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Techniques in Tissue Metabolism. 1. A Mechanical Chopper

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The apparatus now described was devised to chop small and irregular fragments of animal tissues for metabolic experiments, with as little disruption as possible of cell structure. It will also cut larger pieces of tissue. Small and irregular specimens were being examined because they are often the only ones available at biopsy or when small organs, or parts of them, are being investigated. Cell structure was required to be relatively intact in order to disturb as little as possible the many metabolic characteristics on which such structure exerts a controlling influence. These include levels of metabolites and metabolic rates, concentration or exclusion of added substances, and response to inhibiting and stimulating agents. Several instances of the importance of such structural factors in preparations from the central nervous system have been encountered recently (McIlwain & Grinyer, 1950; McIlwain, Buchel & Cheshire, 1951; McIlwain, 1951*b*). In some of these studies (see also McIlwain, Ayres & Forda, 1952) specimens had been reduced to a size small enough for diffusion of metabolites to the tissue, by a method consisting of 'chopping' free-hand, by repeatedly passing a blade vertically into the tissues. The machine now described was built to do this in a regular and reproducible fashion.

EXPERIMENTAL

Preliminary observations

At least three different types of treatment have been used to fragment tissues.

Cutting. A blade with a relatively small cutting angle is moved both across and into the specimen simultaneously. Its motion is in the plane of the blade and has components both parallel and perpendicular to its cutting edge. Efficacy in cutting does not markedly vary with the speed of motion of the blade.

Shearing with scissors. Opposed cutting edges with large cutting angles are made to approach the specimen in directions perpendicular to their cutting edges.

Chopping. This motion, as of an axe or of a knife in chopping vegetables, has been applied little in dissection. It is, however, applied to hardened or supported tissues in microtomes. A sharp blade with relatively small cutting angle is used and approaches the tissue directly without necessarily any motion parallel to the cutting edge. The result, when applied to soft tissues, depends greatly on the speed of approach of the blade.

It appeared that the static rigidity of embedded tissues might be simulated by the resilience and inertia which they show, while still fresh, to a sudden blow. If a scalpel about the size and weight of a pocket knife is held by the extremity of its handle and its blade swung against a piece of liver lying on a pad of filter paper, the tissue can easily be cut (chopped) cleanly and suffers little depression at the point of entry of the blade. On the other hand, if the blade is laid on the tissue and pressed directly into it, appreciable force is needed for the cut and the tissue is distorted. (If a cutting motion is given to the blade, then again, much less force is required.)

There appeared to be relatively little advantage, when 'chopping' with a scalpel, in giving the blade an additional motion parallel to the cutting edge, as in ordinary cutting. A simple chopping motion was therefore made the basis of the machine described.

Description of the chopper

Fig. 1, Pl. 1, is a view of the chopper from the position of a person using it, and Fig. 2, Pl. 1, a view from a position at his left-hand side. Fig. 3 gives constructional details and omits the motor and rheostat which are at the back of Fig. 1, Pl. 1.

Motion of the blade. Chopping is by the blade *B*, held in arm *A*. This is lifted by the trigger cam *C*1 and then released, when it is drawn by its own weight and by the spring *Sp*1 into the specimen on the cutting table *T*. This is the only motion of the blade; it is lifted and released at each revolution of the shaft *Sh*1.

While the machine is cutting, shaft *Sh*1 is rotated by an electric motor, being continuous with the shaft of a helical

reduction-gear built to the motor framework. A handwheel $H1$ is provided on shaft 1 so that the blade can be lifted if, at the end of a cut, when the motor has been switched off, the blade has stopped in an inconvenient position (in the specimen and not in the air). With a little practice, however, the motor can be switched off at a moment such that the blade remains out of the specimen without further adjustment.

spring $Sp3$ against the large ratchet wheel. The ratchet wheel is thus partly rotated each time the lever rises, and turns with it the shaft $Sh2$. This shaft carries a large lead-screw (1 in. diam., Whitworth, 8 threads/in.) which passes through a corresponding thread attached to the base of the table T carrying the specimen. The specimen is thus moved to the right (viewing the machine as in Fig. 1, Pl. 1) at each revolution of shaft 1. The cams are arranged so that the

