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A New Instrument for the Rapid Preparation of Tissue Slices

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A manually operated microtome for the rapid preparation of live tissue slices is described. The instrument operates submerged in an isotonic solution and incorporates an automatic mechanism for the removal of the cut slices which are gently carried by a stream of fluid to a strainer reservoir. An inexperienced operator can obtain circular slices of nearly identical thickness at a rate of one every 3 or 4 s. Mechanical trauma to the tissues is minimized and the temperature and oxygenation of the medium are easily controlled.

Since Otto Warburg introduced the tissue slice technique more than 50 years ago (1), this simple method for the study of the metabolism of tissues has been in continuous use. More recently it has been demonstrated that mammalian brain slices can be kept alive and electrically excitable *in vitro* (2), extending the applications of the tissue slice technique into the domains of neurophysiology and neuropharmacology (3–5). Despite its applicability a major obstacle in the use of the technique is that it is quite difficult to rapidly prepare thin tissue slices of uniform thickness and dimensions under conditions that minimize trauma to the live tissues. In this article, we describe a simple, manually operated microtome designed to rapidly produce slices of nearly identical thickness and of very similar shape and dimensions in a controlled environment with minimal tissue damage. The instrument can be successfully used by an inexperienced operator.

DESCRIPTION AND OPERATION OF THE INSTRUMENT

Figure 1 shows front and rear views of the microtome. Figure 2 shows scale drawings of the instrument in several projections. The slicer is constructed of acrylic plastic except for the blade holder and counterweights which are made of brass and stainless steel.

The microtome is operated partially submerged in an appropriate isotonic medium contained in the box shown in Fig. 3. The level of submersion is indicated by (a) in Fig. 2-I. Figure 3 shows the instrument on its back in the "load" position with the door (b) open. The sample well (c) is automatically opened by the counterweighted lever (d) (Figs. 2-I, II, V) which swings in the direction indicated by the open arrow (see Fig. 2-I) allowing plunger (e) to drop down. An approximately cylindrical piece of tissue of about 12 mm in diameter, cut by means of a sharp punch, is then placed in the sample well, and the spring-loaded door (b) closed. The desired slice thickness is set by the position of the screw-adjustable rest (f) (Figs. 2-I, II, V) with respect to the plane of the slot (g) that guides the movement of the blade (h). For the sake of clarity, the width of the guiding slot (g) is not drawn to scale in Fig. 2. In actuality it measures only 0.3 mm in width, allowing the blade (h) to move freely but with minimal play. Since the screw-adjustable rest (f) has 20 threads per inch, one full turn changes slice thickness 1.28 mm. The pointer (i) and a simple protractor grid (in 45 degree increments) inscribed on the face of door (b) (Figs. 1 and 2-IV) facilitate intermediate settings that differ by 160 μm for each 45 degrees advanced. The blades used (Thomas tissue

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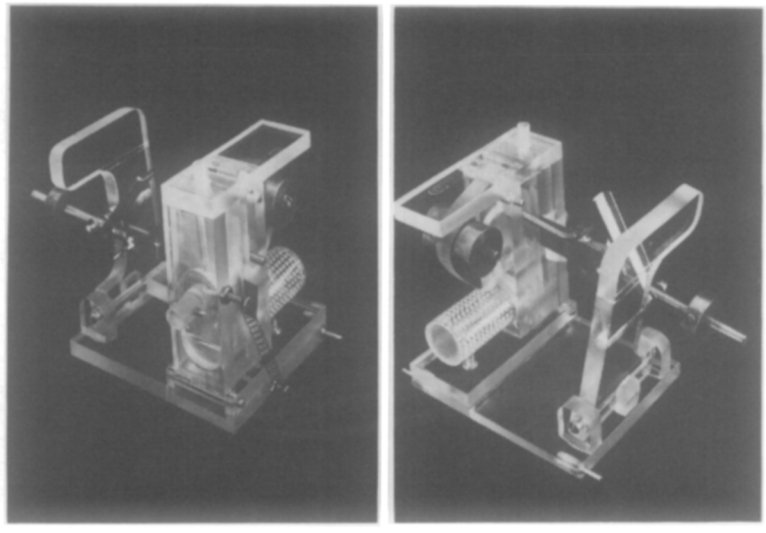

FIG. 1. Front and rear view of the microtome.

slicer blades) are available from Arthur Thomas Company, Philadelphia. As supplied, these blades measure ¾ × 45⁄8 in. and had to be broken to a length of 3 in. A small circulating pump (not shown), connected between outlet (j) and inlet (k) (Fig. 3), provides a flow of liquid of approximately 1 liter per minute and establishes a current

