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A Hand-operated Tissue Chopper

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(*Received* 11 *May* 1955)

The mechanical chopper devised by McIlwain & Buddle (1953) for the preparation of tissue slices marks an important advance in manometric technique. The machine prepares slices more rapidly and more uniformly than any previous device; furthermore, it allows small and cheap animals to be used; for example, mouse liver and kidney may be chopped as easily as those of the rat. It seems likely that the tissue chopper in one form or another will be widely adopted.

The chopper of McIlwain & Buddle (1953) was designed to allow quick adjustments, a principle leading to an ingenious but intricate design. In consequence their machine is available only to those laboratories which command the services of skilled mechanics. In practice, frequent adjustment of the tissue chopper is not essential; once the machine has been set to cut slices of a given thickness, for example, months may elapse before it is desired to alter the slice thickness; further, there is little virtue in being able to make an adjustment in 1 sec. instead of, say, 10. This paper describes a chopping machine, based on the principle of McIlwain & Buddle (1953), but designed to be as simple as possible, so that skilled labour is not required for its construction, and of heavy construction, so that many years of trouble-free service will be obtained. The first requirement has been achieved at the expense of simple and rapid adjustment. The second principle leads to the use of ferrous metals and brass throughout, so that, apart from being of assured durability, the machine absorbs its own vibration.

One feature of the technique of using the chopper merited investigation. In their instructions McIlwain & Buddle (1953) recommend that the tissue be weighed, chopped and then transferred to the experimental vessel. If, as a general principle, the rate of oxygen consumption of tissue slices is referred to the wet weight before rather than after chopping, it is implied that a negligible proportion of the cells are injured in chopping; this seemed unlikely so the point was investigated.

CONSTRUCTION OF THE MACHINE

The main features of the machine are shown in Figs. 1–5.

The body is of bright mild-steel flats, sides $3 \times \frac{5}{16} \times 8$ in., ends $3 \times \frac{1}{4} \times 3\frac{3}{8}$ in. The base is a selected cast-iron retort-stand base 9×12 in. All shafts are of $\frac{3}{8}$ in. diameter silver steel. The steel screw, obtained from a government surplus dealer, is $\frac{5}{16}$ in. $\times$ 10 threads/in., square thread, with a brass nut $\frac{1}{2} \times \frac{1}{2} \times \frac{7}{8}$ in.; the brass ratchet wheel is 64 diametrical pitch, 100 teeth, so that each tooth corresponds to 0·001 in. (25·6 μ.) movement of the table; the driving wheel was made from a 3 in. diameter model locomotive-wheel casting. The table and connecting-rod ends were made from brass, B.S. 249:1940; the cam and plates of the ratchet mechanism were brass, B.S. 251:1940. Fixings were high-tensile steel hexagon head B.A. bolts with spring washers. The chopping lever was forged from a bright mild-steel bar $\frac{3}{4} \times \frac{1}{4}$ in. The drawings are largely self-explanatory. Provision was made for the lubrication of all bearings on the principle shown in Fig. 5; the oil holes (not generally shown) were 0·066 in. in diameter, tapped 8 B.A. to receive $\frac{1}{4}$ in. screws as dust excluders. Other features not indicated in the drawings are: (i) the fixing of the body to the base, by means of six 4 B.A. bolts; (ii) the attachment of the ratchet wheel to the screw spindle by a $\frac{1}{32}$ in. silver-steel pin; (iii) the connecting-rod halves were brazed into their ends; (iv) the driving pawl was brazed to its spindle; (v) the silver-steel pin for the razor blade was press-fitted into the lever. Extensive use has been made of the principle of the clip boss, which was used to attach not only the wheels but also the levers and collars to shafts.

Potential difficulties have been eliminated as follows: (i) the necessity for aligning the connecting-rod ends and obtaining the optimum length was avoided by making the connecting rod in two parts joined by a pair of bosses, so that it is fully adjustable; (ii) the requirement of perfect alignment of the table guides and screw was circumvented by not fixing the nut to the table, while retaining a positive location of the nut by a spring.