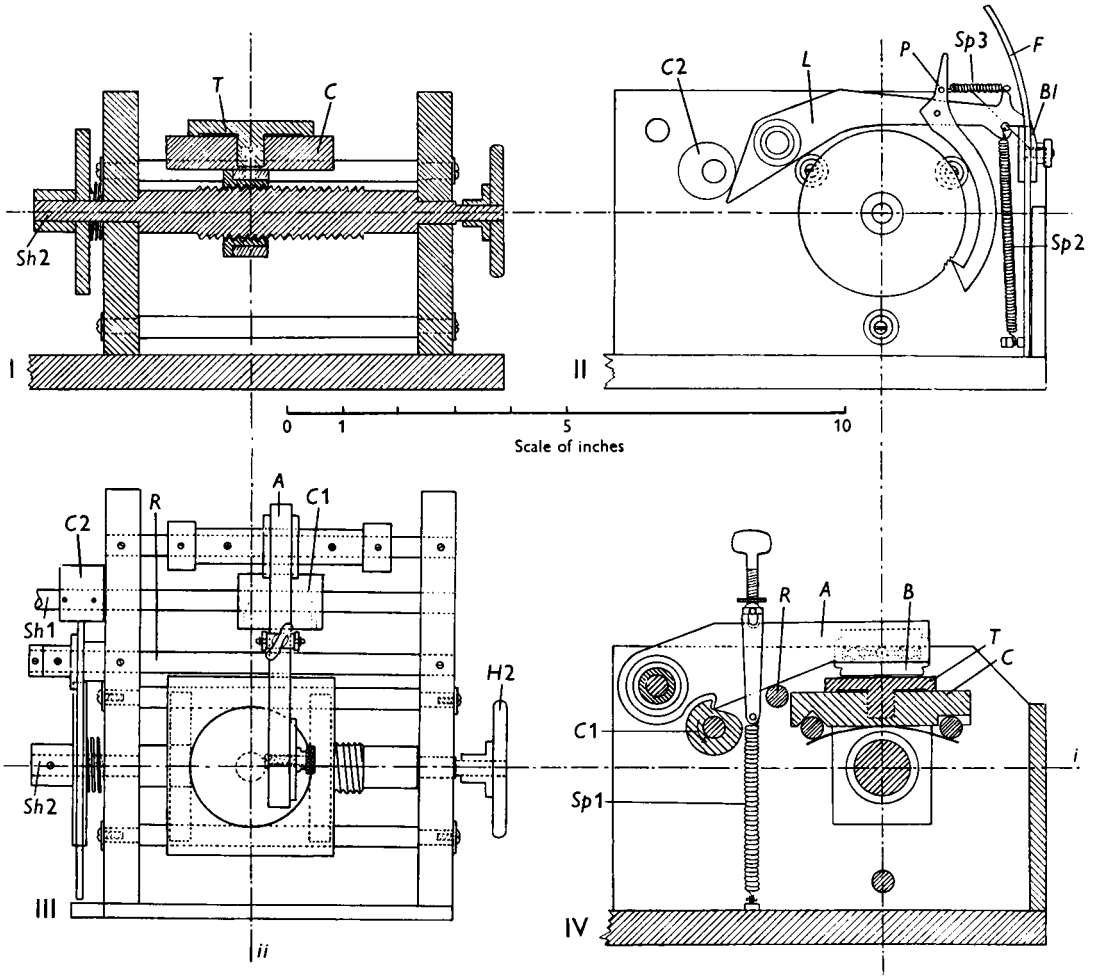

Fig. 3. Constructional details of the workshop-made parts of the chopper. Motor, rheostat, switch and most of the baseboard are omitted. I: sectional elevation through centre line (i), omitting cutting arm. II: end elevation from left-hand side of I, omitting cutting arm and part of frame F; a few teeth only are shown on the ratchet wheel. III: plan, omitting baseboard, pawl P and frame F. IV: sectional side elevation on centre-line (ii) from left-hand side. A, cutting arm; B, blade; Bl, block in frame; C, carriage for cutting table; $C1$, trigger-cam; $C2$, cam; F, frame within which moves the lever L which turns $Sh2$; L, lever moved by $C2$; P, pawl; R, rod on which the cutting arm falls; $Sp1$, spring which draws down the cutting arm; $Sp2$, spring which keeps L against $C2$; $Sp3$, spring which keeps pawl against the ratchet wheel; T, cutting table on which specimen is placed.

Motion of the specimen. Shaft 1 carries, as well as the trigger cam, an eccentric cam $C2$ which operates a lever L which is held against it by a spring $Sp2$. At each revolution of shaft 1, the end of the lever opposite to that bearing on $C2$ is raised, and carries with it the pawl P. This is held by a specimen moves only while the blade is withdrawn from it. The blade falls each time in a different part of the specimen, and thus cuts it to a series of slices. This is the second basic movement of the machine. In addition to these movements, certain adjustments are provided.

Adjustments. (1) The thickness of the slices cut is governed by the extent to which the specimen moves between each cut of the blade. Provision is made for altering this by limiting the extent which lever *L* moves at each rotation of shaft 1. The end of the lever farthest from the shaft rises and falls within a slit in a frame *F*. The slit carries a block *Bl* which can be clamped in various positions by a screw. The relative positions of the lever and frame are such that, when the block is at its highest point, the movement of the lever is enough to move the pawl over one tooth of the ratchet wheel; but, when the block is at its lowest point, it moves over 14 teeth. The wheel has 120 teeth and thus, at the minimum setting of the block, it moves 1/120 rev. and the table moves $\frac{1}{4\,5\,0}$ in. or 0·0264 mm. At the maximum setting the table moves 0·356 mm.

After a specimen has been sliced, the table is returned manually by lifting the pawl from its ratchet and rotating shaft 2 by a handwheel *H*2. As the pitch of the leadscrew is large, this can be done reasonably rapidly (the table moved 1 in. in 4 sec.) and other arrangements for returning the carriage were not considered necessary.

(2) The table rotates about a central, vertical pin. The vertical plane in which the specimen is cut is thus continuously variable. After the specimen has been cut to a series of slices, it can be rotated 90° and the slices cut to a series of prisms. For this purpose, there are cut in the rim of table *T* four aligning grooves which can be opposed to a groove in the carriage *C*.

(3) The extent to which the blade descends on its release is governed by contact between the arm *A* and the rod *R*. These are not adjustable, but the blade (an ordinary double-edged safety-razor blade) can be adjusted in relation to the arm. It is held to the arm by a single screw which passes through the centre hole or slit of the blade and carries a knurled nut. This bears on a strip of fabric bakelite, 4·5 × 2 cm., which is shaped to a shallow arc so that when the nut is tightened by the fingers, the blade is gripped over a large part of its surface, against the arm *A*.

The position of the blade in the arm is chosen as follows. Three moist filter papers are placed on the table, a blade loosely clamped to the arm, and the arm brought to rest against rod *R* by rotating handwheel 1. The blade is pressed by the fingers so that it is in contact with the paper along the whole of its lower cutting edge and clamped in this position by the knurled nut. The machine is then switched on. It will probably just cut the upper filter paper. This is removed. That below will probably be intact and ready to receive a specimen. During the cutting of a series of specimens, it may be necessary to add or remove a filter paper to bring the specimen to its correct height.

(4) The speed and force with which the blade descends can be adjusted by the spring 1. This is connected to a bridle with an adjustable screw. Suitable springs for use in this position were found empirically, and the following measurements were then made by temporarily attaching a spring balance to the arm at a point corresponding to the centre of the blade: to lift the blade without an attached spring, 50 g.; to lift the arm, while minimum tension was applied by a light spring, 200 g.; to lift it while maximum tension was applied to the same spring, 300 g.; to lift it while maximum tension was applied to a stronger spring, 650 g. The lighter spring has been used in most of the work described in this paper.

