be expected that the concentration of cytidine diphosphate choline would be higher in young than in adult animals, but no evidence for this was obtained. The phosphorylcholine concentrations in young rat brain (Ansell & Spanner, 1959) did not differ significantly from those in adult animals (Dawson, 1955*a*; Porcellati, 1958).

In view of the rapid incorporation of [^{32}P]orthophosphate into cerebral phosphorylcholine *in vivo* (Ansell & Spanner, 1959) and the steady formation of phosphatidylcholine (Ansell & Dohmen, 1957; Ansell & Spanner, 1959), there should be a very high turnover of the cytidine diphosphate choline to account for the transfer of phosphate to the D-$\alpha\beta$-diglyceride. It also seems likely that either the enzymic reaction leading to the formation of cytidine diphosphate choline or the reaction leading to the formation of the complete phosphatidylcholine molecule is affected in insulin hypoglycaemia (Ansell & Spanner, 1959) and thiopentone anaesthesia (Ansell, 1960). In any event the role of those cytidine molecules concerned with phospholipid synthesis in cerebral tissues would appear to be a central one, although turnover studies *in vivo* are difficult in view of the small amount present.

SUMMARY

1. A method for the separation of cytidine diphosphate choline from brain tissue is described.

2. Recovery of added cytidine diphosphate choline amounted to about 60% and the amount present in rat brain was calculated to be about 0·03 μmole/g. of fresh tissue.

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Techniques in Tissue Metabolism

5. CHOPPING AND SLICING TISSUE SAMPLES*

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Chopping and slicing tissue samples for metabolic work have as their objective the preparation of portions small enough to receive metabolites by diffusion, but with minimal damage to cell structure. They are therefore distinct from the blending, grinding and homogenizing which typically yield cell-free systems. Description of apparatus and procedures for chopping and slicing are relatively rare, in comparison with those for blending and homogenizing (Allfrey, 1959, includes over 40 of

* Part 4: Heald & McIlwain (1956).

these). For chopping, the only machine described is that of McIlwain & Buddle (1953) with modifications claimed by Sproull (1956) to make for ease of manufacture. Some 10 years' experience with the machine is summarized below.

Cutting animal tissues free-hand with a razor or razor blade does not yield slices as large and uniform as is desirable when they are to be manipulated during or after a metabolic experiment, and transferred individually for analysis. Deutsch & Raper (1936) described how an ordinary microscope slide, or one with a surface roughened by an abrasive, could be used to aid the cutting of tissue with a razor blade. A heavy, carefully machined plastic apparatus was introduced by Stadie & Riggs (1944) to keep the blade at a fixed distance from the tissue surface during such cutting. Experience has indicated the Stadie & Riggs cutter to be unsatisfactory in that it holds the whole of the blade rigidly parallel to the upper plate of the cutter. The tissue slice which is being cut lies between the blade and the upper plate and in cutting it is compressed by the blade against the plate (Fig. 1*B*); the part which has been cut is subject to shearing forces from the whole width of the blade as it advances in cutting. For this reason a procedure had been adopted which used a simple glass guide (McIlwain, 1951) but which was not described in detail. As it is much simpler and more versatile than the method of Stadie & Riggs (1944) and also than that subsequently reported by Majno & Bunker (1957), it is now described below.

In cutting tissue by all the methods mentioned above, the tissue or blade is normally moistened with an iso-osmotic aqueous fluid, which acts as a lubricant and minimizes distortion or 'smearing' of the tissue during the cutting. Such use of fluid is occasionally undesirable, as for example when the extracellular space of the tissue is being studied or experiments are being carried out in non-aqueous fluids. Cutting soft tissues without fluid becomes feasible if a much narrower blade is used (Rodnight & McIlwain, 1954), and it has now been found possible to use a blade only 1·2–1·5 mm. wide by means of the bow cutter illustrated in Fig. 3.

