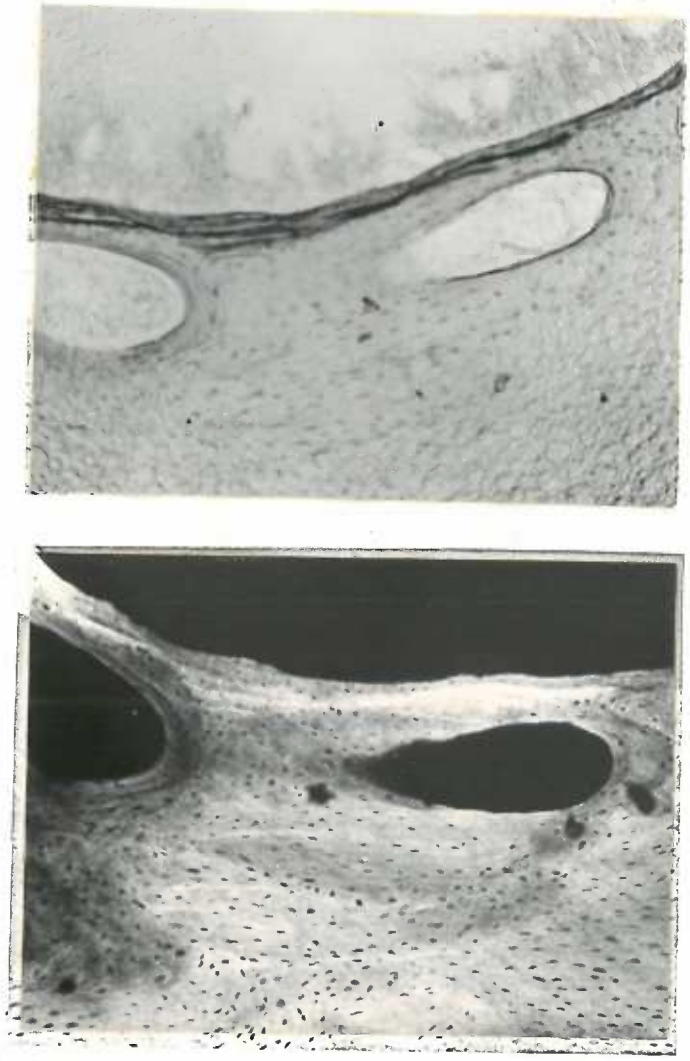

Fig. 4. H and E stained section (top) show darkly stained resting lines next to the socket of a tooth. The microradiograph (below) shows the area of this line to be quite radiopaque. 55μ section with no backing, 90 seconds exposure at 25 ma and 5 kv.