The blade falls a distance of 1·6 cm. at its centre point. The shape of the eccentric cam and its relationship to the arm and shaft 2 are such that after the fall of the blade, shaft 1 turns 45° before commencing to lift the arm. With the light spring at its minimum tension, the arm just failed to strike rod *R* when shaft 1 was running at 220 rev./min. The 1·6 cm. was, therefore, covered in 0·034 sec.; average velocity, 47 cm./sec. Assuming uniform acceleration, the final velocity was thus about 95 cm./sec. With the spring 1 at a maximum tension, this rose to about 150 cm./sec.

(5) A rheostat connected in series with the motor gave continuous variation in speed. A normal speed of operation has been 150 cuts/min. The machine can operate at more than twice this speed. The speed of operation does not affect the speed of fall of the blade, but does affect its rate of rise. Presumably because of this, a tissue which tends to stick to the blade is most successfully cut at slower speeds.

Manner of using the chopper

Small specimens. The specimen of tissue, or a portion of suitable size cut from it, is weighed on a torsion balance and placed on a sheet of hard filter paper (Whatman no. 50) already moistened with saline by quickly passing it through a dish of saline and sharply shaking off the excess. The filter paper and tissue are placed on the table of the machine. The table has previously been moved to the left of the blade, the blade adjusted in height, and the ratchet arm set to give the necessary thickness of section. The machine is switched on so that it cuts the specimen to strips. At this point the specimen can be removed, or the table can be turned through 90°, the specimen again brought to the left of the cutting blade, and the machine again switched on. After cutting, it is stopped with the blade out of the specimen.

The paper and chopped specimen are removed from the table, the paper held in the hand and the specimen lifted from it with a spatula moist with a drop of saline. When the specimen is on the spatula, the saline is allowed to drain into the paper and the specimen on the end of the spatula transferred to the experimental vessel, usually by shaking it under the fluid which is being used.

A typical size of specimen handled in this way, has been 15–200 mg. No difficulty has been found in transferring the whole of such specimens, after chopping, with a stainless-steel spatula.

Larger specimens. Specimens of several tissues up to 3 × 3 × 0·5 cm. or about 4 g. in weight, can be cut in a single loading of the machine. These have been handled either (*a*) by weighing beforehand and transferring entirely to a single experimental vessel, (*b*) by transferring to several vessels and weighing the tissue afterwards, or (*c*) distributing volumetrically, if the material permits handling as a suspension. In this case, either the whole or a portion can be weighed.

Distribution volumetrically can be as successful with chopped tissue as with the mince from a Latapie machine, but is not as successful as with the suspension from a Potter-Elvehjem homogenizer. It has been most successful with tissue placed on the table of the machine as a block not more than 2 mm. in thickness, and then cut to prisms not more than 0·2 × 0·2 mm. square in section. A pipette with a wide orifice close to the bulb has been employed.

Handling specimens before and after chopping. Little if any pretreatment is needed for many tissues, but the following three points should be noted: (1) A tough sheath of markedly different consistency from the rest of a specimen should be removed; e.g. that around the kidney, although equally

tough material such as skin can be successfully chopped alone. (2) The simplicity of operation with most tissues depends on their adhering to moist filter paper and not to the chopping blade. This can be aided in various ways. Passage of the blade is facilitated by moistening the blade or the top of the specimen with a camel-hair brush previously dipped in saline and squeezed between filter paper to the desired extent. It is best to moisten most specimens in this way. (3) An approximately flat surface is required on the specimen to cause it to adhere to the filter paper on the cutting table. Thus, a kidney must be cut in half and the portions laid on the cut surfaces, but a lobe of liver needs no preparation.

After cutting a specimen and transferring it to fluid, the individual fragments, although completely cut, need shaking or stirring to separate them. The shaking normally given during a metabolic experiment may be sufficient for this. Separation may also be done by the spatula used in transference, by jerking the vessel containing the fluid and specimen, or by holding the vessel for a second or so against the moving rubber-covered jaws of a Microid flask shaker or similar agitator.

Notes on construction and components

The base which is 14 in. (35 cm.) square has four rubber cushions on its under surface. It and the two main uprights which carry all shafts, are of $\frac{5}{8}$ in. (1·6 cm.) bakelized paper. The shaft holes are close running fits without bushes. Shaft *Sh* 2 which moves the carriage is held to the left (Fig. 3, I and III) by a flat helical spring. The carriage is guided on two rails *R* of round silver steel, by a V and flat machined in its under surface. It is held to the rails by flat springs of phosphor-bronze. The lever *L* and the pawl are of fabric bakelite and are held by springs at tensions of respectively 150 and 250 g. The cutting arm is also of bakelite, and the cams and ratchet wheel of brass. The cutting arm must be rigid and not tend to vibrate. For this reason it has long bushes (see Fig. 3, III) which are close fits on the shaft on which it pivots, and the arm itself is $\frac{3}{8}$ in. (0·95 cm.) thick.

The motor is a universal series-wound type of $\frac{1}{3}$ h.p., built to run at its driving shaft (after a reduction gear), under load, at 300 rev./min. with a torque of 9 lb. in. Its speed is controlled by a resistance (280 Ω) in series with the motor. Also in series with the motor is a two-pole switch mounted on the front panel and seen in Figs. 1 and 2, Pl. 1. The model of motor shown in these photographs may not be generally available, but an equivalent is Type SA 90-W 50 supplied by Fractional H.P. Motors Ltd., London, N.W. 9. (this would require a resistance of 490 Ω).

Ordinary safety-razor blades with two cutting edges of 3·7 cm. were used, after removing their waxy coating. Each edge sufficed for several thousand chopping blows, or for cutting some scores of specimens, without deteriorating. They were kept from day to day by drying with filter paper after use. In practice, a new blade has been used every few days as their cost is small. Stainless-steel blades, kindly given by Prof. A. St G. Huggett, have the advantage of being packed without wax and probably last longer. Blades which are thicker than the type illustrated in Fig. 3, IV, and which project farther from the cutting arm, can be made from strips of blading supplied by manufacturers of the types of safety-razor blades which have single cutting edges.

In an earlier model of the machine, the blade was given an oscillating motion by a driving wheel and a link mounted eccentrically on it. This proved adequate for cutting certain tissues only; the downward speed of the blade was dependent on the speed of operating the machine as a whole, and was minimal at the base of the specimen.

