PRO EXPERIMENTIS

An Ultramicrotome with Cone-shaped Bearings

The production of ultrathin tissue sections for electron microscopy has, until quite recently, been a very difficult problem. But improvements of the fixating, embedding and sectioning technique have now made it possible to obtain tissue sections that permit a very high resolution in the electron microscope. However, at the same time as the electron microscope results have improved the demands for investigation of larger materials, quantitative recording and serial sectioning have increased and this creates a situation where the sectioning must be largely a routine work performed by the technical assistants. This fact increases the demands on the microtome, which must not only give an almost 100% yield of thin sections but must also be simple, easily operated and not easily damaged. Our purpose has therefore been to construct such an ultramicrotome.

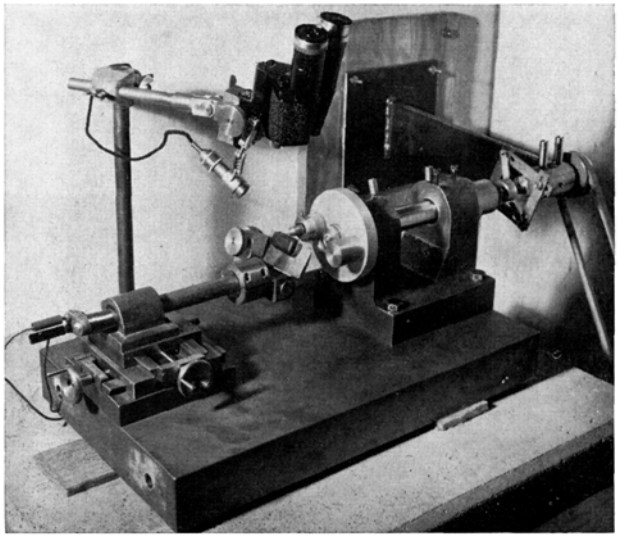

Fig. 1.

The principles laid down in constructing the microtome are essentially the following:

(1) The specimen should pass the knife edge only once during a cutting cycle. The simplest way to obtain this is to use a circular movement of the specimen in relation to the knife[1].

(2) The bearings of the rotating axle of the microtome should be made in such a way that both the axial and radial play is as small as possible. The ideal bearings in this case with comparatively low speed of rotation seem to be those of conical shape.

(3) The cutting should be performed at a constant and given speed. Therefore, the microtome must be motor driven. Care must be taken to avoid vibration in power transmission and this can be accomplished by a rubber shaft coupling.

[1] The first microtome working according to this principle is the one constructed by F. SJÖSTRAND, Exper. *9*, 114 (1953).

(4) The specimen feed should be continuous and free of variations for periods of about 10 min and the supply between two consecutive cuttings should be of the order of at most 200 Å. This is brought about in a simple manner by making use of the thermal expansion of a metal rod.

A microtome built according to these principles has the appearance shown in Figure 1. The construction is clearly seen in the schematic drawing in Figure 2.

The different parts of the microtome are mounted on a carefully machined cast iron base (*A* in Fig. 2) of the dimension of 300 × 500 × 60 mm. In the plummer block (*B*) bushings (*C*) of copper-base bearing metal are accurately fitted. They are both conical in shape and the one nearest to the driving device is adjustable in axial direction. The adjustment contrivance (*D*) consists of a coil spring affected by a screw and pressing on the bushing. The bearing is locked in adequate position by locating screws (*E*). The microtome axle of steel (*F*), extending through the plummer block in both directions, has conical ground in bearings. The lubricating material consists of a low viscid mineral oil.

The axle extension nearest to the driving device is provided with two cross bars (*G*). These are connected by rubber strops to two similar bars mounted on the driving shaft (*H*), which is centered with the microtome axle. The driving shaft is connected to the motor with a V-belt.

On the other extension of the microtome axle a circular aluminium disc (*I*) is fixed. The specimen holder (*J*) is mounted on this at a distance from the centre of 35 mm. Furthermore, the aluminium disc is provided with a counter weight.

Opposite the plummer block an assembly is placed with the purpose of making the knife adjustable and to feed the knife against the specimen. A table (*K*) consisting of three plates with two pairs of guide bars is movable in two perpendicular directions. On the top of this table a soft iron rod (*L*) is mounted. The one end of this expansion rod is equipped with a heating coil (*M*) of manganin. The other end of the rod has the holder (*N*) for the microtome knife (*O*). The expansion rod is heat-insulated from the table as well as from the knife holder. In order to avoid vibrations the knife-end of the rod is provided with a tiltable rubber absorber (*P*).

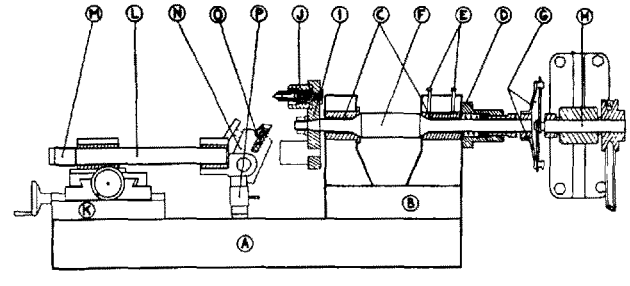

Fig. 2.

On a separate support a binocular low-magnifying microscope (not indicated in Fig. 2) is placed, so that the adjustment of the knife in relation to the specimen and the sectioning may be controlled.

As cutting edge a microtome knife of stainless steel is used. The sharpening of knife is carried out by a special

method described earlier[2]. When sectioning, a small plastic trough, in which the sections are collected on a liquid surface, is attached to the knife.