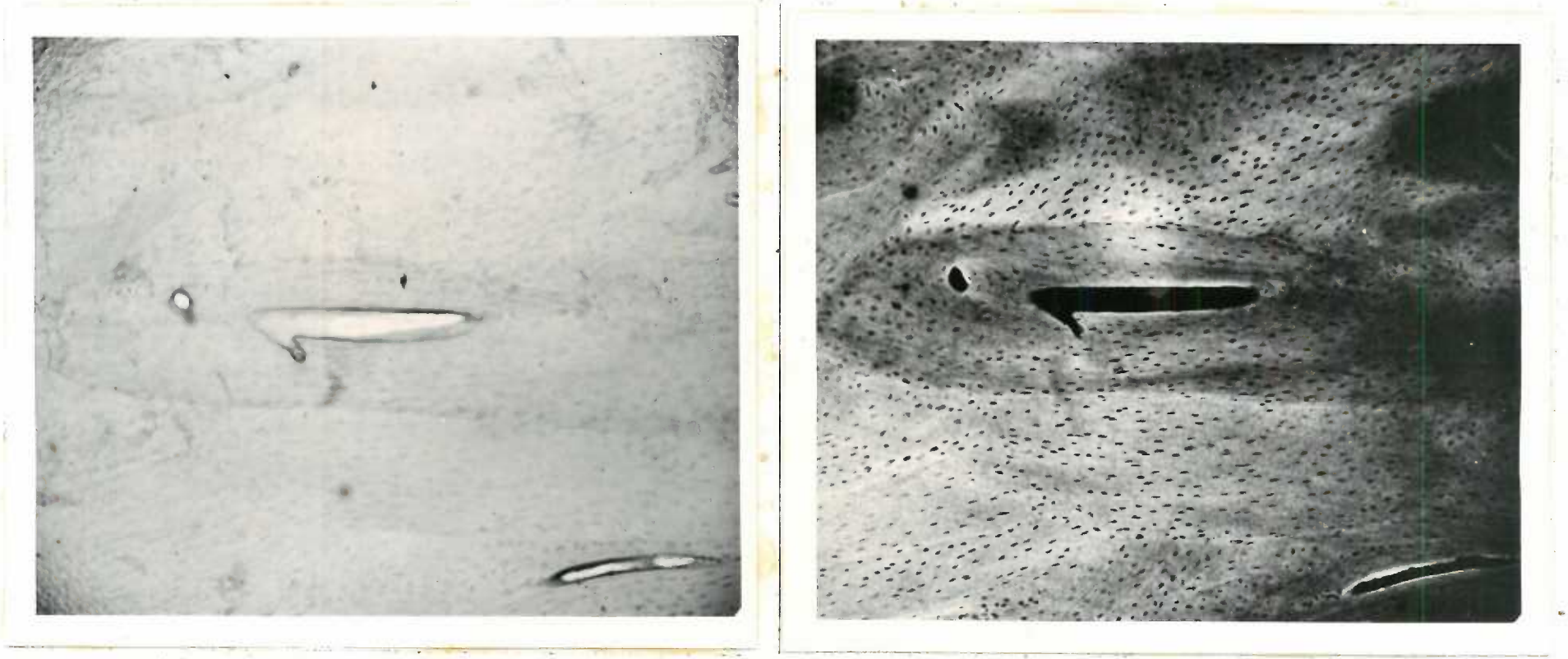

Fig. 5. These pictures show an osteone which has been cut diagonally. The H and E section (left) shows some difference in staining in the bone around the canal. The microradiograph shows that the osteone is less dense than the surrounding bone. 55 μ section, no backing, 90 seconds exposure.