Metabolic and electrical techniques

In the examples quoted in the tables, conventional manometric apparatus was used with the following additions. Electrode vessels were pattern *E* of McIlwain (1951*b*). Electrical stimulation was by diphasic condenser pulses, 100/sec., of time-constant 0·35–0·5 msec. (McIlwain, 1951*a*) from a mains-operated stimulator (McIlwain & Ayres, unpublished). The glucose-phosphate and glucose-bicarbonate salines were those described by McIlwain (1951*a*). Experiments were run at 37°, in O_2. Tissues were obtained and prepared as described by McIlwain (1951*a*), and McIlwain *et al.* (1952), and were mostly from guinea pigs and rats. We are greatly indebted to Mr Murray Falconer, Director of the Guy's-Maudsley Neurosurgical Unit, for specimens of human cerebral tissues which, though removed for therapeutic purposes, were macroscopically normal.

RESULTS

Qualitative observations

Most animal tissues and many organs were cut with the machine and yielded preparations suitable for metabolic work. Tissues satisfactorily cut included spinal cord, which is soft; skin, which is tough; and lung which has a loose texture and fibrous elements. Brain, liver, kidney and heart muscle presented no difficulties.

Central nervous system. Specimens from the brain were among the easiest to cut with the machine. No preparation, apart from choice of the area to be cut, was necessary with specimens from small laboratory animals, but membranes and blood vessels closely adhering to the surface were removed from human specimens prior to chopping. White and grey matter were cut together in the same specimen without distortion or tendency to separate. This can be seen in the cerebellar slices included in Pl. 2. Slices made by setting the machine at 0·21 mm. thickness of cut are remarkably uniform in thickness. This can be seen in the photograph of cerebral cortex, chopped in two directions at right angles and with this thickness of cut. The initial specimen in this case was about 3 mm. thick, so that it yielded prisms 0·21 × 0·21 × 3 mm., of which the long sides were formed entirely by the cutting. They are seen to be uniform. Each prism carries at one end a portion of the subcortical white matter. Although the cut tissue has been shaken with moderate vigour to separate its individual sections, these have not markedly fragmented.

Specimens were cut equally well in planes bearing various relationships to the direction of the fibres they contained. Thus spinal cord, after removing its sheath, could be prepared for cutting as disks of

2 or 3 mm. in thickness made by cross-sectioning, and then chopped parallel to the direction of the main fibres; or 1 or 2 cm. of the cord could be split longitudinally by extending the medial fissures, laid on the table on its cut surfaces and chopped perpendicularly to the fibres. Portions of the brain stem of a guinea pig could be dissected out, weighed, chopped and transferred without loss to an experimental vessel. So also could the brain of a mouse, or the complete cerebral hemisphere of a guinea pig.

The downward speed of the blade was not found critical in cutting specimens from the central nervous system. Such specimens could also be cut with the earlier model of the machine. Quantitative data are given below regarding the metabolic behaviour of chopped cerebral tissues.

Liver. Specimens from any position in the organ were chopped to slices or prisms by cuts at least as small as 0·2 mm. When a worn blade was used, it was evident that material from the edge of a lobe cut more easily than from the centre, where vascular or fibrous elements were larger. In material from laboratory animals these presented no difficulty. Their presence, however, made the slices or prisms less regular than those obtainable from cerebral tissues. This is shown in Fig. 6 (Pl. 2), in which chopped guinea pig liver is clearly not so uniform as is chopped cerebral cortex. The specimen was seen to spread during chopping so that it occupied a slightly larger area on the cutting table after the chopping than it did before.

A complete lobe of guinea pig liver without initial preparation (some 3 × 2 cm. and 3 g. in weight) was accommodated on the table and chopped at 0·32 mm. intervals in two directions in 1 min.

Spleen. This behaved much as did liver.

Kidney. A kidney of up to 2 g. from a guinea pig or smaller animal was accommodated in the machine. It was prepared by removing the transparent outer sheath, cutting in half by a longitudinal cut through the hilus, and placing on the paper of the machine with the cut surfaces downwards. When so prepared, the medulla was chopped nearly as cleanly as the cortex. This is very difficult to achieve by cutting (as distinct from chopping), with a blade either free-hand or with a template. When the medulla was freed from cortex before chopping, it tended to be picked up by the blade. Cortex alone presented no difficulty. Fig. 7 (Pl. 2) shows slices obtained by chopping a portion of guinea pig kidney. The majority, but not all, are complete cross-sections of the specimen. When chopped to prisms, these were of a regularity intermediate between those shown for cerebral cortex and liver.

Adrenal. Outer sheaths were removed from guinea pig or rabbit adrenals, they were cut in half along their largest plane and placed on the cutting table on the cut surfaces. Sections of 0·2 or 0·3 mm. were made successfully and included both the cortex and medulla. If they were suspended in saline and shaken strongly, the medulla dispersed. Glands were also cut on several occasions by laying them on their largest flattish surface, without preliminary section.

Lung. Portions of guinea pig lung, without preparation, were cut to 0·2 or 0·35 mm. slices, but these tended to remain connected by fine fibres in that part of the specimen which had been applied to the filter paper on the cutting table. They could then be detached by scissors or a scalpel. The tendency to remain attached could be minimized by allowing the blade to chop through not only the specimen but also a layer of the filter paper on which it was placed. When part of the outer surface of the specimen, especially the angles running the length of the lobes, was removed with scissors before chopping, the attachment was minimized. Slices obtained by chopping were much more regular than can normally be prepared from lung by cutting with a blade.

Heart. The muscular wall of the heart, taken from various positions, was cut satisfactorily to sheets 0·2 or 0·35 mm. in thickness. The sheets curled after suspending in salines, but not to such an extent as would be expected to hinder diffusion to their interior.

Abdominal wall. The specimens examined were 2–3 mm. in thickness with two main layers between which lateral motion could occur. No difficulty was encountered in cutting them with a fairly fresh blade. After suspending in saline, the strips curled.

