A "MICRO" HOMOGENIZER FOR SMALL-VOLUME SAMPLES*

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INTRODUCTION

This paper describes the design, construction and performance of a micro "blendor" type homogenizer constructed and used in this laboratory. It was designed for uniform homogenization, for purposes of biochemical analysis, of materials such as sea urchin embryos that are not readily dispersed in a piston-type homogenizer. The nature of the material to be homogenized and the analysis to be performed on the homogenates required that specific conditions be realized before the homogenizer could be considered satisfactory. They were (1) small sample size, (2) quantitative recovery, (3) easy maintenance of low temperature, and (4) complete absence of air. The last point is unquestionably the most important since the primary hazard of "blendor" homogenization is frothing and the concomitant surface denaturation of proteins.

Basically the instrument consists of two main units: a stainless steel stopper assembly bearing the shaft which rotates in a nylon bushing, and a stainless steel chamber assembly (Fig. 2) into which the stopper assembly fits variably. The design, described below, permits filling the chamber with any volume from 2.5 ml to 7 ml, with the exclusion of air and minimal loss of material. The shaft bears two sets of blades at one end and a rubber-tired drive wheel at the other and is driven by touching the drive wheel to the revolving head of an angle centrifuge. Speeds of 25,000 to 30,000 r.p.m. have been achieved with no difficulty, using the Servall SS-1 centrifuge.

DESIGN AND CONSTRUCTION

The most critical feature, in terms of performance of the instrument, was the bearing for the shaft. A nylon bearing (bored out from a rod) was used since it would operate satisfactorily with no other lubricant than the suspending medium itself, thus avoiding contamination of the homogenate by any other lubricant.

Experience indicated that appreciable (0.003 in.) clearance between the shaft and the nylon bushing was necessary to avoid having the shaft "freeze" during prolonged high speed runs. Owing to the dimensions of the blendor, it proved more desirable and practicable to machine the blades and shaft from a single piece of stock.

The blades spin within a fluted chamber (Figs. 1 and 2); the clearance being about 0.02 in. The pitch (30°) of the two sets of blades opposed each other in such a manner that the fluid was drawn into the space between them, thereby creating tremendous turbulence.

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The drive wheel was constructed of two pieces of stainless steel (B and D, Fig. 1) between which could be clamped the "O" ring (C, Fig. 1) that served as a tire. This permitted greater speeds than could be achieved with a drive wheel which depended only upon the elasticity of the tire to hold it in position. This design also allowed easy replacement of worn "O" rings.

Construction of the chamber with a removable bottom simplified machining and facilitated cleaning. A gasket was not necessary.

A small groove milled in the stopper, which could be aligned with a small hole (K, Fig. 1) in the chamber, provided passage for the escape of air as the stopper assembly was pushed into position. After the sample had been placed in the chamber, the latter was inclined at an angle with the small hole facing upward and the stopper assembly was cautiously advanced, being sure that the groove was properly aligned

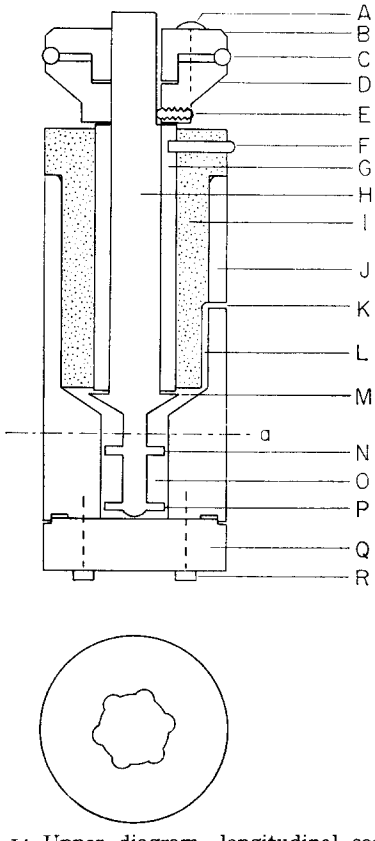

Fig. 1: Upper diagram, longitudinal section through the homogenizer. Lower diagram, cross-section through *a*: A, Screw (one of three) holding the two halves of the drive wheel together; *B* and *D*, Upper and lower halves respectively of the drive wheel; *C*, "O" ring; *E*, Set screw; *F*, Pin securing nylon bushing; *G*, Nylon bushing; *H*, Shaft; *I*, Stopper; *J*, Chamber; *K*, Air escape hole; *L*, Air escape groove; *M*, Slinger; *N* and *P*, Upper and lower sets of blades respectively; *O*, Fluted portion of chamber; *Q*, Bottom plate; *R*, Cap screw holding bottom plate in position (total of six).