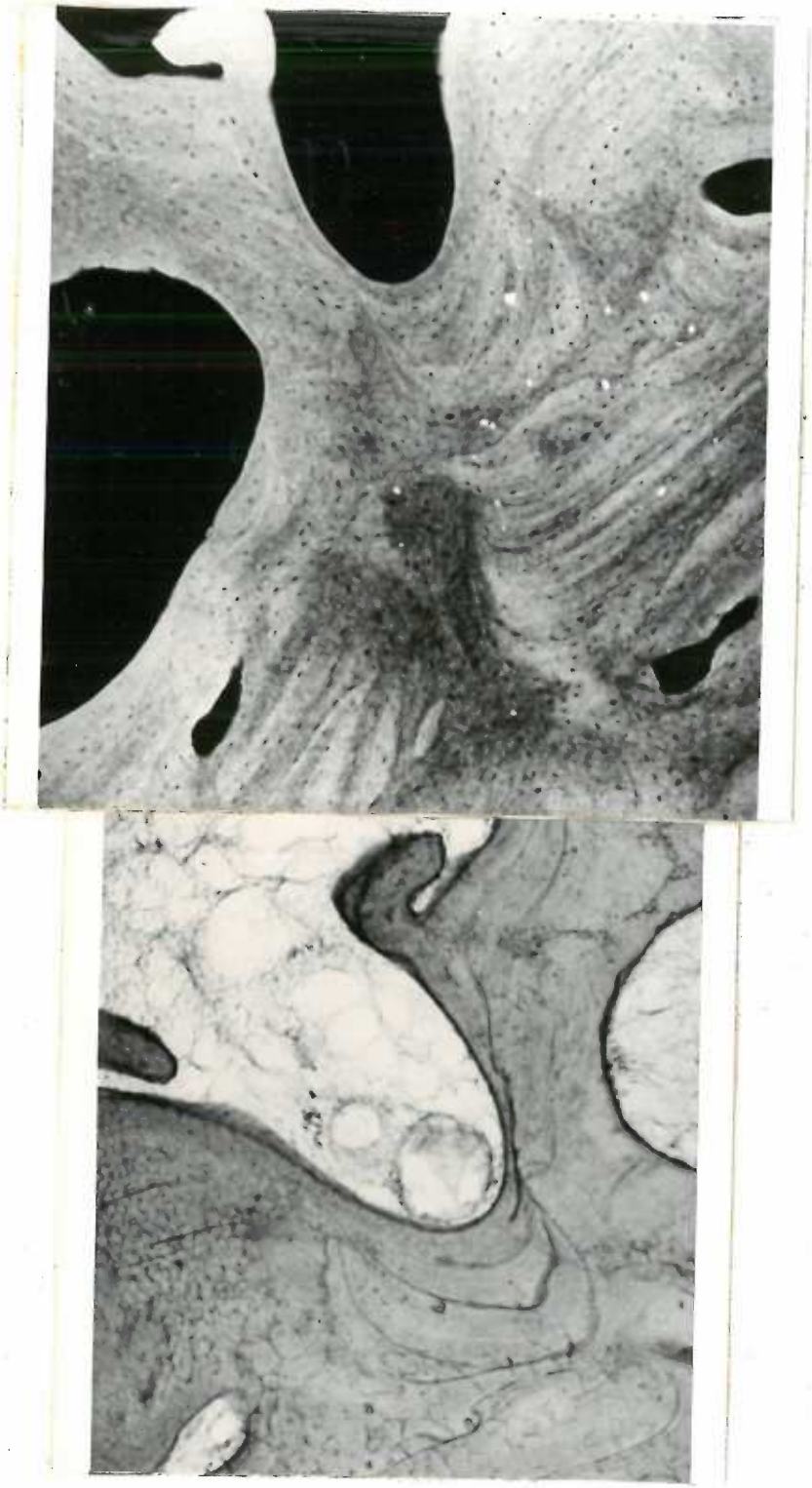

Fig. 6. The microdadiograph (top) shows an area of cortical bone next to an area of trabeculated bone. The cortical bone (lower left of the field) shows considerable variation in density. The trabeculated area next to the marrow spaces is much more uniform. The H and E stained section is below. 45μ section, no backing, 25 seconds exposure.

Fig. 7. Microradiograph on the left shows the variable density of cortical bone, only a few small resting lines are seen on the H and E. 25 μ section, no backing, 25 seconds exposure.