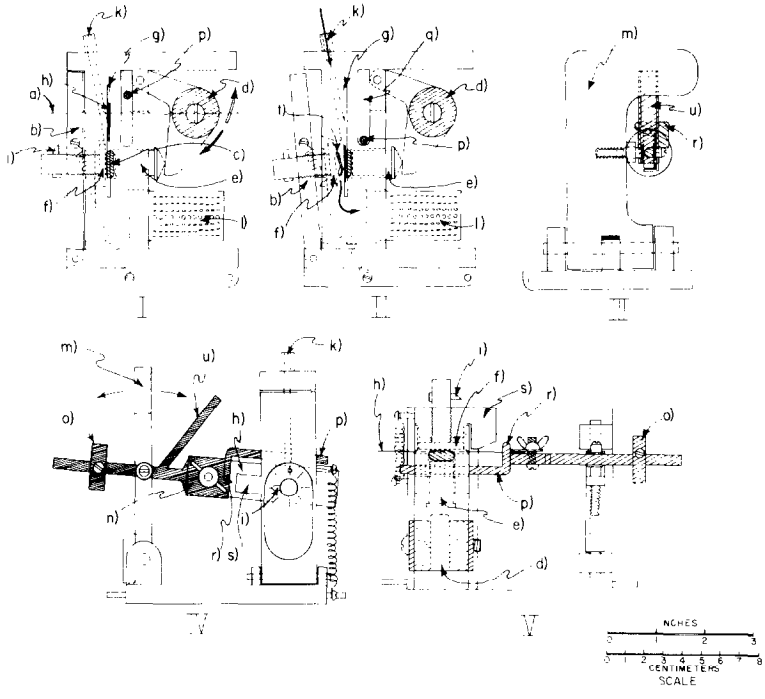

FIG. 2. Scale drawings: (I) Left side view. (II) Left side view—detail of removal of the cut slice. (III) Right side view. (IV) Top view: (a) level of submersion, (b) spring loaded door, (c) sample well with tissue sample, (d) counterweighted lever, (e) plunger, (f) screw-adjustable rest, (g) blade-guiding slot (not drawn to scale), (h) blade, (i) pointer, (j) outlet (Fig. 3), (k) inlet, (l) strainer–reservoir, (m) blade handle, (n) blade holder, (o) counterweight, (p) rod to limit downward travel of blade, (q) slot, (r) cam, (s) wedge, (t) cut slice, (u) lever.

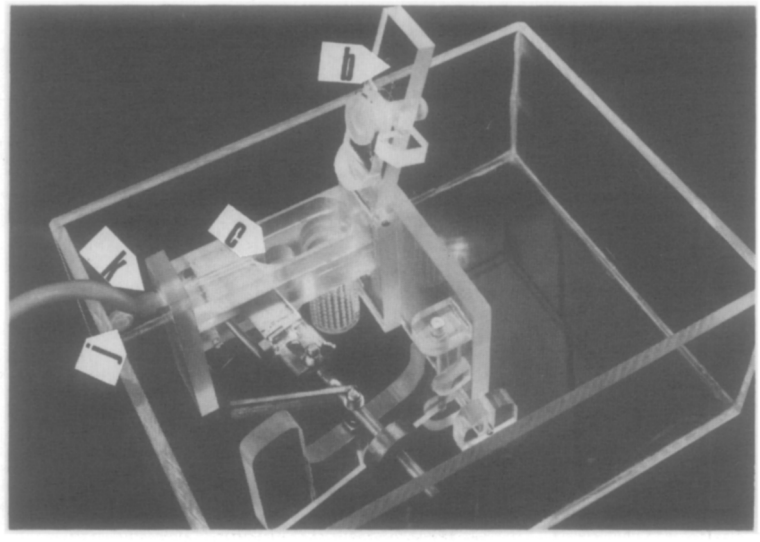

FIG. 3. Microtome in "load" position inside box for isotonic medium: See (b), spring-loaded door; (c) sample well.

that exits the instrument through the strainer–reservoir (l) (Figs. 2-I, II). The circulating pump is turned on immediately after closing door (b). The instrument then is turned 90 degrees to its upright "cutting" position. In this position the counterweighted lever (d) moves in the direction indicated by the heavy solid arrow in (Fig. 2-I) and through plunger (e) exerts a constant pressure on the block of tissue held against the adjustable rest (f).