DESCRIPTION OF APPARATUS

Tissue chopper

Behaviour of original model. The machine previously described (McIlwain & Buddle, 1953; the letters below refer to Fig. 3 of that paper) has continued in use for 8 years without repair and with only minor adjustments. Current examination of its moving parts gave the following results. Arm *A*: no wear; its knife edge bearing on cam *C*1 was formed at 0·013 in. radius to avoid chipping. Arm *L* and pawl *P*: no wear. Trigger cam *C*1: very slight rounding of its knife-edge; such wear was envisaged in the original design and the cam was made 1·5 in. long and with locking screws, so that three different parts of its knife-edge could be used successively. The machine is now operating on the last of these positions: when necessary the cam can easily be removed and refaced. Cam *C*2: approx. 0·003 in. wear at the point of maximum pressure; this has not affected the thickness of slices cut by the machine. Ratchet wheel, the lead-screw and nut; shafts *Sh*1 and *Sh*2 and their bearings; the bearings of arm *A*, carriage *C* and its guides: no wear.

It is evident that the design and materials are satisfactory; the brass employed was British Standard Specification 249, the fabric Bakelite B.S.S. 972 type A, and the black Bakelite and silver steel were the best commercial qualities.

Modifications. Based on manufacturing experience (Mr H. Mickle, Mill Works, Gomshall, Surrey) and on comments from this and other laboratories (especially from Dr Ian Leslie, The Queen's University, Belfast), the following modifications have been introduced. The lead screw has been made of a larger pitch so that slices up to 0·8 mm. in thickness can be cut, and a cut-out switch has been provided so that the carriage *C* cannot over-run its free path. The part of the arm *A* which carries the blade has been made detachable so that it and the cutting table *T*, which is already removable, can be sterilized apart from the rest of the machine and easily reaffixed for cutting; this is especially valuable in preparing tissue for tissue-culture studies, and in addition Rinaldini (1959) suggests two projecting arms at the circumference of the table *T*, so that it can be rotated with minimal microbial contamination. Bearing surfaces previously made of bakelite have been replaced by light-alloy castings fitted with brass brushes, and a ball race has been fitted to the bearing surface of cam *C*2. Silver-steel rods have been replaced by rods of stainless steel, grade EN.56.AM. Many moving parts have been shielded, and the motor and the rheostat rehoused. Fig. 3 of McIlwain & Buddle (1953) still represents accurately the form and centre-to-centre distances of the moving parts and their supports, in the manufactured machines.

Further applications of the chopper to plant and animal tissues. At the suggestion of Mr N. W. Pirie, tobacco-plant leaves were found to be readily cut by the chopper with little if any bruising. Three to five leaves or portions of leaves up to 4 cm. in width were dipped in a salt solution for a subsequent experiment and put one on top of another on a filter pad, similarly dipped, on the cutting table *T*. They were held loosely in place

with a spatula and the machine started. During the cutting the solution entered the air-spaces of the leaves so that, after cutting, the strips produced were darker in colour than the original leaves, and when placed in water the strips sank while the original leaves floated.

In addition to the uses of the chopper with animal tissues which were previously described (McIlwain & Buddle, 1953) the chopper has been applied to lobes of the thyroid gland from guinea pigs (Bottari & Donovan, 1958 and personal communication) in producing uniform fragments for assay of thyrotropic hormone. It has been used to produce uniform, cell-containing suspensions of guinea-pig-lung tissue for immunological experiments (Mongar & Schild, 1960). Application to mammary gland and liver for metabolic experiments is described by Abraham & Chaikoff (1959) and by Bassham, Birt, Hems & Loening (1959). Its use in preparing tissues for culture *in vitro* is described by Leslie & Paul (1954), Paul (1959) and Rinaldini (1958, 1959), including especially the chopping of the chicken-embryo heart.

