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A NEW DEVICE FOR MICRORADIOGRAPHY AND A SIMPLIFIED TECHNIQUE FOR THE DETERMINATION OF THE MASS OF CYTOLOGICAL STRUCTURES

by

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Microradiography is increasingly becoming a tool of importance for several research fields such as biology, medicine, mineralogy and metallurgy. In the field of histochemistry quantitative microradiography has given new information on composition and function of cells and tissues[1].

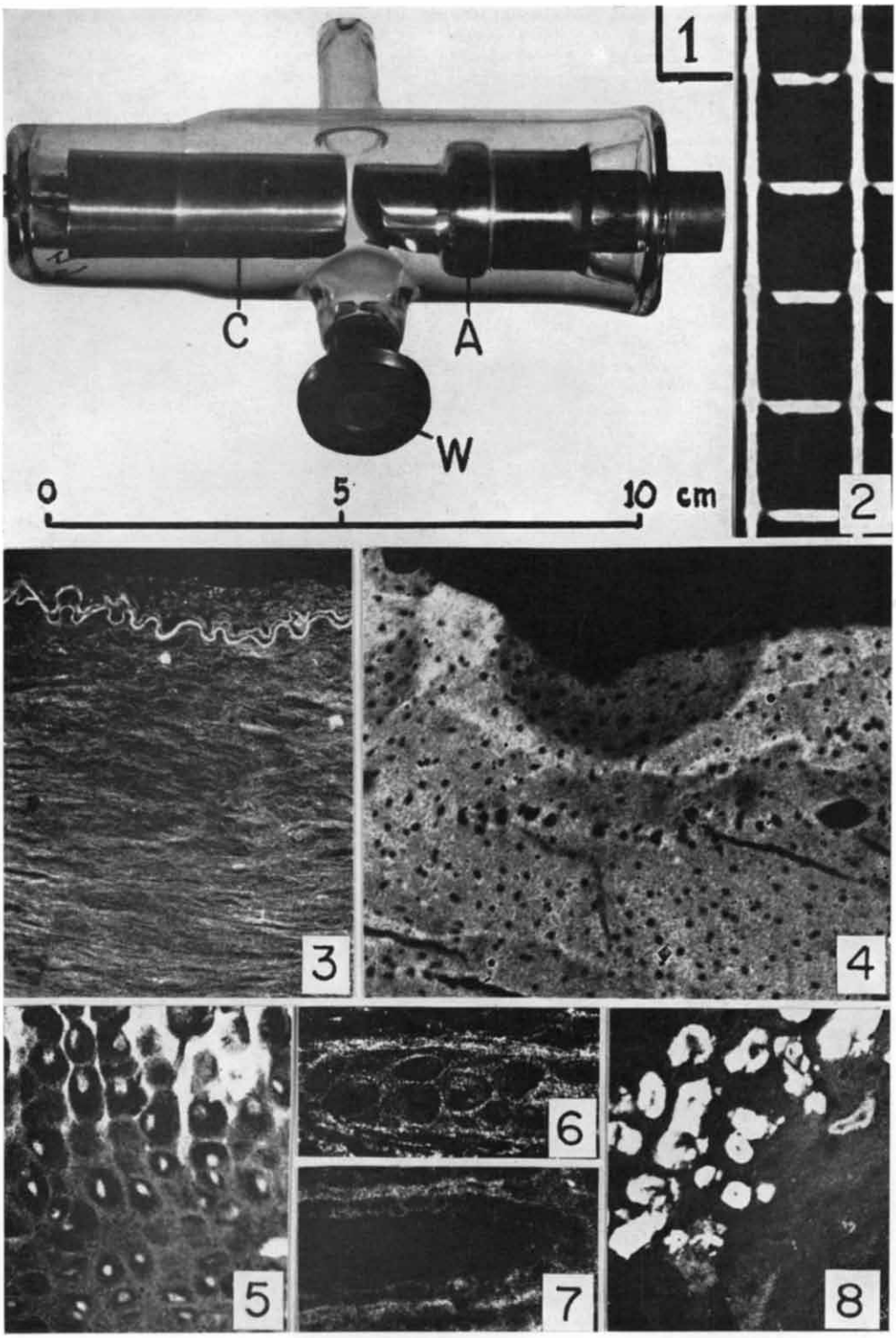
The dry weight (mass) of a histological or cytological structure in a biological tissue can be determined from its capacity to attenuate extremely soft X-rays[2]. The method is based on measurement of the photographic density in a microradiogram of the specimen and a simultaneously microradiographed reference system. The microradiogram is registered on a fine grained photographic emulsion such as Lippmann emulsion, Kodak Maximum Resolution Plate or Eastman Kodak Spectroscopic Plates 548 or 649. The resolution of the best of these fine grained emulsions is more than 1000 lines per mm, but granularity varies from one batch to another. Quantitative microradiography permits the determination of the absorption of soft X-rays in cytological structures as small as a few microns in diameter. The theory for the cytological X-ray weighing procedure has been described elsewhere[2].

In order to calculate the dry weight of a cytological structure the microradiogram has to be recorded with extremely soft X-rays[1,2]. The first equipment for such weight determination worked with 8 A and softer X-rays and the specimen was in the high vacuum of the X-ray tube. The tube was evacuated during the exposure. Other types of apparatus for weight analysis have been described[3,4,5] and they all have in common relatively complicated X-ray tubes that have to be pumped continuously.