It is not necessary that the chopping lever should be geometrically true, but experiment showed that the performance of the machine was considerably improved if the razor blade lay at an angle to its plane of movement, so that the bevel facing the unchopped tissue was collinear with this plane, as shown in Fig. 5 *B*. The precise angle will depend on the make of razor blade, and in this case was 5° 30′. This requirement is easily met by judicious forging of the lever between the bend and the hole for its shaft. To prevent lateral movement of the chopping-lever shaft, it was held by a pair of adjustable collars.

Use of the machine follows the principles laid down by McIlwain & Buddle (1953). The table is returned to the starting position by a turnscrew inserted in the slot provided in the ratchet assembly. To ensure that the filter paper under the tissue could not slip, it was held by a clip and bolt which was simply tightened by the fingers. Adjustment of slice thickness is obtained by varying the eccentricity of the driving connecting-rod end. The height of rise of the chopping lever is controlled by altering the rotational position of the lever *L*2 which the cam actuates. The force with which the chopping lever descends is not variable since it was found that a very wide range of lever spring tensions were acceptable, and the necessity for adjustment does not arise; in practice the lever-spring tension was chosen empirically with regard to the speed at

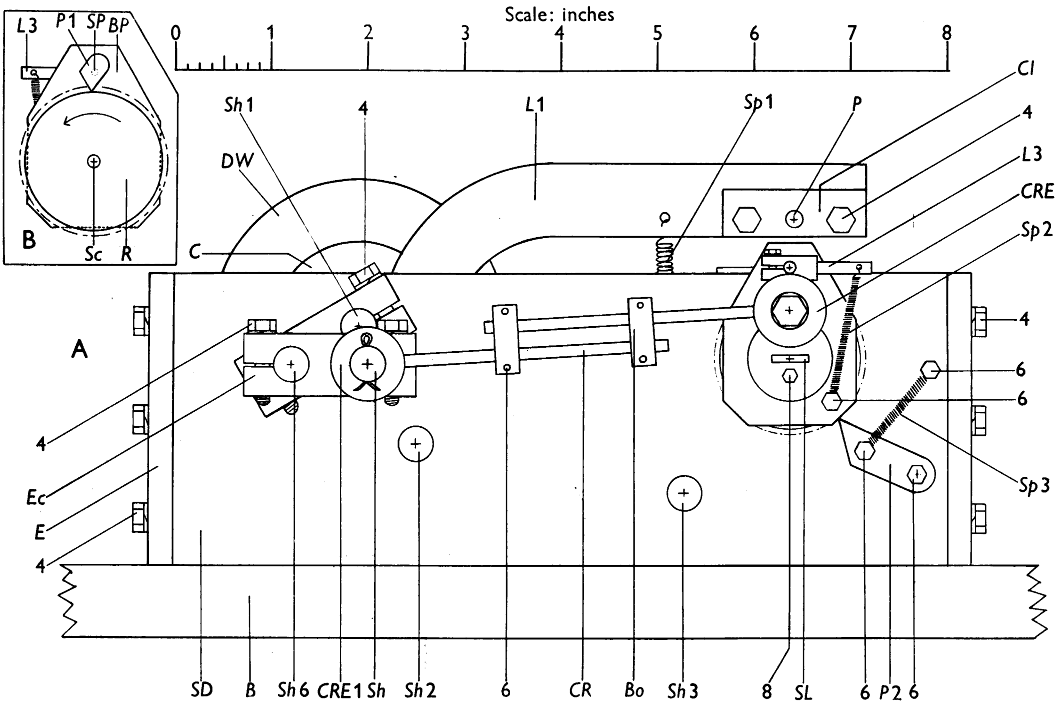

Fig. 1. A: side elevation showing reciprocating and ratchet mechanisms. Omitted: washer between split pin and connecting-rod end; chopping-lever stop; razor blade. B: elevation of ratchet mechanism, viewed from inside. The arrow shows the direction in which the ratchet wheel is driven.