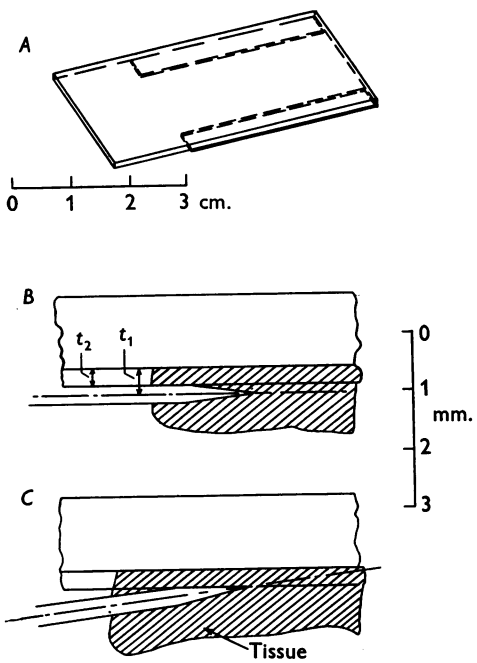

Fig. 1. Guide and blade for tissue slicing. *A*: The glass guide described in the text. *B*: Relationship between guide, blade and tissue in slicing, when the blade is held in the same plane as the guide; this is the position in the Stadie–Riggs cutter. Note that on moving forward in cutting, the slice is compressed from thickness t_1 to t_2. *C*: As *B*, with an angle of 6° between blade and guide.

Slicing tissues with blade and guide

The recessed glass guide, which has been found preferable to other apparatus, is illustrated in Fig. 1. It consists of a microscope slide, 3 in. × 1½ in., with ridges on its long edges which leave the centre of the slide as a recess. It is normally used in conjunction with a strip of razor blading 118 mm. × 18 mm., obtainable as a stage in the manufacture of the type of safety razor blades which have one cutting edge (Valet 3-blade strip, Gillette Industries Ltd., Syon Lane, Osterley, Middlesex). There is a particular manoeuvre in the use of the blade and guide which minimizes the rubbing of the blade on the tissue being cut; it is as follows.

The tissue is placed on a heavy table which raises it about 7 cm. above the bench (Fig. 2). The guide and blade are dipped in the fluid medium to be used

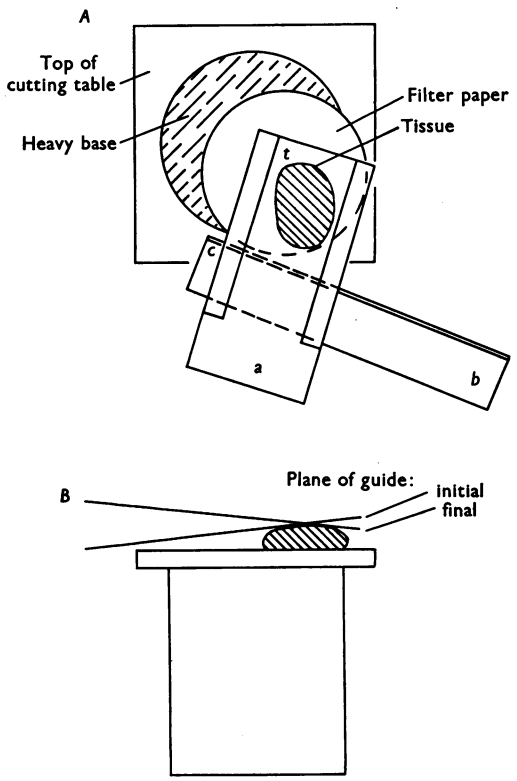

Fig. 2. Slicing tissue with blade and guide. *A*: In plan; the tissue lies on a circle of moist filter paper on a cutting table. The guide is held at *a* between the left thumb and forefinger, and the blade at *b* with the right thumb and fingers; *t*, initial position of the corner *c* of the blade when sweeping out air bubbles (see text). *B*: Elevation of cutting table and tissue showing the change in the plane of the guide during cutting, as seen from the left-hand side of *A*.