Skin. This offers a good example of how qualitative observation with a swung scalpel (see p. 412) agrees with the performance of the machine. Skin lying on moist filter paper is relatively difficult to cut with a scalpel in the ordinary way, unless tension is applied to the skin, but it can easily be cut with a sharp blow of the scalpel. For cutting in the machine, skin from the neck or abdomen of a guinea pig was plucked free of most of its hairs, cut out with scissors at approximately the level of the attached muscles, and laid outer surface uppermost on the paper of the machine. It was cut to strips 0·35 or 0·2 mm. in thickness. These tended to collect on the blade rather than to remain on the paper.

Metabolic measurements

The metabolic characteristics which are dependent on cell structure and which it was desired to preserve in the chopped tissue, are exhibited by sliced, but not by 'homogenized', tissues. In the following studies, the chopped preparations were therefore compared with 'homogenates' and, especially, with slices from the same material. Some data regarding the relative size and area of the preparations used are given in Table 1.

Table 1. *Weight and area of different tissue preparations*

(Tissue density is taken as 1. 70 mg. is the weight of tissue used in typical experiments, suspended in 3·5 ml. fluid.)

Preparation (mm.)	No. of pieces in a specimen of wt. 70 mg.	Wt. of individual fragments (mg.)	Area of individual fragments (sq.mm.)	Area of pieces in 70 mg. sample (sq.mm.)
Slice 0·35 × 20 × 10	1	70	421	421
Slice fragments 0·35 × 2·5 × 4	20	3·5	24·6	491
Chopped as prisms 0·2 × 0·2 × 2	875	0·08	1·68	1470
Chopped as prisms 0·067 × 0·067 × 2	7875	0·009	0·545	4292

Table 2. *Level and persistence of respiration in preparations of guinea pig cerebral cortex*

(The tissue was cut or 'homogenized' about 3 min. after death of the animals, and respiratory measurements began 25 min. after death, using glucose-phosphate saline. The 'homogenate' was made in a glass apparatus (Potter & Elvehjem, 1936) with 1 part of tissue and 3 parts of the same saline, 'homogenizing' for 10 sec. Slices were 0·35 mm. thick and cut by hand with a blade and template. Chopped tissue was prepared with the machine set to give prisms 0·21 × 0·21 mm. square in section.)

Preparation	Respiration (μmoles O_2/g./hr.) at different times after placing in thermostat			
	0–30 min.	30–60 min.	60–90 min.	100–130 min.
Sliced 18–22 mg./ml.	63, 57, 61	—	63, 59, 60	65, 62, 60
Chopped 18–22 mg./ml.	55, 61, 59	—	57, 62, 57	57, 59, 56
'Homogenized' 83 mg./ml.	33	27	24	—
'Homogenized' 42 mg./ml.	23	19	24	—
'Homogenized' 21 mg./ml.	24	18	13	—

Respiration and aerobic glycolysis of preparations from cerebral cortex. Measurements were made with glucose as substrate in a balanced saline which supported respiratory rates of 60–75 μmoles/g./hr. for slices of guinea pig or rat cortex. Table 2 and the first column of Table 3 show that tissue chopped in the machine respired at rates comparable to those of slices made in the ordinary fashion. Sufficient data are available in Tables 2 and 3 to suggest that the chopped tissue has rates of respiration only some 1–3 % lower than that of slices. 'Homogenized' tissue, on the other hand, gave initial values some 60 % lower than slices when examined at comparable tissue concentrations. Much higher rates of respiration can be obtained for 'homogenates' of cerebral tissues when these are examined at higher concentrations and in enriched media (see, for example, Elliott & Libet, 1942; Case & McIlwain, 1951), but such conditions are not relevant to the present comparison. Moreover, the respiration of chopped tissue was practically as stable with time as was that of the slices normally used in metabolic studies (Table 2). Whereas respiration of 'homogenates' had fallen to 50–60 % of its original rate in 90 min., that of the chopped tissue was stable for periods of over 2 hr.

The lactic acid which accumulated during aerobic metabolism with glucose as substrate was also determined (Table 3). The accumulation in experiments with machine-chopped tissue and with hand-cut slices was similar, but significantly greater with the chopped tissue. Such accumulation is a relatively labile property of the tissue, and changes considerably during the progress of an experiment with sliced tissue (McIlwain & Grinyer, 1950). The difference between the accumulation with chopped and sliced tissue is less than that which occurred spontaneously with time, during experiments with slices.

In a few instances, human cerebral tissues were available in sufficient quantity for slices to be compared with chopped preparations (Table 3). The respiratory rates were in all cases lower than those with rat or guinea pig (cf. Elliott & Penfield, 1948; McIlwain *et al.* 1952). Comparison between sliced and chopped tissue gave results similar in all respects to those with animal tissues.

Metabolic response to electrical stimulation

Little response to electrical stimulation is shown in simple 'homogenates' in the present medium (McIlwain, 1951*a*) in distinction to the effect clearly shown by slices. Mechanically chopped tissue behaved in this respect like slices (Tables 2 and 3). Respiration of both preparations of guinea pig cerebral cortex nearly doubled with the greatest stimulus. With the lesser stimulus, response of the chopped preparation was significantly lower than the response of slices. With preparations from rat, the same tendencies were evident. The respiration of human cerebral cortex, after chopping, also responded well to stimulation.

The aerobic formation of lactic acid by cerebral tissues also increased on application of electrical

Table 3. *Metabolic response to electrical impulses in mechanically chopped and in hand-sliced cerebral cortex*

(Experiments were in vessels *E* (McIlwain, 1951*b*) with concentric electrodes between which the tissue floated. About 70 mg. of tissue were used in each case, with 3·5 ml. of glucose-phosphate saline. The slices always consisted of approx. 20 pieces (thickness 0·35 mm.) in order that they, like the chopped tissue, should be distributed between the electrodes. Respiration was determined from 5 min. manometric readings. The values which are summarized in column *a* were derived from vessels observed for periods of at least 30 min., and in columns *b* and *c* are derived from subsequent periods of observations with some of the same vessels (not every vessel being subjected to the two voltage ranges). Accumulation of lactic acid was determined by Barker & Summerson's (1941) method in the vessel contents at the end of the manometric experiments. The values of column *d* are derived from vessels not exposed to impulses, and of column *e* from vessels which were so exposed and of which the respiration furnished data incorporated in columns *b* and *c*.