Key to Figs. 1–5: *B*, base, cast iron 10 × 12 in.; *Bo*, boss for connecting-rod halves; *BP*, brass plate; *C*, cam; *CB*, detachable clip boss; *Cl*, razor-blade clamp; *Co*, adjustable collar; *Cp*, clip to hold filter paper; *CR*, connecting rod; *CRE*1, connecting rod, driving end; *CRE*2, connecting rod, driven end; *DW*, driving wheel; *E*, end of body; *Ec*, variable eccentric mechanism; *H*, handle of driving wheel; *HL*, hole for lubrication of bearing; *L*1, chopping lever; *L*2, lever actuated by cam; *L*3, lever for spring of driving pawl; *LN*, lock nut and distance piece; *N*, nut; *P*, silver-steel pin; *P*1, driving pawl; *P*2, second pawl, to prevent table from moving back during return movement of *P*1; *R*, ratchet wheel, brass, 64 diametrical pitch, 100 teeth; *RC*, ratchet mechanism cap; *S*, driving pawl spindle; *Sc*, screw, $\frac{5}{16}$ in. × 10 threads/in. square thread; *SD*, side of body; *SL*, slot for turnscrew, to return table to starting position; *Sh*, eccentric shaft, bearing *CRE*1; *Sh*1, driving shaft; *Sh*2, lever shaft; *Sh*3, shaft for lower attachment of *Sp*1; *Sh*4 and *Sh*5, table guides; *Sh*6, eccentric mechanism shaft; *SP*, driving-pawl spindle; *Sp*1, chopping-lever spring; *Sp*2, driving pawl spring; *Sp*3, second pawl spring; *Sp*4, lock spring; *ST*, lever stop, a $\frac{1}{4}$ in. B.S.F. bolt; *T*, table; *T*4, holes tapped 4 B.A.; *W*, washer; 2*A*, steel bolt 2 B.A. Arabic numerals 4, 6 and 8 refer to B.A. sizes of bolts, which are not drawn to scale.

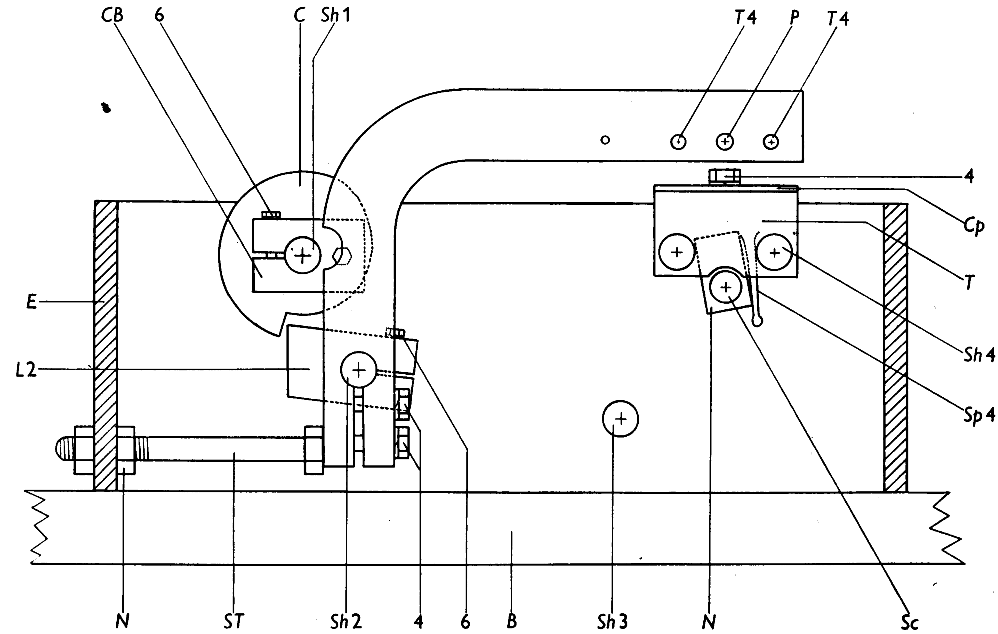

Fig. 2. Side elevation showing lever mechanism; reciprocating and ratchet mechanisms, near side of body, proximal collar and washer of lever shaft, razor blade and clamp removed. (For Key, see p. 373.)