Recently, sealed-off miniature X-ray tubes with very thin Be-windows have been developed at Philips X-ray laboratories, Eindhoven. Earlier technical development of these tubes for therapeutic treatment has been described[6]. The purpose of this note is to show how a newly developed X-ray tube provides us with very simple equipment for quantitative historadiography. Fig. 1 shows a photograph of one the models of this small tube. It is provided with a Be-window 50 μ in thickness and has a 0.3 mm focal spot. This tube could be energized with max. 3 mA at 5 kV using a simple small high voltage generator. The sample-film holder (camera) is attached directly to the tube; the advantage is that the sample is outside the high vacuum of the tube. If necessary the camera can be evacuated. The output of this particular tube is such that the exposure time varies with the present technique between 2 and 20 minutes in the 2000 to 5000 volts range.

The resolution of the microradiographic technique using this sealed-off miniature tube is highly satisfactory. Fig. 2 shows an optical enlargement of the microradiogram of a silver grid kindly supplied by Dr. V. E. COSSLETT. The width of each bar is 3 μ and it is clearly demonstrated that structures even smaller than 0.5 μ are resolved. Of course this resolution is not reached for objects

Fig. 1. Laboratory model of a miniature X-ray tube. A, anode; C, cathode; W, window.
Fig. 2. Microradiogram of a silver grid. Each bar is 3 μ thick.
Fig. 3. Microradiogram of a microtome section of a blood vessel (2500 volts, 50 μ Be-window).
Fig. 4. Microradiogram of a thin ground section of bone showing the distribution of mineral salts (5000 volts, 200 μ Be-window).
Fig. 5. Microradiogram of a section of epiphyseal cartilage with beginning calcification (top of the fig.) of the cartilage cells (3000 volts, 50 μ Be-window).
Fig. 6 and 7. Microradiograms of sections from a rabbit ear and a pathological kidney. In Fig. 7 the individual cells of the tubuli are clearly seen (2500 volts, 50 μ Be-window).
Fig. 8. Microradiogram of a section through a kidney with pathological calcification (4000 volts, 200 μ Be-window).


1
C
A
W
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5
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with less contrast, such as biological specimens. The microradiogram (2500 volt) of a 5 μ thick section of a blood vessel wall is shown in Fig. 3. It is clear that the contrast is good, and so is the resolution. Figs. 5, 6 and 7 show microradiograms of microtome sections from rabbit epiphyseal cartilage, rabbit ear and pathological kidney respectively. Fig. 4 taken with a similar tube with 200 μ Be-window at 5 kV illustrates that the tube also can be used to study the distribution of calcium salts in a ground section of bone tissue. Another example of this type of application is Fig. 8 which shows calcifications in a kidney (5 μ thick section).

It was originally suggested that a small reference system should be radiographed simultaneously with the sample[2] when quantitative measurements have to be performed in the microradiogram. This procedure requires a relatively large homogeneous field of X-rays. The reference system can be made of thin nitrocellulose foils. By comparing the density of a biological structure with that of the steps in the reference system the weight of the structure can be computed[2]. There are certain difficulties in preparing a good reference system and also in determining the mass of each step[3,7]. Several methods have been proposed both for the preparation of the reference system and the measurement of the mass of each step[2,3,7]. The simple construction of the scaled off tube as compared with that of continuously pumped tubes enables us, however, to eliminate the necessity of a reference system. Instead of exposing only one specimen on each film, more, *e.g.* six, different samples can easily be registered on the same film. Each sample, however, is exposed differently, *i.e.* the time is varied with constant X-ray intensity causing different densities. The density of the X-ray image of the structure to be investigated and the density corresponding to the incident X-ray intensity are measured in each of the microradiograms. As all samples are registered on the same film it is possible from the measurement of the densities caused by the incident beam to construct the density-exposure curve for each individual photographic film, and no standard processing is necessary. From this density curve the X-ray transmission of any cytological structure in any of the six different samples can be determined. In order to convert X-ray transmission into weight per unit area, the nitrogen mass absorption coefficient for the radiation must be known, and this coefficient is determined for each voltage used by measuring the absorption of the X-rays in a certain distance of air.

Summarizing, the new tube described in this communication makes quantitative microradiography simple. The main advantages over old equipment for historadiography are: 1. No continuous pumping; 2. Small and compact construction; 3. Simple high voltage generator of small size; 4. No special reference system required if the procedure indicated above is followed; 5. High image resolution due to very small focal spot.

The tube is now being further tested for historadiographic work and most probably several modifications of the equipment and technical procedure will be introduced. The goal is to get an equipment as easy to use as an ordinary light microscope.

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GUANOSINE TRIPHOSPHATE, THE PRIMARY PRODUCT OF PHOSPHORYLATION COUPLED TO THE BREAKDOWN OF SUCCINYL COENZYME A*

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The enzyme system that catalyzes the phosphorylation of ADP** coupled to the breakdown of succinyl CoA consists of at least two enzymes[1] and an additional coenzyme[2]. The latter has been

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** The following abbreviations will be used: adenosine di-, and triphosphates, ADP and ATP;