in a subsequent experiment, and the excess of medium is removed by shaking. The guide is held nearly horizontally with its ridges below, and the blade brought up from underneath it so that the two ridges of the guide touch the blade, but only at points near its cutting edge; the plane of the blade is a few degrees different from that of the guide (Fig. 1*C*; see below). The pressure of guide on blade is only a little greater than the weight of the guide, which is 8–9 g. Maintaining this relative position of blade and guide, both are lowered on to the tissue as shown in Fig. 2*A*. They rest lightly on the tissue, with a pressure about equivalent to the weight of the guide. The blade is then advanced into the tissue, cutting it with the usual combination of forward and side-to-side movements. As this is done the plane of the guide is altered as shown in Fig. 2*B*, while still keeping about the same angle between blade and guide. Thus at first the 'handle' end of the guide is lowest, while at the end of the cut it is raised above the rest of the guide. After cutting, the tissue is transferred with the blade or the guide (to whichever it is adhering) into a shallow dish of medium, and by jerking the blade or guide the slice is floated free.

The procedure described should be found satisfactory in cutting slices from a tissue surface which is slightly convex; but with a tissue surface which is flat or slightly concave, air bubbles are likely to be trapped when the guide is first placed on the tissue, and to cause holes in the slices which are cut. In such cases, the blade and guide are first applied to the tissue while the tip *c* of the blade is in the position indicated in Fig. 2*A*, and the blade drawn towards the operator until it occupies the normal starting position. Its movement sweeps out air which would otherwise be trapped, and brings the guide into complete contact with the tissue. It can readily be seen through the transparent glass guide whether this has been done; the surface of the guide should not be ground.

Preparation of the recessed glass guide. Glass microscope slides 76 mm. × 38 mm. (3 in. × 1·5 in.) and 1–1·2 mm. thick, with their edges ground smooth, and also no. 0 or no. 1 coverslips, 64 mm. × 44 mm. (2·5 in. × 1·75 in.) are put one by one into chromic acid–sulphuric acid cleaning mixture and after some hours washed well and dried in an oven at 105°. The coverslips are placed on a flat clean glass surface [Opalite: James Clark and Eaton Ltd., London, S.E. 1] and cut to strips 5–6 mm. × 64 mm., with a steel rule and a diamond. Between each cutting the Opalite and the rule must be wiped free of fine glass slivers with a soft paper tissue or cloth.

A Canada balsam–xylene solution (approx. 2:1, v/v) is prepared and with a glass rod four small spots of the solution are placed at each edge of a slide in the positions to be occupied by the strips of coverslip (Fig. 1*A*). A strip is taken in forceps and lowered on to each set of balsam spots. These should flow to a complete film between slide and strip, with a little excess of fluid appearing at the edges. Further spots of balsam–xylene are placed on the affixed strips, and further strips placed above them. The spots of fluid are transferred with a rod already drained from balsam and are not added as drops. The strips of coverslip are aligned with each other and with the edge of the slide, and the slide carrying them is put horizontally in an oven at 105° overnight. Excess of balsam is then wiped off with a tissue moist with xylene. If the balsam has retracted and left a gap under a strip of coverslip, the gap is filled with xylene–balsam solution. The slides are left for a further day at 105°. Measurement has shown the thickness of their ridges to be stable for a year or more after this treatment.

The guides are conveniently made in batches from six to twelve, and are then calibrated with an engineer's micrometer. Measurements are made to 0·005 mm., of the thickness of the slide at its centre, and of the thickness of the slide plus coverslips at three points on each ridge. The difference between measurements at the centre and at the ridge gives the thickness of the ridge and is the main factor conditioning the thickness of slices cut with the guide. The six measurements of thickness of ridge should agree to within 0·01–0·02 mm. in a satisfactory guide. No. 0 coverslips have been found about 0·10 mm. in thickness, and no. 1, 0·125 mm. Guides (*a*) made from two no. 0 coverslips have averaged 0·24 mm. in depth; those (*b*) from one no. 0 and one no. 1, 0·265 mm.; and those (*c*) from two no. 1 coverslips, 0·285 mm.