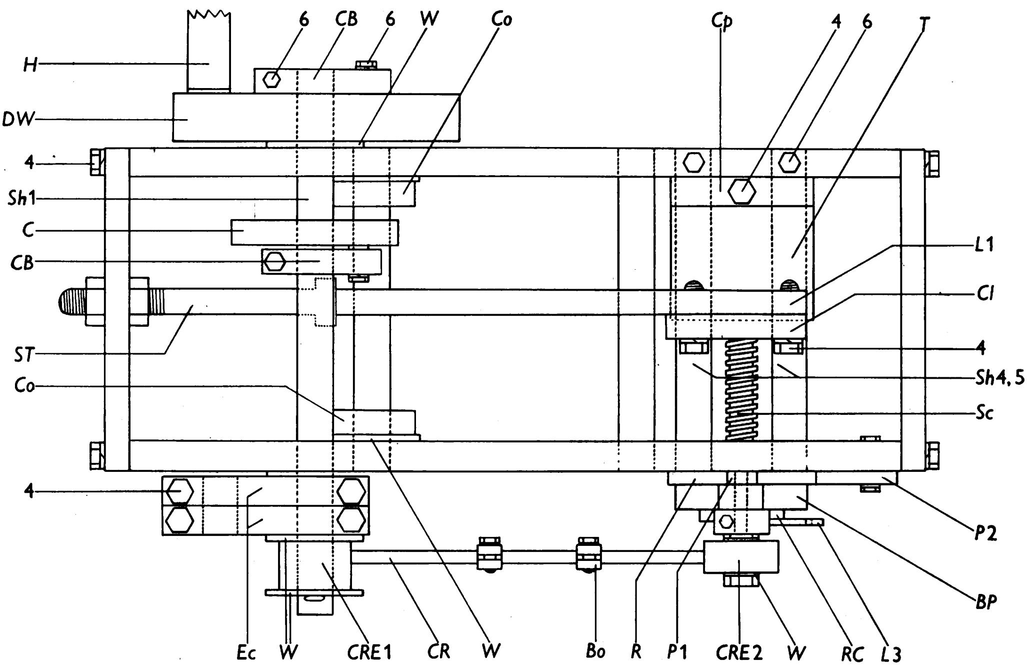

Fig. 3. Plan. Omitted: all springs and lever $L2$; the eccentricity of the connecting-rod end $CRE\,1$ has been made zero for simplicity. (For Key, see p. 373.)

which the machine would be operated. The depth of descent of the chopping lever is controlled by the stop bolt; it was found advantageous to stick a piece of $\frac{1}{8}$ in. thick rubber on the head of this bolt, so that the machine operates almost silently.

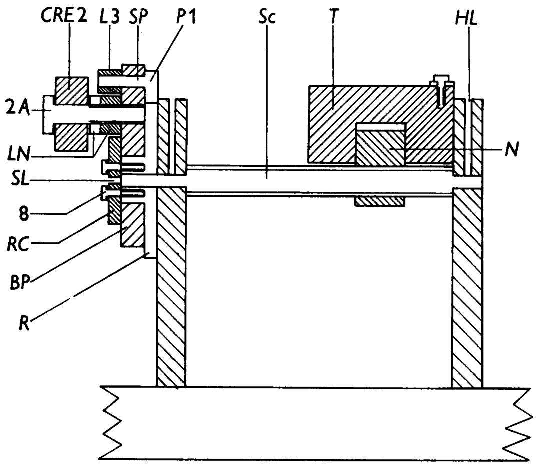

Fig. 4. Vertical section through screw. (For Key, see p. 373.)

