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A Tissue Homogenizer

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For the separation and isolation of the particulate fractions existing within cells, the cells must be disrupted. In studies of the action of toxic substances upon mitochondria it was important that we should use a method of homogenization which was both efficient and reproducible. It was also essential that if the homogenizer was broken it could be readily replaced by one with an identical performance. The above criteria are not all fulfilled by previously described homogenizers.

The homogenizer described has been designed on the general principles of that of Potter & Elvehjem (1936) and the constructional details are given in Fig. 1. A Perspex pestle rotates in a smooth precision-bore glass tube. Since Perspex is easy to machine a precise gap between pestle and mortar may be readily obtained. Instead of the usual spherical end, the end of the pestle is a cone of 90° angle; this fits accurately into the base cavity and also allows the shape to be accurately repeated when required.

It is essential to use a motor with sufficient power ($\frac{1}{4}$ h.p.) to prevent slowing of the pestle when the tissue and fluid is being forced through the gap between the pestle and mortar. Under

these conditions the gap between pestle and mortar is likely to remain constant, thus maintaining constant the shearing forces in the fluid. The lack of contact between the two surfaces is indicated by the very slight wear in the pestle; after use for 18 months the diameter of the pestle had decreased by only 0.001 in. at the bottom and less at the top.

For the preparation of mitochondria the pestle has been rotated at 1100 rev./min. and the gap between the pestle and mortar varied for different tissues (0.01 in. for liver and 0.005 in. for brain). The properties of the liver and brain mitochondria so prepared have been previously described (Aldridge, 1957, 1958; Aldridge & Parker, 1960). Preliminary experiments have shown that with a gap of 0.005 in. mitochondria may be isolated from rat kidney. The oxidation of pyruvate and fumarate by this preparation is stimulated threefold by 2:4-dinitrophenol and potato apyrase and has an activity of 300 μ l. of O_2 /mg. of protein/hr. (for experimental details cf. Aldridge, 1958). Heart mitochondria could only be isolated (though in poor yield) when the tissue had been chopped (McIlwain & Buddle, 1953) before homogenization with a gap of 0.005 in. Such preparations were stimulated

Fig. 1. Constructional details of the tissue homogenizer. *A*, Glass tube (Veridia tubing, manufactured by Chance Bros. Ltd.) with 30 mm. bore and 2 mm. wall thickness. *B*, Rubber O ring (Edwards High Vacuum Ltd., catalogue no. OR. 17). *C*, Two mated Perspex blocks, grooved as shown (*B*₁) to hold the rubber O ring and clamped just sufficiently to the glass tube to take the pressure of the rotating pestle. *D*, Perspex base, turned and shaped to the same angle (90°) as the Perspex pestle to be a sliding fit in the Veridia tubing; this is cemented to the glass tube with Bostik D and held in position by screws to the Perspex block *C*. *E*, Perspex block machined to shape with the necessary clearance between pestle and tubing. *F*, Stainless-steel rod (0.5 in. diam.) turned down at one end (*F*₁) and threaded ($\frac{7}{16}$ in. B.S.F.) to fit the pestle.

1.5–2.0 times by 2:4-dinitrophenol and apyrase and had an oxidative activity of 400–500 μ l. of O₂/mg. of protein/hr.

The design of this homogenizer is in principle very similar to the rotating concentric-cylinder apparatus for the determination of viscosity. The relation between the shearing forces in the fluid, the radii of the cylinders and the angular velocity is known when the angular velocity is low (Poynting, Thomson & Todd, 1947). However, for homogenization, the angular velocity is high and the flow turbulent. Under these conditions it is impossible to calculate the shearing forces. It is therefore necessary with homogenizers of different dimensions to test empirically the best combination of gap and speed of rotation of the pestle. However,

it has been our experience that changes in speed of rotation produce bigger changes in the proportion of cells broken than do changes in the gap between pestle and mortar.

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