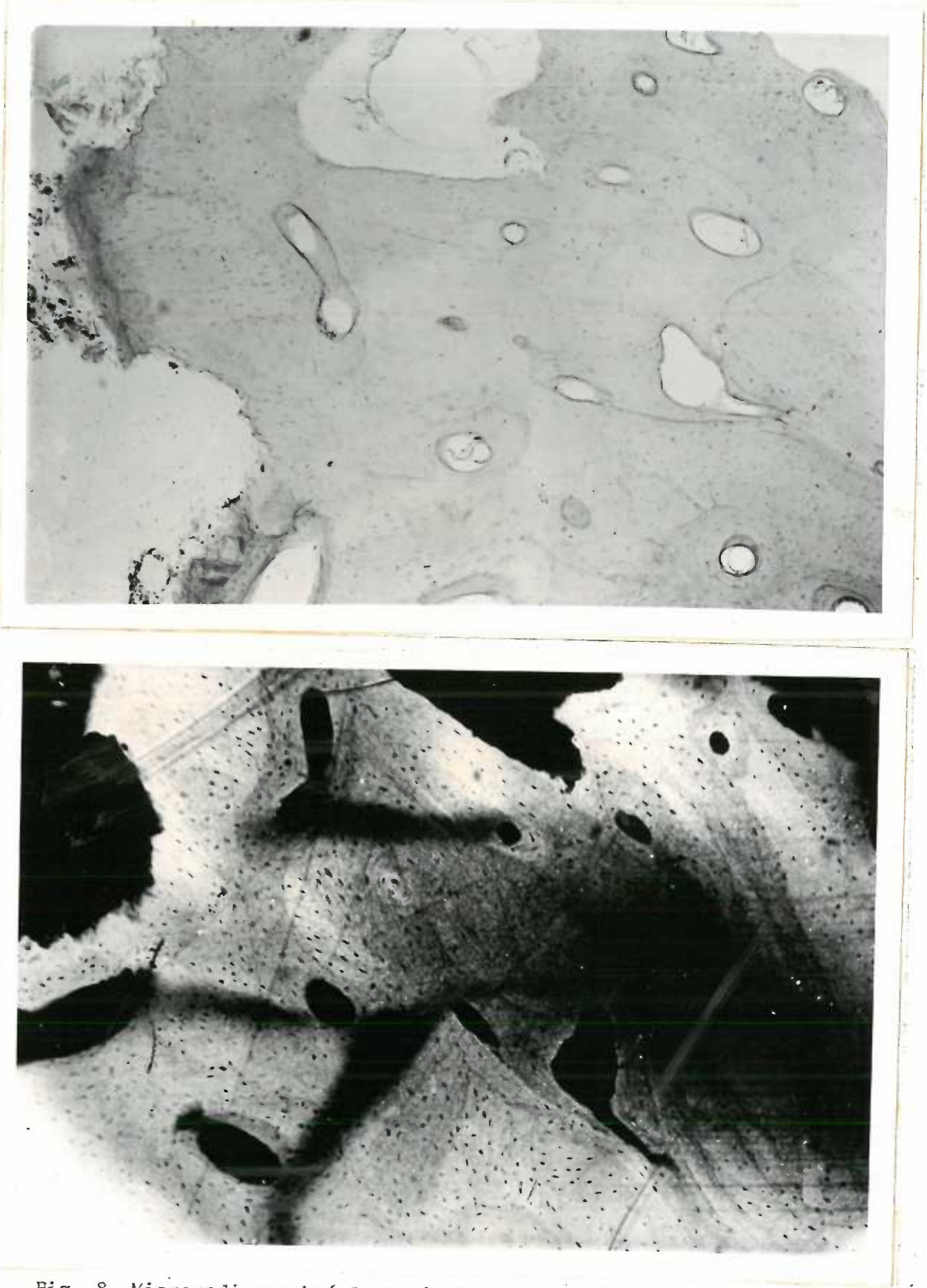

Fig. 8. Microradiograph (lower) shows variation in density due to grinding (no backing used) and side canals which the H and E section does not show. 25μ section, 20 seconds exposure.

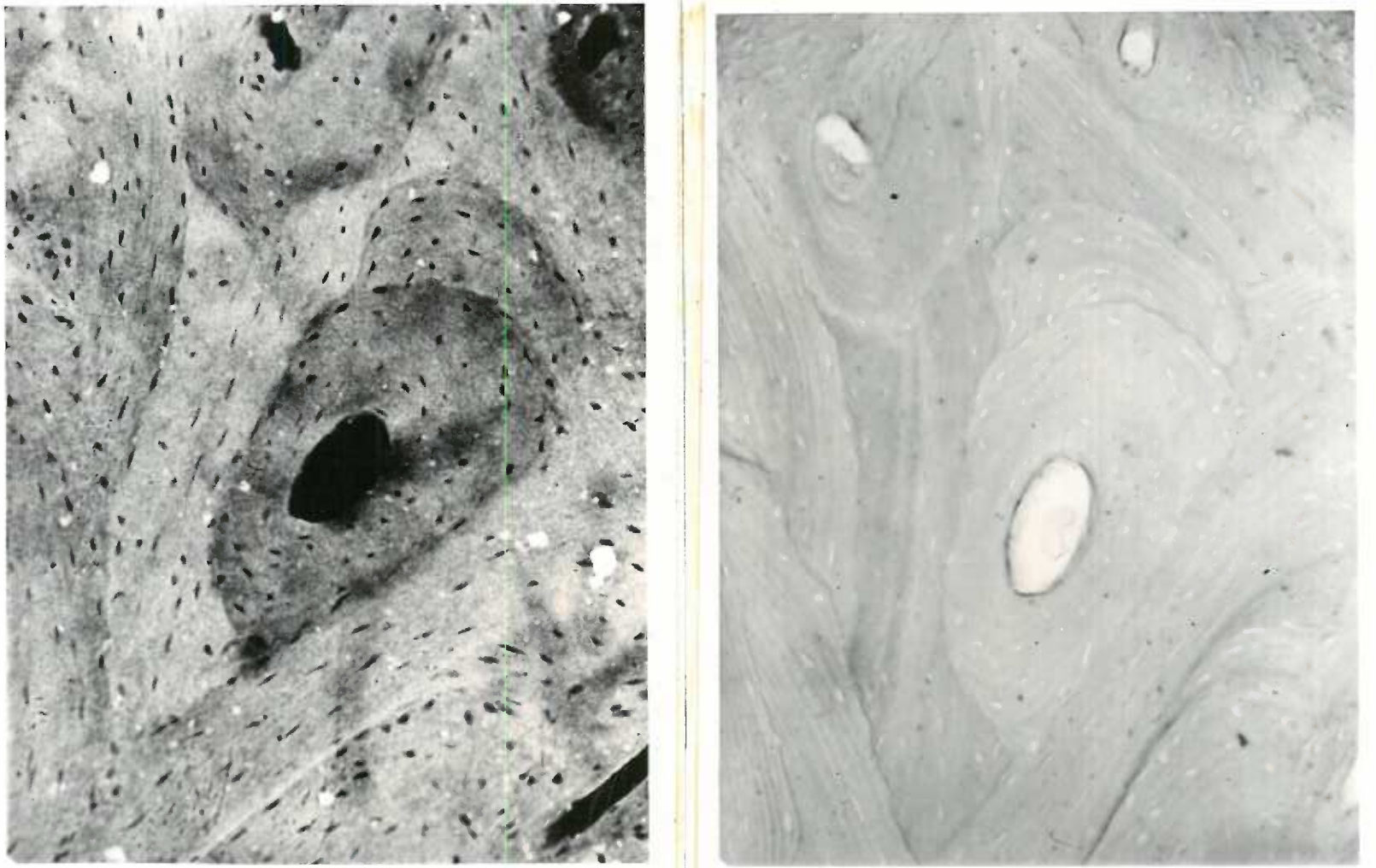

Fig. 9. Higher magnification picture of an osteone. The H and E stained section (left) shows variation in staining which shows the architecture of the bone very nicely. The microradiograph (right) shows the variations in density. 25 μ section, 45 μ backing, 5 minutes exposure.