Thickness of slices cut. The thickness of slices cut with the guide and blade can be judged by Fig. 1*B* and *C*, to be conditioned by the depth of the recess in the guide and by the thickness of the blade. However, it is less than the sum of the depth and half the thickness of the blade by an amount which depends on the angle between the blade and guide. This, and the pressure exerted by the guide on the tissue, are individually-conditioned matters which should be standardized as far as possible by an investigator, who should then measure the thickness of the slices which he cuts. The following measurements were made according to Warburg (1923) with slices of guinea-pig cerebral cortex, specific gravity 1·03, cut with the blades of thickness 0·26 mm. in the fashion recommended and with guides (*b*) and (*c*) above. Immediately after the slices were cut, they were washed from the guide, drained from fluid medium and were weighed on a torsion balance to the nearest mg.; they were then floated in a Petri dish of phosphate–

glucose saline (McIlwain, 1951) above paper ruled in millimetre squares. The data showed that with guide (*b*), slices were 0·32 mm. and with guide (*c*), 0·34 mm. in thickness. By keeping the blade parallel to the guide, slices were about 0·06 mm. thicker.

Bow cutter. The bow cutter was designed to enable soft tissues to be cut without aqueous fluid; this depends on its having a narrow blade kept rigid by tension (Fig. 3). The narrow blades are obtained, from the strips of razor blading described above, by one of the following methods. (i) The cutting edge of the blade is gripped in the centre of the carefully cleaned jaws of a sheet-metal bending machine (an Edwards swing-beam sheet-metal folder). The machine is operated as though a right-angle bend were to be made in the blade; as the blade is hard and brittle, it breaks much before this degree of bending has been achieved, and in a proportion of cases gives the narrow blade desired. The yield of successful blades with a new bending machine was about 50 % of those bent, but slight irregularities in the jaws reduce this yield; a piece 7 cm. or more in length is required. (ii) A strip of the blade 1·2 mm. wide including the cutting edge is gripped between metal bars in a vice. A strip of copper sheeting about 15·4 cm. × 3·75 cm. (6 in. × 1·5 in.) is bevelled at one of its long edges, and this edge used with emery to cut through the blade by abrasion. The narrow blade when released from the bars is intact and 1·2–1·5 mm. wide.

The frame of the bow cutter (Fig. 3) is made from Stubbs's silver-steel wire, $\frac{1}{16}$ in. (1·6 mm.) in diameter. A length of about 22 cm. is bent to a shape which will become that shown in Fig. 3 when compressed (see below). It is tinned with solder at its two ends. The narrow strip of blading, about 7 cm. long, is also tinned at its ends. The frame is fixed between nails on a block of wood so that its ends are under compression of rather more than 500 g., but not under torsion. The blade and frame are brought together and soldered. The frame is released when cold, and the blade should then be quite flat (not twisted) and require a weight of about 500 g. applied to its ends to cause it to bend.

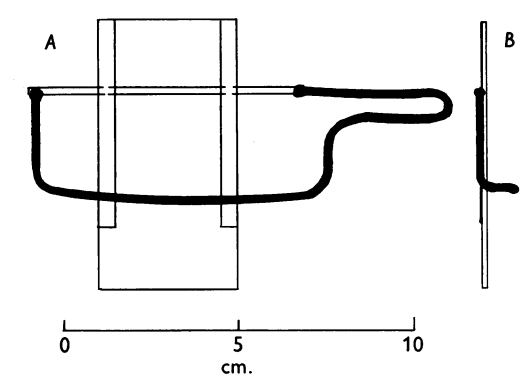

Fig. 3. The bow cutter. *A*, Plan; *B*, end elevation, showing its position in relation to a glass guide when both are used in slicing tissue.

The tension on the narrow blade in the bow cutter is approx. 20 kg./cm.^{2} and keeps the blade sufficiently rigid for it to be handled in much the same way as one of the wide blades from which it was made. In particular it can be used with the recessed glass slide to give slices of defined thickness from soft tissues without the use of saline. Metabolic experiments with slices prepared in this way from cerebral cortex, liver and kidney have been described by Rodnight & McIlwain (1954). The bow cutter weighs 3·5–4 g., and the original blades 4·2 g.