The tissue is cut by manually moving the blade handle (m) back and forth (Figs. 2-III, IV). The downward pressure of the cutting edge is determined by the weight of the blade holder (n) (Fig. 2-IV) and can be varied by sliding the counterweight (o). This facilitates slicing tissues of different consistencies. Once the blade has completed cutting the tissue, its downward movement is limited by rod (p) (Figs. 2-I, II, IV, V) reaching the bottom of the slot (q) (Fig. 2, II; also see Fig. 1). After completing the cut, the blade is left between the cut slice and the remaining tissue as shown in Fig. 2-II. At this point the last forward movement of handle (m) will make the cam (r) of the blade holder automatically engage wedge (s) (Figs. 2, IV, V) and crack open door (b) leaving the cut slice (t) (Fig. 2-II) unsupported. The slice is instantly swept into the strainer–reservoir (l) by the current of fluid indicated by the heavy arrows in Fig. 2-II. The operator brings the blade back up by means of lever (u) (Figs. 2-III, IV). This motion simultaneously closes the spring-loaded door (b) and allows the remaining tissue to be moved forward into cutting position by plunger (e). After cutting the desired number of slices, the operator removes the strainer–reservoir to retrieve the slices.

RESULTS

With minimal practice, slices can be cut in rapid succession at a rate of one every 3 or 4 s.

The use of a cylindrical block of tissue results in nearly identical circular slices of very similar weights (See Table 1). Figure 4 shows histological preparations of liver slices obtained at four different settings of the screw-adjustable rest (f) (Figs. 2-I, II, V). The settings were changed in 160-μm increments from 160 (Fig. 4-A) to 640 μm (Fig. 4-D). There is good agreement between the setting of the instrument and the thickness of the slices obtained. The histo-

TABLE 1

WEIGHT (mg) OF CONSECUTIVE LIVER SLICES OF DIFFERENT THICKNESS

Section No.	Setting of the instrument (μm)			
	160	320	480	640
1	35.2	66.8	82.2	121.3
2	33.5	64.3	86.0	117.3
3	37.3	68.5	91.3	133.7
4	37.6	68.8	86.2	122.8
5	39.2	67.3	82.3	123.2
6	37.5	64.3	107.7	141.9
7	32.5	64.5	82.9	126.8
8	31.9	64.3	94.2	134.4
9	34.5	62.8	86.5	102.9
10	31.4		102.4	117.2
Mean ± SE	35.1 ± 0.8	65.7 ± 0.7	90.2 ± 2.7	124.1 ± 3.4
Range	31.4–39.2	62.8–68.8	82.2–107.7	102.9–141.9

logical sections also show that the slices have parallel surfaces and that there is minimal disruption of the architecture of the tissue at the cut surface. Aside from the liver, slices of brain, spleen, kidney, heart, skin, and adipose tissue (rat epididimal fat pad) have been successfully prepared.

Slices prepared with this instrument have been used for the past 2 years by the authors to study some aspects of the hormonal regulation of hepatic cholesterol synthesis in the rat. The viability of the slices is demonstrated by the data (Table 2) showing the linearity of incorporation of [2-^{14}C]acetate into cholesterol and into CO_2 over a 90-min period of observation. The results shown are the means of four separate experiments. Twelve flasks (four for each time period) containing two slices per flask were used in each experiment.

DISCUSSION

In developing the microtome herein described we sought to make an instrument that: (i) could be successfully used by an unskilled operator, (ii) would yield reproducible results, (iii) would minimize the time required for the preparation of the slices, (iv) would minimize mechanical trauma to the tissues, and (v) would operate in a controlled environment. To satisfy requirements (i) and (ii) the instrument was designed so that the forces applied to the tissue during slicing (i.e., compression to support the block of tissue and the downward pressure of the cutting blade) are both set by weights and held constant during operation. The back and forth movement of the blade imparted by the operator has no effect on the forces directly applied to the tissue being sliced. This is in contrast with previously described slicers such as the time-honored Stadie–Riggs microtome (6) and several others of more recent design (7–9) that have hand-held knives and/or where the tissue block is held in place during slicing by manually exerted pressure.