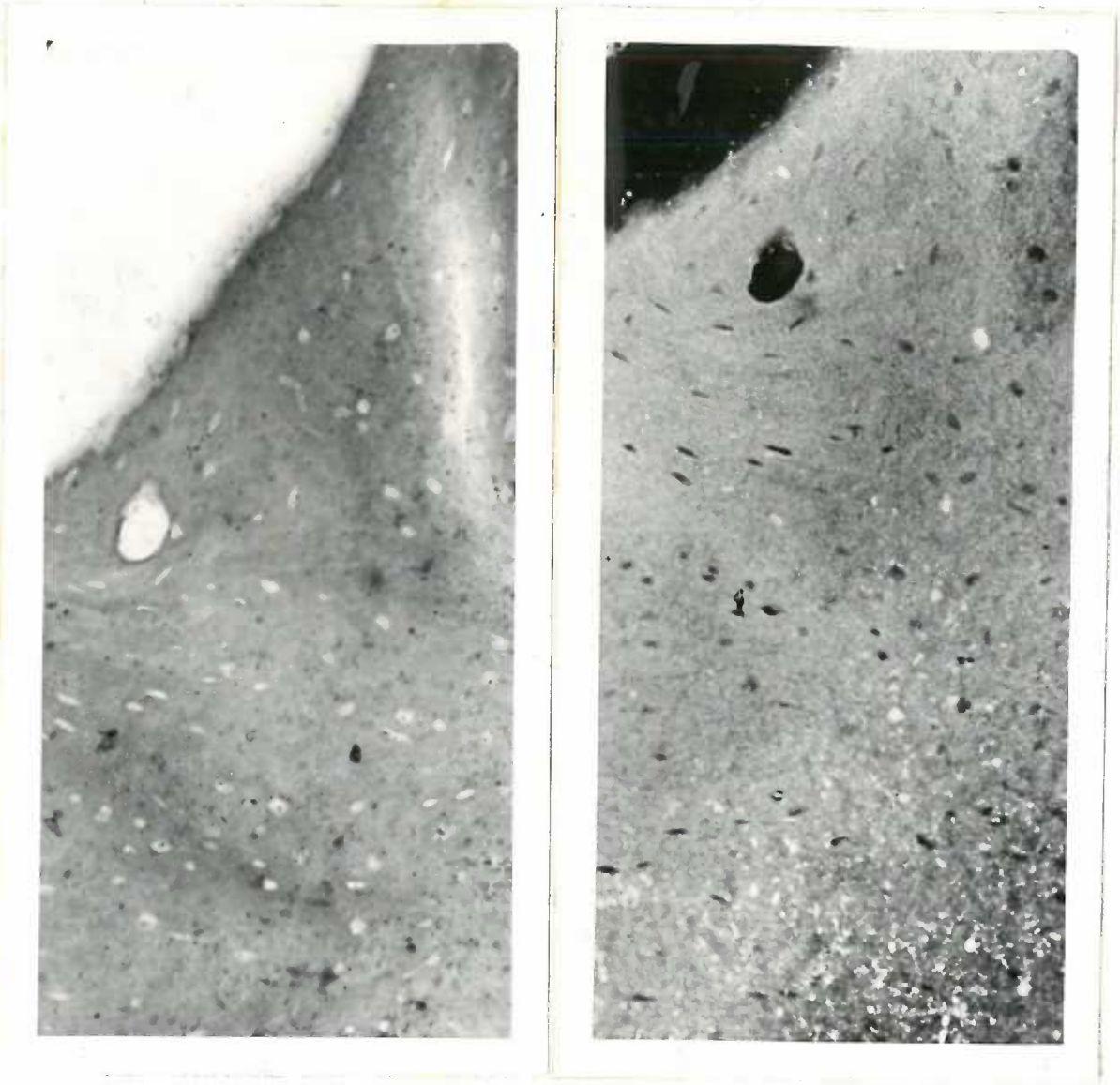

Fig. 10. This very thin section (8μ) shows very little variation in density in the microradiograph (right). 70μ backing, 90 seconds exposure.