DISCUSSION

Comment may be made on the choice of chopping or slicing methods in the preparation of tissues, for their relative merits are not always understood. The tissue chopper was designed to give cell-containing preparations from small and irregular specimens, obtained for example at biopsy or from small organs or small parts of organs. It remains valuable in this and other situations in which a cell-containing preparation is required from the whole of a tissue sample, without leaving a residue and thus introducing the likelihood that the material used is not representative of the whole sample. For these purposes the tissue is chopped in one plane only, to a series of slices. The chopper has also been found valuable in sampling heterogeneous tissues, for after chopping in two planes to a series of prisms, the tissue can be suspended in a fluid, mixed and representative samples taken for each of a number of vessels. Also, when finely subdivided, it can be suspended and dispensed by pipette though remaining a cell-containing tissue. The chopper has a further use in cutting, mechanically, larger specimens of such a size that slicing by hand would be tedious.

Slicing remains the most suitable process when a moderate amount of relatively uniform tissue is to be prepared. Slicing can yield relatively large sheets of tissue, larger than those available by chopping, and thus with a proportionally smaller degree of damage. Also, it can yield up to about 150 mg. of tissue as a single sheet thin enough to meet the requirements of metabolic experiments. It is advantageous to have the tissue as a single sheet when it is to be transferred and analysed.

Slices of these dimensions cannot be cut free-hand but are readily prepared with blade and guide as described above. After this method of

slicing had been devised and used for some years, Majno & Bunker (1957) described a more elaborate apparatus for slicing in which a blade is fixed horizontally on a stand and the tissue moved by hand across and above the blade. This apparatus also yields such slices. Two models of the apparatus have been examined, one with the mounted microtome blade described by Majno & Bunker (1957) and another employing a strip of razor blading. With both, the cutting process could not be watched; the slice was more difficult to transfer, and an aqueous medium was always required in cutting. [*Added August* (1960). In the apparatus of Hultin, Arrhenius, Löw & Magee (1960), also, the slice is not visible during cutting and is again subject to shearing forces from the whole width of a cutting blade.] The guide described here allows the progress of the cutting to be seen and thus to be better controlled; with the bow cutter it allows slicing without added aqueous fluids. For these reasons the simple recessed guide used as described above appears the more satisfactory and versatile instrument.

SUMMARY

1. The tissue chopper previously described has undergone development and has been further applied.

2. For slicing tissues, the preparation and use of a simple guide is described.

3. A bow cutter is described for use with the guide when cutting tissue in the absence of added aqueous fluid.

I acknowledge with gratitude help and information from Mr H. L. Buddle and Mr H. Mickle regarding the tissue chopper; from Mr A. McNeil regarding the cutting guide and from Mr H. L. Buddle and Dr R. Rodnight regarding the bow cutter.

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Use of Carbon Monoxide for Detecting Denaturation in Haemoproteins

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Through the work of Hoppe-Seyler (1889) it became known that haemoglobin forms a compound with carbon monoxide. Hill (1926, 1930) observed that combination with carbon monoxide takes place also with the isolated prosthetic group of haemoglobin in the form either of haem or of its pyridine compound pyridine haemochromogen. The reactivity towards carbon monoxide observed both with haemoglobin and its prosthetic group is by no means characteristic of haem compounds in general. On the contrary, many haem compounds, among them the important class of intracellular respiratory pigments known as cytochromes, appear as a rule to be inert towards carbon monoxide. Keilin, in his pioneer investigations on these respiratory pigments, encountered only one exception to this rule in animal tissues, namely cytochrome a_3 (Keilin & Hartree, 1939).

As soon, however, as their native structure is disorganized by exposure to alkali, or by other influences, cytochromes as a rule can become reactive towards carbon monoxide. It thus appears that the reaction with carbon monoxide in the neutral pH range provides a convenient means of detecting denaturation. In numerous publications, however, investigators failed to report the behaviour of their preparations towards carbon monoxide, leaving it open to doubt whether their

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