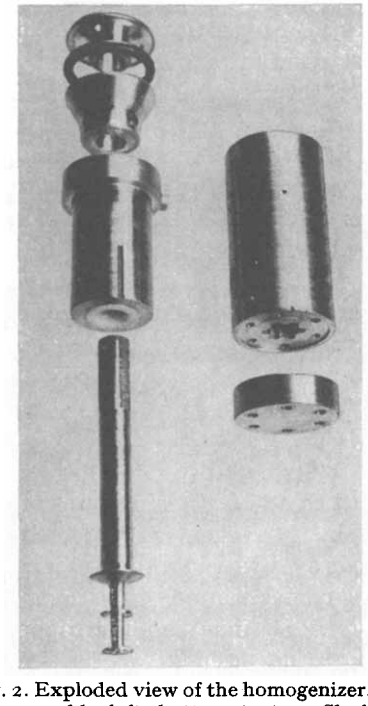

Fig. 2. Exploded view of the homogenizer. Stopper assembly left, bottom to top: Shaft with blades, stopper showing nylon bushing and air exhaust groove, lower half of drive wheel, "O" ring, upper half of drive wheel. Chamber assembly right, bottom to top: bottom plate, chamber showing fluted portion and air exhaust hole.

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with the hole, until a drop of fluid appeared. At this moment all the air had been expelled from the chamber assembly and the stopper assembly was rotated through 45°, sealing the homogenizer, which was now ready for operation.

During the actual homogenization, the drive wheel was touched *intermittently* to the spinning centrifuge head for periods of no longer than five to ten seconds alternated with shorter periods of coasting. The maximum speed at which the homogenizer could be operated without the tire flying off was about 33,000 r.p.m.

The omission of specific dimensions is deliberate since dimensional details of design are probably not very critical and are dictated by the specific problem to be solved.

EXPERIMENTAL

A variety of cells and tissues have been tested. *Tetrahymena pyriformis*, a ciliate that is ordinarily somewhat difficult to disrupt, was uniformly dispersed in 20 seconds at about 28,000 r.p.m., with only 0.1% of the cells remaining unbroken. The resultant homogenate could reduce methylene blue in the presence of 1.5% proteose-peptone at least as rapidly as a control suspension of intact cells.

Rat skeletal muscle (thigh and leg), when dispersed in 7.3% polyvinylpyrrolidone in 0.25 *M* sucrose adjusted to pH 7[1] for 45 seconds at about 30,000 r.p.m., gave a homogenate consisting mostly of granules and fibers of a few micra in size and some larger chunks about 40–80 μ^2. When the homogenate was centrifugally fractionated into a "heavy" and a "light" fraction, each was able to reduce methylene blue in phosphate buffered 0.1 *M* sodium-succinate medium at comparable rates.

Sea urchin embryos fixed in 30% ethanol at —10° C were completely homogenized in 0.1 *M* KCl in 15 seconds at 30,000 r.p.m. Fresh material in sea water was more difficult to disperse.

Yeast cells (store purchased cake yeast) and the green alga *Chlorella* were, for practical purposes, completely refractile to the homogenizer. Two minutes at maximum speed disrupted no more than 0.5% of the yeast cells and none of the algal cells. Perhaps a superiorly designed and constructed blendor could effectively disrupt these cell types.

There is no evidence to indicate a significant temperature increase of the suspension during homogenization.

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SUMMARY

This paper describes the general design, construction and performance of a blendor capable of homogenizing a variety of biological samples of 2.5 ml to 7 ml volume in the complete absence of a liquid-gas interface.

REFERENCES

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