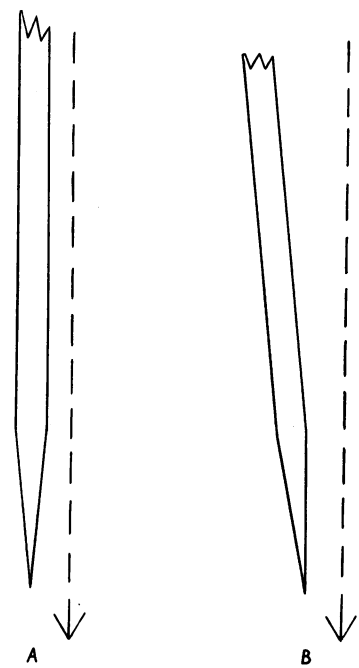

Fig. 5. A: razor blade held collinear with its plane of movement, which is indicated by the arrow. B: optimum position of razor blade, which is inclined so that the bevel nearer the uncut tissue is collinear with the plane of movement.

METHODS

CBA mouse-liver slices were chopped nominally 0·33 mm. thick. The rate of oxygen consumption of 2 ml. portions of the 15 ml. of glucose–phosphate–Ringer solution (pH 7·4) which was used to separate the slices obtained by chopping 400–500 mg. of liver was determined by the direct method of Warburg in runs of 30 min. duration.

RESULTS

The proportion of tissue lost in chopping mouse liver was determined as follows. From each mouse two pieces of liver were obtained. The wet weight (a, mg.) of one piece was determined; this piece was then dried to constant weight (b, mg.) at 110°. The wet weight (m, mg.) of the second piece was determined; this piece was then chopped into slices nominally 0·33 mm. thick; the slices were separated by shaking in 15 ml. of Ringer solution and were then recovered and their dry weight (n, mg.) was determined. From the dry weight of the sliced liver the wet weight of the slices was estimated, by using the ratio of wet to dry weight found for the unchopped tissue; the difference between the wet weight of the slices and the weight of the tissue before chopping was found, and this difference, the loss of weight in chopping, was finally expressed as x, mg./100 mg. of initial tissue; i.e. $x = (m - na/b)\,100/m$. All operations on the second piece of tissue were carried out as quickly as possible; the weighings were performed on a torsion balance.

The hypothesis $H_0: \mu < D\sigma$, where σ is the standard deviation and μ the mean x, was tested sequentially (Wald, 1947; Arnold, 1951); the value assigned to D was 1, and to α and β 0·01, where α is the probability of rejecting H_0, when in fact $\mu < D\sigma$, and β is the probability of accepting H_0, when in fact $\mu \geqslant D\sigma$. H_0 was rejected after eight trials. Particulars of the distribution of x were: range 11–26; mean $\pm$ 95% confidence limits $16{\cdot}0 \pm 4{\cdot}2$.

These results show that a substantial proportion of the tissue was lost in the chopping. Microscopic examination of the Ringer solution used to separate slices revealed an abundance of cellular debris; it was found that this Ringer solution had a negligible rate of oxygen uptake; the mean of twelve manometer readings was less than 0·5 mm., which was insufficient for the missing mass of tissue to be accounted for as small aggregates of intact cells which, although not recoverable as slices, were capable of respiration as cells.

DISCUSSION

The chopper described above was made by unskilled labour with a minimum of machine tools, and gave a satisfactory performance. The chopper has been presented as made, so that there is scope for improvement; for example, it would have been better to have procured a screw with a longer end-spindle to take a wheel for returning the table; again, if the chopping lever had been bent to about 75° instead of 90° it would not have been necessary to scribe the lever to the driving shaft. It is difficult to conceive of a simpler design for a satisfactory tissue chopper. Although many refinements could be added to the machine, this would be contrary to our purpose.