With previously described slicers the removal of the cut slice has always been a slow process, very dependent on the skill of the operator and requiring the use of forceps, wire loops, hair brushes, and the like. Designing our instrument to operate submerged in an appropriate isotonic medium presented the opportunity of using an internal current to rapidly remove the cut slice and gently carry it to a reservoir. This simplifies the task of the operator and greatly speeds up the functioning of the

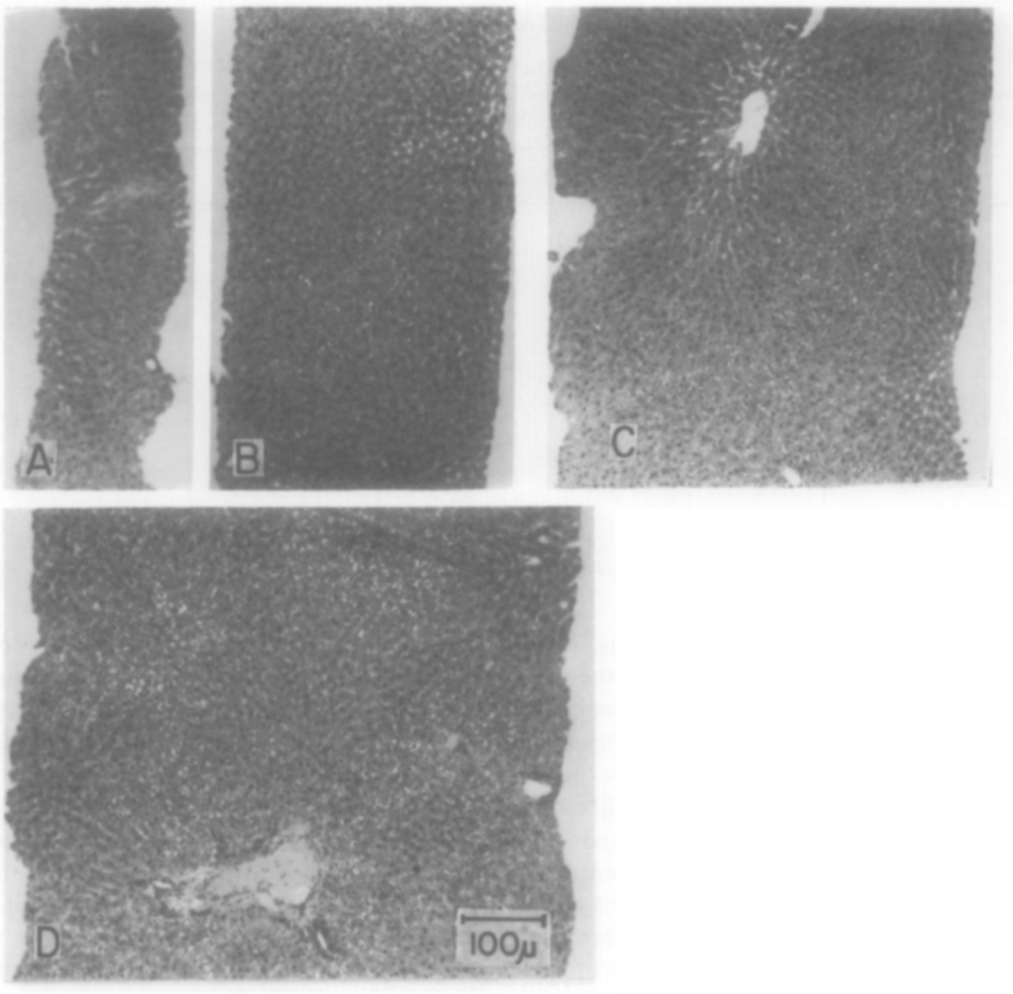

FIG. 4. Histological sections of rat liver slices prepared at setting of: (A) 160 μm; (B) 320 μm; (C) 480 μm, and (D) 640 μm.

instrument, making it easy to obtain 10 to 12 slices in less than 1 min. This is an important advantage when working with tissues, such as nerve tissue, which can tolerate only short periods of anoxia. By operating the instrument submerged, several other advantages are realized. Adhesion of the tissue to the blade and the resulting wear and tear are eliminated by the continuous lubrication provided; tissue temperature is easily controlled; dessication and osmotic insult are prevented; and, when desired, optimum oxygenation of the slices can be achieved by bubbling a mixture of 95% oxygen and 5% carbon dioxide into the system.

TABLE 2

TIME COURSE OF INCORPORATION OF [2-^{14}C]ACETATE INTO CHOLESTEROL AND CO_2 BY RAT LIVER SLICES 480 μm THICK[a]

Time (min)	Cholesterol[b] (nmol/g liver/h)	CO_2[b] (nmol/g liver/h)
30	56.6 ± 4.2	553.2 ± 42.3
60	128.2 ± 4.8	1400.4 ± 110.8
90	206.6 ± 11.1	1893.3 ± 124.4

[a] The incubation conditions, analytical procedures and calculations were as described by Weiss and Dietschy (10).

[b] Values are means ± standard errors of the means of four experiments.

In brief, the instrument described allows an inexperienced operator to rapidly prepare minimally traumatized tissue slices of very uniform shape and dimensions and excellent viability.

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