With a given species, the individual experiments from which are derived the values for tissues 'sliced' and 'chopped at 0·21 mm.' were matched with respect to length of metabolism and impulse characteristics so that the mean values for the groups are comparable. The length of experiments and impulse characteristics varied between different experiments in the same group, and this contributed to the variation in values for lactic acid (see McIlwain, Anguiano & Cheshire, 1951).

Under 'respiration' and 'accumulation of lactic acid', mean values are in bold type, with below, the number of individual values and S.D. Individual values marked by an asterisk (*).)

		Respiration			Accumulation of lactic acid		
		a	*b*	*c*	*d*	*e*	*f*
Species	Preparation	Unstimulated (μmoles O_2/ g./hr.)	Stimulated at 10–12 V. (% of *a*)	Stimulated at 18–22 V. (% of *a*)	Unstimulated (μmoles/ g./hr.)	Increase (μmoles/ g./hr.)	Stimulated rate (% of *d*)
Rat	Sliced by hand	**75** (12; 4·0)	**145** (3; 4·9)	**180** (6; 18)	**31** (4; 12·3)	**48** (5; 17·9)	**274** (5; 54)
Rat	Chopped at 0·21 × 0·21 mm. by machine	**72·3** (12; 6·9)	**131** (3; 8·5)	**168** (6; 16)	**35** (4; 12·5)	**31** (5; 9·2)	**202** (5; 35)
Rat	Chopped at 0·1 × 0·1 mm. by machine	**56** (4; 5·3)	**139** (4; 14·1)	**181** (4; 15)	24*, 28*	56*, 38*	332*, 236*
Guinea pig	Sliced by hand	**60·8** (18; 6·0)	**157** (8; 15)	**193** (7; 25)	**21** (7; 5)	**39** (9; 12·3)	**288** (9; 51)
Guinea pig	Chopped at 0·21 × 0·21 mm. by machine	**59·7** (18; 5·5)	**133** (8; 14)	**192** (7; 22)	**35** (7; 5·6)	**37** (9; 17·8)	**203** (9; 43)
Guinea pig	Chopped at 0·067 × 0·067 mm. by machine	**57·0** (4; 8·9)	117*	153*	21*, 26*	16*, 28*	176*, 218*
Man	Sliced by hand	**36·0** (6; 4·0)	126*, 149*	197*, 188*	33*	25*	176*
Man	Chopped at 0·21 × 0·21 mm. by machine	**38·7** (6; 6·0)	166*, 129*	206*, 188*	44*	41*	193*

impulses (Table 3). The increase was large, and corresponded to a doubling or trebling of the rate of accumulation of the acid during the periods of stimulation. The increase with chopped tissue approached that with sliced tissue, when measured by the increase in lactic acid formed. When the rate of formation of lactic acid during stimulation was expressed as a percentage of its unstimulated rate of formation, values for the chopped tissue were, however, smaller. The more rapid formation of lactic acid in unstimulated chopped tissue contributed to this.

Effect of fineness of chopping and direction of cut. A few experiments are included in Table 3, in which tissue has been chopped to prisms of cross-section 0·1 × 0·1 mm. and 0·067 × 0·067 mm. Both respiration and aerobic accumulation of lactic acid were somewhat lower than with tissue chopped to pieces of cross-section 0·21 × 0·21 mm. The response to electrical stimulation was not markedly altered in the preparation at 0·1 × 0·1 mm., but was less in the instance examined at 0·067 × 0·067 mm. The individual fragments have then one-quarter or one-ninth of the volume of fragments of the usual size; for other data, see Table 1.

Tissues have normally been chopped in two directions at right angles to each other, and each at right angles to the outer surface of the tissue specimen. With cerebral cortex from experimental animals this was also at right angles to the lateral ventricles. In biopsy specimens such a surface is not always available, and the effect of direction of cut was therefore examined. The usual block was cut with a scalpel to strips about 2 mm. in width, these were turned on their main axis by 90°, and then chopped in the usual manner. Their respiration and its response to electrical stimulation were normal.

Other metabolic observations. The following findings are the results of one or two experiments in each case, and metabolic rates are not quoted. The respiration rates of mechanically chopped (0·21 × 0·21 mm.) and hand-sliced cerebral cortex were compared in

glycylglycine-glucose saline, in the absence and presence of electrical impulses, without finding marked differences between the preparations. The approximate course of aerobic acid formation from glucose in the bicarbonate saline was followed with the same result. The tissue chopped at 0·21 × 0·21 mm. should be fine enough to be adequately oxygenated by air, and rates of respiration were found to be very similar in air and oxygen.

DISCUSSION

Most of the apparatus introduced during the past 20 years for preparing tissues for biochemical work destroys cell structure. This is true of Potter & Elvehjem's (1936) homogenizer, of the Waring Blendor, and of other similar apparatus (e.g. that of Folley & Watson (1948)). A notable exception is Stadie & Riggs's (1944) template for cutting slices (see also Deutsch & Raper, 1936), but this requires larger specimens of tissue than normal slicing techniques and so was not applicable to the present problems.

From time to time, experimental descriptions have included references to tissue preparations largely made manually, but which were probably partly analogous to the present one, without, however, giving full descrptions of the methods or of the properties of the product in comparison with other methods of preparing tissues. Some (but not all) minces, breis, and scissor-cut tissues may have approached the condition here described as chopped. Dickens (1941) briefly reviewed such methods and pointed out that they were then much less used than before. The articles edited by Potter (1948), which include descriptions of a variety of methods of preparing tissues, do not describe a method comparable to chopping. It would appear that the introduction of blenders and homogenizers had largely displaced the manual preparation of breis. This is understandable as Potter & Elvehjem's (1936) apparatus is very convenient, is applicable to small specimens, and was immediately applied with success to problems which required breakdown of cell structure. That it was also applied to problems which would better have been tackled with preparations retaining cell structure, was largely overlooked.