When operated the microtome axle has a speed of 74 rpm. As the specimen is mounted about 35 mm from the centre of the axle the cutting speed is about 0·27 m/s. The intensity of the heating current is 1·0 amp. At this amperage the expansion rod elongates 1·5 micron per min from the second till the eleventh minute after closing the circuit. Thus the theoretical section thickness is about 200 Å.

Provided that the knife edge is of high quality the microtome displays very satisfactory operating properties. The cutting starts about 30 s after the circuit is closed and goes on regularly for about 12 min. By continuous control of a great number of cutting series it has proved that a section is produced every time the specimen passes the edge for periods of 1·5–3 min. Between these periods there is a miss of one or two sections. In the electron microscope the sections prove to be of very uniform thickness. On the basis of the regular cutting and the uniformity of the sections in the electron microscope, we are justified in taking for granted that the divergence from the theoretically calculated section thickness of 200 Å is very slight.

It is a pleasure to acknowledge the interest and skillful workmanship of Mr. V. KUIKKA during the building of the prototype of this microtome.

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Department of Anatomy, University of Gothenburg, December 5, 1955.

Zusammenfassung

Die prinzipielle Konstruktion eines neuen Ultramikrotoms wird beschrieben. In diesem Mikrotom wird das Präparat durch Montieren auf eine konisch gelagerte, motorgetriebene Achse in einer zirkularen Bahn fortbewegt. Die gewünschte Schnittdicke erhält man durch das Ausnutzen der thermischen Verlängerung eines Metallstabes, auf dem das Mikrotommesser befestigt ist. Mit diesem Mikrotom können routinemässig Schnitte von etwa 200 Å Dicke hergestellt werden.

[2] R. EKHOLM, O. HALLÉN, and T. ZELANDER, Exper. *11*, 361 (1955).

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Elektronenmikroskopische Untersuchung der phäochromen (chromaffinen) Granula in den Markzellen der Nebenniere[1]

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Die bekannten Farbreaktionen an den Markzellen der Nebenniere, zum Beispiel mit Chromsalzen, Osmiumtetroxyd, Eisenchlorid, ammoniakalischem Silbernitrat beruhen übereinstimmend auf der reduzierenden Fähigkeit der im Cytoplasma gespeicherten Brenzcatechinamine («catechol amines»): Adrenalin, Noradrenalin bzw. deren Vorstufen. In älteren und neueren Arbeiten[2] wurde nachgewiesen, dass diese Stoffe im Cytoplasma in granulären Gebilden, den chromaffinen oder phäochromen Granula, lokalisiert sind, die neuerdings nach Homogenisierung des Gewebes durch Zentrifugieren weitgehend isoliert und biochemisch studiert wurden[3]. Nähere morphologische Angaben über diese Granula liegen bisher nicht vor.

Für elektronenmikroskopische Dünnschnittuntersuchungen des Nebennierenmarks, die an anderer Stelle ausführlich mitgeteilt werden[4], wurden die Nebennieren der Maus (ferner auch von Meerschweinchen und Katze) in zwei (bzw. mehrere) Stücke zerteilt, in gepufferter isotonischer Osmiumtetroxydlösung fixiert, in bestimmter Orientierung[5] in Methacrylat eingebettet und mit dem von SJÖSTRAND[6] entwickelten Ultra-Mikrotom geschnitten. Die etwa 200 Å dicken Schnitte wurden ohne Entfernung des Einbettungsmittels in Elektronenmikroskopen verschiedener Typen untersucht.

Bei der Maus erscheinen die phäochromen Granula im Schnitt als annähernd kreisrunde Scheiben von unterschiedlicher Grösse (siehe unten) und hoher elektronenmikroskopischer Dichte (Abb. 1 und 2). Beim normalen Tier reduzieren die gespeicherten Brenzcatechinamine soviel Osmiumtetroxyd, dass die meisten Granula ein

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[1] Ausgeführt mit Unterstützung der Friedrich-Baur-Stiftung und der Deutschen Forschungsgemeinschaft.

[2] R. BACHMANN, *Die Nebenniere*, Handbuch der mikroskopischen Anatomie des Menschen, Bd. 6, 5. Teil (Springer-Verlag Berlin-Göttingen-Heidelberg 1954). – N.-Å. HILLARP und B. NILSON, Kungl. fysiogr. Sällsk. Förh., Lund *23*, 29 (1953). – N.-Å. HILLARP, B. HÖKFELT und B. NILSON, Acta anat. *21*, 155 (1954). – H. BLASCHKO und A. D. WELCH, Arch. exper. Path. Pharmak. *219*, 17 (1953).

[3] N.-Å. HILLARP und B. NILSON, Kungl. fysiogr. Sällsk. Förh., Lund *23*, 29 (1953). – N.-Å. HILLARP, B. HÖKFELT und B. NILSON, Acta anat. *21*, 155 (1954). – H. BLASCHKO und A. D. WELCH, Arch. exper. Path. Pharmak. *219*, 17 (1953). – N.-Å. HILLARP und B. NILSON, Acta physiol. Scand. *31*, Suppl. *113*, 79 (1954); *32*, 11 (1951). – H. BLASCHKO, P. HAGEN und A.D. WELCH, J. Physiol. *129*, 27 (1955).

[4] R. WETZSTEIN (in Vorbereitung).

[5] R. WETZSTEIN, Mikroskopie (Wien *10*, Heft 9/10 (1955) (im Druck).

[6] F. S. SJÖSTRAND, Exper. *9*, 114 (1953).