It is thought that the present machine is superior, for the routine preparation of tissue slices, to that of McIlwain & Buddle (1953) in the following respects: (i) simplicity, (ii) absence of plastics and other materials whose durability may be questioned, (iii) positive location of the table, and larger bearing area of the table on its guides, (iv) greater weight (18 lb. gross), and consequent freedom from vibration.

Particular importance is attached to the angle between the razor blade and its plane of movement. If this was zero, as shown in Fig. 5*A*, it was found that although semi-fluid tissues, e.g. brain, were cut perfectly, more rubbery tissues, e.g. kidney, were cut with irregularity of slice thickness; this was attributed to the tendency of the blade to push the tissue aside by half its thickness as it entered the tissue. When the razor blade was tilted so that the bevel nearer the unchopped tissue was collinear with its plane of movement, as shown in Fig. 5*B*, it was found that, contrary to previous experience, mouse kidney was sliced as easily as brain; in this case any displacement would occur at the expense of the already sliced part of the tissue.

Results have been presented concerning the recommendation of McIlwain & Buddle (1953) that tissue be weighed, then chopped and transferred to the experimental vessel, and the rate of oxygen consumption apparently be expressed in terms of the wet weight of the unchopped tissue. That tissue is lost in the chopping and subsequent separation of the slices may seem obvious. Our concern is rather with extent of the loss and how it may be accounted for. The cut surfaces of a tissue slice cannot be conceived as plane, especially for a tissue with large cells, such as liver. If we suppose that the cutting instrument is perfect, i.e. that its edge has no thickness, then the plane of section will pass through cells: some will be halved, others divided in the ratios 1:2, 1:3, etc., all ratios between the limits 0:1 and 1:0 being equally frequent if the cells be considered infinite in number and arranged at random in space. The remains of the sectioned cells will be washed away when the slices are separated. The cut surface of a slice will then be irregular, like a roughcast rendering, and the distance between the highest peak and lowest depression measured normally to the plane of section will be the cell diameter. Although the cut surface of a slice is not visualized as a plane, it can obviously be referred to a hypothetical plane, related to the real surface, by, say, the principle of least squares. This concept implies that reference cannot be made without qualification to slice thickness, for this will vary from point to point, up to twice the maximum cell diameter.

The results presented above are consistent with this view. It was found that in cutting mouse-liver slices nominally 0·33 mm. thick, an average of 16% of the initial weight was lost, and the minimum observed was 11%. Now the diameter of a mouse-liver cell is about 20–30 μ. (P. R. Peacock, personal communication); thus if the razor blade had a perfect cutting edge, each cut would injure a zone of tissue whose width at any point would be 20–30 μ., so that the mean thickness and weight of the slices would be reduced by a minimum of 6–9%. This figure is in reasonable agreement with the findings for slice weight, in view of the assumptions implicit in the theoretical calculation. This interpretation of the experimental data suggests that a liver slice whose nominal thickness was 0·33 mm. had a mean thickness of about 0·28 mm.

Since the mass of slices is substantially less than that of the tissue before chopping, if the rate of oxygen consumption is expressed in terms of the weight of the tissue before chopping rather than, for example, the weight of the slices, lower values will be obtained. This could be a source of serious error, the extent of which will depend on (i) the nature of the tissue (i.e. cell size) and (ii) the slice thickness.

In conclusion, although the respiratory rate of tissue slices may be arbitrarily referred to the wet weight of the tissue before chopping, the present results emphasize that the weight of the slices may be substantially less than that of the tissue before chopping, and also that the mean thickness of the slices may be less than their nominal thickness.

SUMMARY

1. A simplified machine for the routine preparation of tissue slices by an ordinary safety-razor blade is described; its manufacture did not require skilled labour.

2. Attention is drawn to the advantage of inclining the razor blade so that the bevel nearer the uncut tissue is collinear with the plane of chopping.

3. It was found that an average of 16% of the mass of mouse liver was lost in chopping it into slices 0·33 mm. thick.

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