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X-RAY MICRORADIOGRAPHY OF TISSUE SECTIONS WITH MAGNESIUM RADIATION

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In a recent note[1] we showed that by using aluminum foil as target and by circulating helium in the enclosed specimen and photographic chamber, projection X-ray microradiographs giving good contrast could be made from sections of soft tissue. We have now found that still better contrast, sufficient to reveal even more structural detail, can be obtained by employing magnesium foil as target and evacuating the space between target and photographic plate. Though the two elements are adjacent in the periodic table, the K radiation of magnesium, with a wavelength of *ca.* 10 A, has an absorption considerably higher than that of aluminum. Furthermore, the low

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yield of white radiation makes it possible to employ excitation voltages as high as 10 KV to provide an efficient production of the K radiation without loss of the desired absorbability. Both projection and contact microradiographs have been made in this manner with a Cosslett-Nixon X-ray microscope[2] modified for vacuum photography.

When resolution within the limits set by the grain of high resolution emulsions is adequate, contact microradiographs of tissue sections are easier to make, and

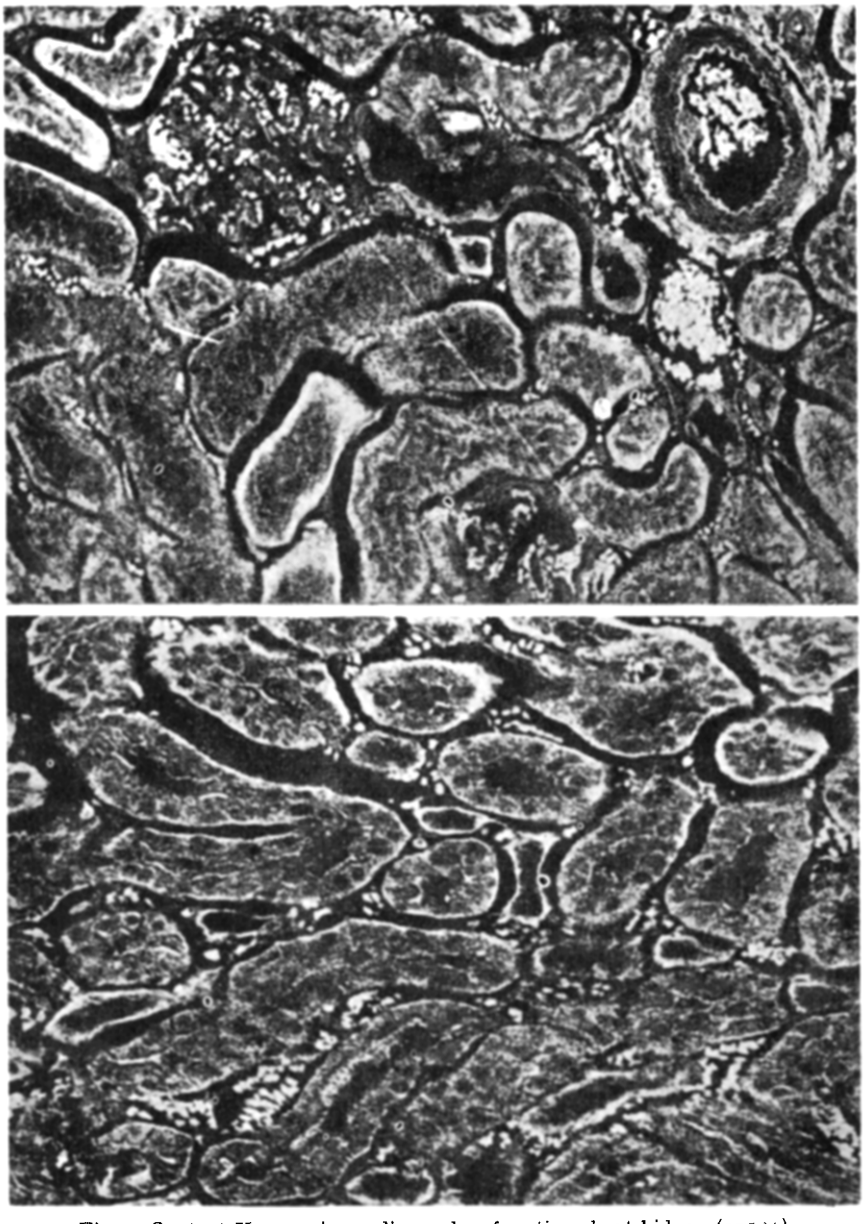

Fig. 1. Contact X-ray microradiographs of sectioned rat kidney (375 ×).

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appear to be as satisfactory as those made by projection. With these thin sections, however, special care must be taken to insure intimate contact between the specimen and the photographic emulsion throughout the exposure. Sharply defined detail has not been obtained in microradiographs of sections merely laid flat on the emulsion. We have found the following mounting technique satisfactory with sections cut from standard paraffin embeddings. The section as cut is first flattened by the usual method of flotation for a few minutes on warm water. It is next transferred with a glass slide to the surface of absolute alcohol and is then picked up from underneath on the emulsion side of the photographic plate. When alcohol is used the hygroscopic emulsion is protected against the damage which otherwise results from immersion in water. To facilitate the later removal of the section it is convenient, though not necessary, to coat the plate with an exceedingly thin film of formvar before mounting the section on it. This is done by dipping the plate in a 0.2% solution of formvar in ethylene dichloride, draining it and allowing it to dry, as in making substrate films for electron microscopy. After the section has been picked up it can be firmly attached to the emulsion or formvar film by a slight warming over a hot plate. As a next step, the paraffin is removed by immersing the photographic plate with its section for a few minutes in xylene. The exposure is then made and, before development of the plate, the section is removed by wiping gently with absorbent cotton saturated with ethylene dichloride. In the event that formvar has been used as a separating medium, the section is more easily dislodged as the plastic dissolves. By using Eastman 649-O Spectroscopic emulsion on glass, the foregoing steps can be carried out under orange light without inconvenience.

Fig. 1 shows two typical enlarged contact microradiographs of 6 μ thick unstained sections of embedded normal rat kidney which was fixed and dehydrated by freezing and drying. It is evident that the contrast produced by the passage of magnesium K-X-rays through such sections of soft tissue is sufficient to reveal many details of the cellular structure. Especially noteworthy in these photographs are the relatively transparent nuclei of the cells seen in the lower photograph and the detailed structure visible in the wall of the arteriole at the upper right of the top photograph.

SUMMARY

Excellent contrast revealing detail within and between cells can be obtained in X-ray microradiographs of standard 6 μ sections of soft tissues when a magnesium target is used and photographs are made in vacuum.

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