The relatively indefinite nature of manually prepared 'breis' and analogous preparations also contributed to their replacement, though it was later realized that 'homogenates' also vary considerably in biochemical properties according to whether they are 'fine' or 'coarse'—properties usually not adjusted quantitatively but assessed according to an approximate estimate of the degree of fit of the homogenizer, the force exerted during its use or the length of time for which it was run. By contrast, fineness of cut in the present machine is predetermined and reasonably precise. Moreover, it can be made in planes selected in relation to structural elements in the tissue being cut, and in many cases yields suspensions which can be pipetted and the tissue distributed by volume of suspending fluid. Blenders are akin to the present machine in depending on speed of the cutter and inertia of the tissue for their disintegrating effect, which is, however, random and very destructive of cells.

The tables indicate that fragments of cerebral cortex of only a few micrograms in weight behave metabolically in a fashion radically different from 'homogenates' of the same tissue. It is especially noteworthy that metabolic response to electrical impulses is retained in such minute fragments. In general, machines of this type would appear to have a wide range of potential uses, much beyond those for which the present model was designed.

SUMMARY

1. A machine is described which produces slices and suspensions of fresh tissues with minimum disruption of their cellular structure. It reduced specimens of several animal tissues to slices down to 0·2 mm. in thickness, and to prisms down to 0·067 × 0·067 mm. in cross-section. It has been applied to liver, spleen, kidney, adrenal, lung, heart, abdominal wall, skin, and especially to tissues of the central nervous system.

2. A convenient preparation which can be made from many tissues in 30 sec. consists of prisms 0·2 × 0·2 mm. in section and 2 mm. long. This can be suspended in salines, and sampled as a suspension.

3. Many such suspensions were made from rat, guinea pig, and human cerebral hemispheres, and compared metabolically with slices or 'homogenates' from the same tissues. In a glucose saline suitable for respiration of tissues, the chopped material respired at rates within 3% of those of ordinary hand-cut slices, and markedly more rapidly than 'homogenates'. Respiration by the mechanically chopped tissue was stable with time over a period of at least 2 hr., during which that of 'homogenates' fell considerably. Aerobic accumulation of lactic acid in systems with the chopped tissue was similar to, but in some cases rather greater than, that in systems with ordinary slices.

4. The rates at which mechanically chopped cerebral cortex respired and formed lactic acid were doubled or trebled by the electrical impulses which induced such changes in sliced cerebral cortex.

5. The properties described in paragraphs 3 and 4 indicate that structure which is lost in 'homogenates' is largely retained in the chopped pre-

parations. The present method is especially applicable to preparing biopsy and other small specimens of tissue for metabolic examination of processes which are controlled by structural factors.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Figs. 1 and 2. The chopper. *A*, cutting-arm carrying blade *B*; *H* 1, handwheel on shaft *Sh* 1 which also carries the two cams; *H* 2, handwheel on shaft *Sh* 2 which also carries the leadscrew. *T*, cutting table on the carriage *C* which is moved by the leadscrew. *Bl*, adjustable block whose position in the frame *F* conditions the thickness of section cut.

PLATE 2

Guinea pig tissues prepared with the chopper and photographed, while indirectly illuminated, in a shallow layer of saline in dishes with black background. The whole of the specimens chopped have been transferred for photographing.

Fig. 4. Cerebellum cut at 0·26 mm. intervals. Note the cortical layers and white matter which show little tendency to separate. There is also little debris.

Fig. 5. Cerebral hemisphere cut at 0·21 mm. intervals in two directions at right angles. The specimen cut was a block 5 mm. square taken from about the middle of the hemisphere, extending from its cortical surface to the lateral ventricle. For cutting, it was laid on the subcortical white matter. A portion of this is seen at one end of each fragment.

Fig. 6. Liver cut at 0·3 mm. intervals in two directions at right angles.

Fig. 7. Kidney cut at 0·26 mm. intervals.

The Occurrence of *m*-Hydroxybenzoic Acid in Urine

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In a study of the excretion of oxidation products of naphthalene by rabbits (Boyland & Wiltshire, 1952), 1-naphthol was estimated in urine as the blue indophenol formed with 2:6-dichloroquinone chloroimide (phenol reagent). Under the conditions described at pH 9·2, this indophenol was completely extracted into butanol. It was noticed that the urine of rats and rabbits dosed with naphthalene or 1-naphthol contained in addition a phenol which reacted to give a blue indophenol which was not extracted by butanol. The compound could not be detected regularly in urine of normal animals by this method, but it could be detected and estimated by chromatography and was isolated from such urine. The amount of the substance appeared to be increased by acid hydrolysis. The compound isolated was shown to be *m*-hydroxybenzoic acid, which Bray, Lake, Thorpe & White (1950) had detected by paper chromatography as a constituent of normal rabbit urine.

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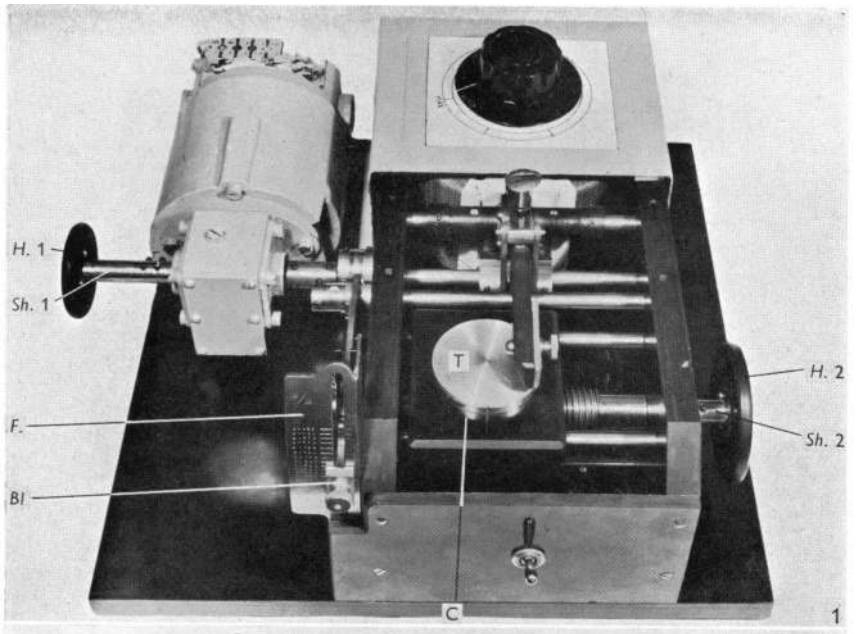

