A Note on "Homogenizers" for Tissue Brei Preparation.* BY NORMAN G. ANDERSON. *(From the Biology Division, Oak Ridge National Laboratory, Oak Ridge.)*‡

Dounce *et al.* (1) have described a new ball type "homogenizer" that is considered to be more gentle and effective than the device described by Potter and Elvehjem (2). The purpose of this note is to show that the shearing fields actually obtained with homogenizers operated in an up-and-down manner (3, 4) are much greater than those obtained when the pestle is rotated at the speeds generally prescribed (*ca.* 1200 R.P.M.).

The rate of shear in the device of Potter and Elvehjem may be calculated from the equation (5),

$$G = \frac{(R_1 + R_2)\pi n}{R_1 - R_2} \qquad (a)$$

in which G is the rate of shear in sec.$^{-1}$, R_1 and R_2 are the radii of the inside of the tube and of the pestle respectively in centimeters, and n is the pestle speed in revolutions per second. In the instrument described (2), $R_1 = 0.7$ cm., $R_1 - R_2 = 0.0115$ cm., and the speed was 1200 R.P.M. The rate of shear is found by Equation (*a*) to be 7600 sec.$^{-1}$. The percentage of the grinding time, per cent T, spent by any one particle in the shearing space is given by the ratio of the volume of fluid in the shearing space to the total volume:

Per cent T

$$= \frac{\pi(R_1 + R_2)(R_1 - R_2)L \times 100}{V} \qquad (b)$$

in which L is the length of the pestle parallel to the tube wall, and V is the total brei volume. When $V = 15$ ml., and $L = 0.65$ cm., the values for the instances given are 0.22 per cent of the time, or 0.53 second during a 4 minute run.

The instrument of the type described by Dounce *et al.* (1) gives a much higher rate of shear even when the pestle is moved slowly. The rate of shear is difficult to evaluate, however, owing to a continuous rate variation caused by the fluid flowing between a cylinder and a sphere. Furthermore, the annular width is so small that the diameter of the suspended particles is an appreciable fraction of it, and the fluid is moving at such a rapid rate that turbulent flow occurs. It should be emphasized that the values for the shearing fields derived here are only approximations since (*a*) the rate of shear does not vary linearly from the midannular point to the wall in the vertically moving pestle device, (*b*) the brei may contain irregularly shaped particles of many different sizes and hence may not behave as a perfect Newtonian fluid, (*c*) the suspended particles may create microturbulences which are difficult to treat, and (*d*) the rates of shear are so high in some instances that laminar flow gives way to turbulence. The approximations are useful, however, in comparing different devices. For these reasons only a simple approximation of the rate of shear in the region of highest shear in the Dounce instrument is given. This is suitable for comparative purposes since it yields a minimum value. The approximation neglects the shear caused by the

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movement of the pestle relative to the tube, and considers the flow of fluid as occurring through an annular space.

The volume of fluid sheared, V, is

$$V = \pi R_2^2 p \qquad (c)$$

in which p is the distance in centimeters that the pestle is moved in the vertical direction. The average velocity h of the fluid in the annular space in centimeters per second is given by

$$h \cong \frac{R_2^2 p}{(R_1 + R_2)(R_1 - R_2)t} \qquad (d)$$

To the first approximation,

$$G \cong \frac{4h}{(R_1 - R_2)} = \frac{4R_2^2 p}{(R_1 + R_2)(R_1 - R_2)^2 t} \qquad (e)$$

If the pestle radius is 0.8715 cm. (R_2) and the tube radius is 0.8740 cm. (R_1), which gives the clearance of Dounce's "loosely fitting" plunger, the rate of shear is found by Equation (*e*) to be approximately 278,000 sec.$^{-1}$ when the plunger is moved at the slow rate of 1 cm./sec. It is evident that the shearing fields in the Dounce instrument are much higher than those obtained in the Potter and Elvehjem device. If the pestle in the latter is moved vertically at the rate of 1 cm./sec., the rate of shear will be approximately 10,000 sec.$^{-1}$, actually *higher* than the rate obtained by rotating the pestle at 1200 R.P.M. It would appear therefore that pestle rotation is unnecessary since slow up-and-down motion gives the same rate of shear with less heating and abrasion. In practice the vertical motion is much greater than 1 cm./sec. The high shearing fields obtained with up-and-down "homogenization" with pestle speeds of approximately 10 cm./sec. have been used by the author for some time (3, 4).

Absence of abrasive ground glass surfaces in the new device is probably one explanation for the superior results reported by Dounce *et al.* (1) in a comparison of the two instruments. Numerous other authors have abandoned ground glass surfaces for polished glass, lucite, teflon, or rubber. Another explanation is that rotating pestles are generally not perfectly centered, with the result that many particles are mashed between solid surfaces. This difficulty has been overcome by precise pestle mounting in the instrument of Lang and Siebert (6). The vertically operated device is superior in that it tends to center itself, thereby allowing very high shearing fields to be utilized with a minimum of wall-to-wall contact. Furthermore, since the shearing field is extremely small, the time during which a particle is actually sheared is almost vanishingly small. It appears therefore that smooth pestles, either short cylinders, half round, or spherical in shape, operated in an up-and-down manner are capable of yielding a much wider range of shearing rates and are more likely to break cells by shearing in a liquid gradient than are the conventional cylindrical pestle designs with the pestle rotated at nominal speeds.

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