LETTERS TO THE EDITORS

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Reproducibility of Differential Centrifugation Experiments in Tissue Fractionation

DURING the past few years, differential centrifugation of tissue homogenates has become one of the major techniques of cytology and biochemistry alike. Most of those who have applied this new technique describe the conditions of centrifugation by mentioning only the duration of operation and the centrifugal field prevailing in the middle (or at the bottom) of the tube; the type of centrifuge used is sometimes also mentioned. However, the information thus supplied is not sufficient to enable other workers to reproduce the conditions, especially when another type of instrument is to be used. This fact may be partly responsible for the wide variations which are sometimes encountered between results obtained in different laboratories.

Since different types of instruments cannot be expected ever to furnish identical results, the definition of a suitable unit raises some difficulties in itself. However, if the approximation is made that angle centrifuges can be regarded as similar to radial centrifuges in which the top and bottom of the fluid are separated from the axis by the same distances¹, different instruments become comparable with each other and the formula of sedimentation in a radial direction can be applied. When used for preparative purposes, a centrifugation is then best described by the characteristics of the lightest spherical particles which are completely sedimented under the conditions used.

The relationship between the variables involved is given by the formula:

$$2.3 \log_{10} \frac{R_{\max.}}{R_{\min.}} = \frac{2r^2 (d_p - d_m)}{9\eta} \int_0^t \omega^2 dt, \quad (1)$$

in which $R_{\max.}$ and $R_{\min.}$ are the distances (in cm.) separating the bottom and the surface of the fluid respectively from the axis during centrifugation; r is the radius of the particle (in cm.); d_p and d_m are the densities of the particle and of the medium respectively (in gm./cm.³); η is the viscosity of the medium (in poises); ω is the angular velocity (in rad./sec.); and t is the time (in sec.).

From this formula, three values are required: $R_{\max.}$, a characteristic of the instrument and, eventually, of the type of tube used; $R_{\min.}$, a value which is dependent on the type of instrument and tube,

and also on the amount of fluid centrifuged; $\int_0^t \omega^2 dt$,

the time integral of the squared angular velocity.

With instruments which accelerate and decelerate rapidly, and which are run during a relatively long time, the latter value is described accurately enough by giving the time during which the power is turned on and the angular velocity at plateau speed. When such is not the case, it is necessary to work out the whole integral. It can then be expressed by means of the actual time of operation and of the equivalent mean angular velocity.

Another alternative would be to give simply the sedimentation constant of the lightest spherical particles which are sedimented completely:

$$s = \frac{2r^2 (d_p - d_m)}{9\eta} = \frac{2.3 \log_{10} \frac{R_{\max.}}{R_{\min.}}}{\int_0^t \omega^2 dt}. \quad (2)$$

It should be pointed out that the use of the average field, expressed in terms of the gravity constant g , rather than the angular velocity, tends to render results less reproducible.

If the average field is defined as the field prevailing at a distance $R_{av.} = 0.5 (R_{\max.} + R_{\min.})$ from the axis:

$$g_{av.} = \frac{1}{981} \omega^2 R_{av.}; \quad (3)$$

equation (2) then becomes:

$$s = \frac{2.3 R_{av.} \log_{10} \frac{R_{\max.}}{R_{\min.}}}{981 \int_0^t g_{av.} dt}. \quad (4)$$

Since individual centrifuges differ more by the absolute values of their radii than by the ratio of these values, they are less comparable, so far as complete sedimentation is concerned, when developing equal average fields than when turning at equal velocities. The converse is obviously true when the rate of sedimentation in the middle of the tube is considered. But in preparative centrifugation, it seems more logical to use complete sedimentation as criterion of comparison.

One can, of course, maintain the convention of expressing the conditions of centrifugation by means of the average field; but it then becomes of special importance to give the values of $R_{\max.}$ and $R_{\min.}$ accurately. In this case, the time integral of the average field can be conveniently expressed in a composite unit, $g_{av.} \text{ min.}$, or alternatively, as the equivalent of x minutes at y times g . If, as is usually the case, the angular velocity is expressed in revolutions per minute (r.p.m.) and the time T in minutes, equations (2) and (4) become:

$$s = 3.5 \frac{\log_{10} \frac{R_{\max.}}{R_{\min.}}}{T \int_0^T (\text{r.p.m.})^2 dT} = 3.9 \times 10^{-5} \frac{R_{av.} \log_{10} \frac{R_{\max.}}{R_{\min.}}}{T \int_0^T g_{av.} dT}. \quad (5)$$

In equations (2), (4) and (5), s is given in sec. it can also be expressed in Svedberg units (10^{-13} sec.) and must then, of course, be multiplied by 10^{13} .

There is little doubt that better reproducibility will be obtained if the conversion from one instrument to another is based on one of these equations. Of course, differences must still be expected, since the equations are based on an approximation in the first place, and also they neglect such factors as diffusion and convection currents, which may be more important in one type of instrument than in another. In addition, the manner in which decantation is performed may be of paramount importance and should be mentioned as well.

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¹ Pickels, E. G., *J. Gen. Physiol.*, **26**, 341 (1943).