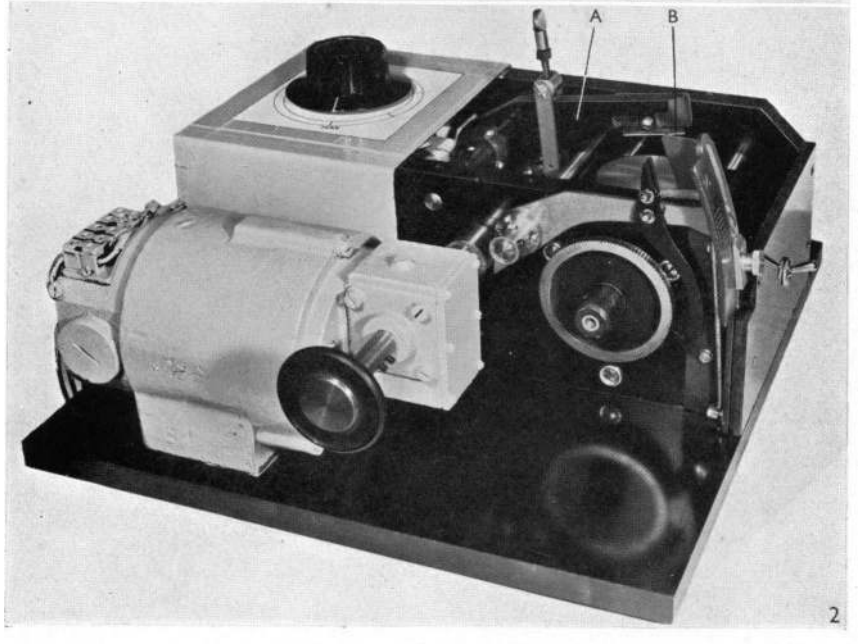

Figs. 1–2.

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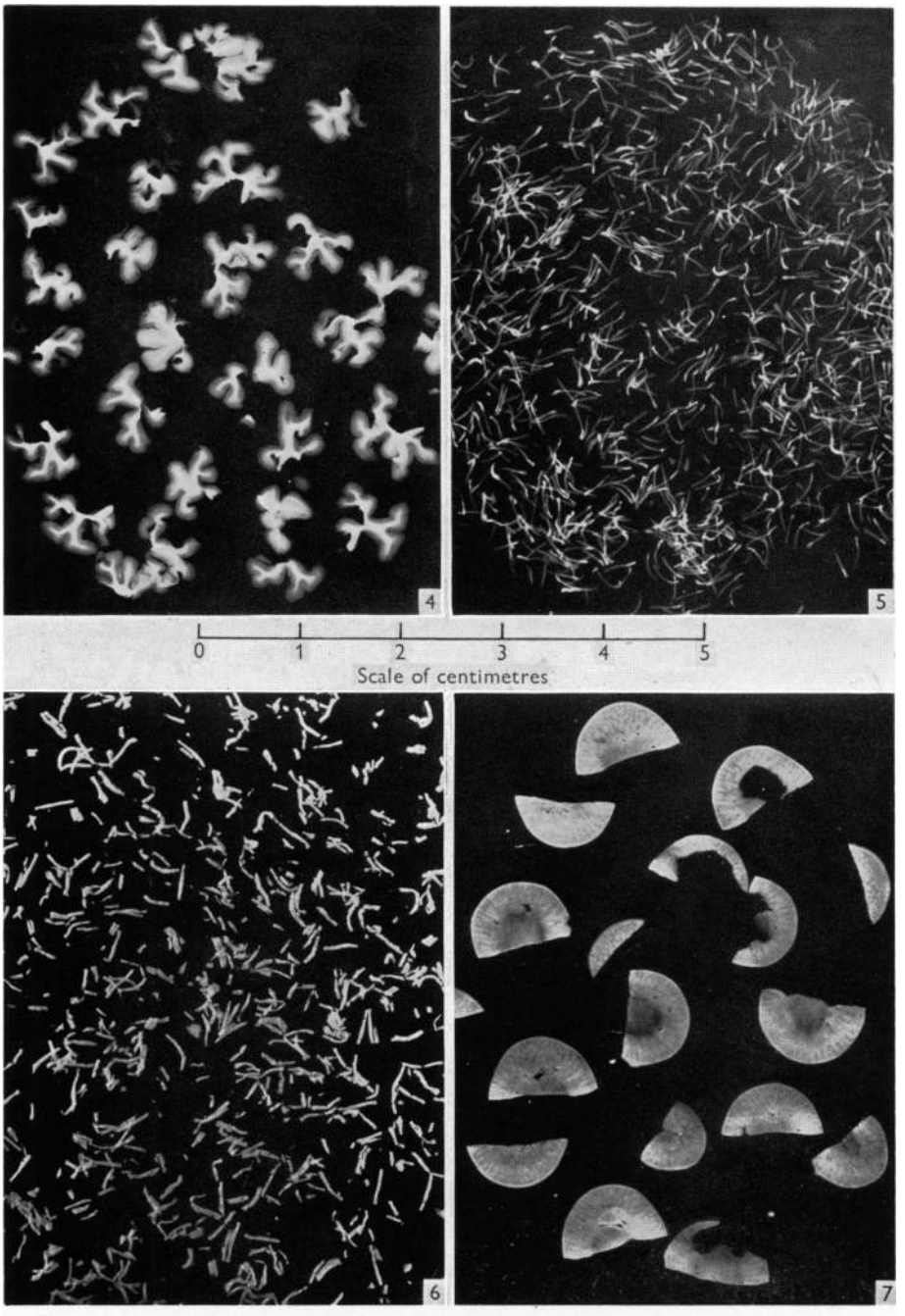
1. A MECHANICAL CHOPPER



Figs. 4–7.

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1. A MECHANICAL CHOPPER