from that of *Trillium*. The influence of temperature is a very real possibility and is presently under investigation.

In conclusion it may be said that for diploid plants a relationship does exist between the minimum mitotic cycle time, the interphase nuclear volume, and the DNA content per cell. Moreover, the relationship is such that if any one of the three cell variables is known, an estimate can be made of the remaining two.

*Summary.*—Experiments were performed to determine the relationship between interphase nuclear volume and DNA content per cell and the minimum mitotic cycle time in several diploid plant species. All measurements were made on meristem cells contained in the terminal 2 mm of the root. The results indicated that linear relationships exist between the interphase nuclear volume and the minimum mitotic cycle time, and between the DNA content per cell and the minimum cycle time. Linearity, however, does not exist if extrapolation is carried out to include the lower forms of life, such as bacteria and viruses. The relationships are to some extent independent of chromosome number and the amount of DNA per chromosome. The data presented enable the estimation of any two of the above three variables, if the third variable is known.

The authors wish to thank Miss Huei-Kuen Ying and Mrs. Anne F. Rogers for their technical assistance, and Mrs. Rhoda C. Sparrow and Mrs. J. Van't Hof for aid in preparing the manuscript.

* Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

11 Van't Hof, J., *Cytologia*, in press.

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**BAND-CENTRIFUGATION OF MACROMOLECULES AND VIRUSES IN SELF-GENERATING DENSITY GRADIENTS***

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Communicated by Norman Davidson, April 24, 1963

This communication presents a new method of carrying out sedimentation velocity experiments. A thin lamella of a solution of macromolecules is layered onto a denser miscible liquid in a rotating ultracentrifuge cell. The macromolecules then sediment through the liquid in a narrow concentration distribution, or band, which is observed photographically as a function of time. The density gradients neces-
sary to stabilize the system against convection are generated during the experiment by the diffusion of small molecules between the lamella and the bulk solution, and in some cases by the sedimentation of the small molecules in the bulk solution. The inhomogeneities in the solvent are usually small enough to have no observable effect on the motion of the macromolecules. Sedimentation coefficients of macromolecules may be evaluated from the motion of bands. Diffusion, hydrodynamic interactions, and chemical reactions affect the shapes of bands.

The method of band-sedimentation velocity\(^1\) differs from the conventional boundary-sedimentation velocity method\(^2\) in that the macromolecules are initially distributed in a narrow band at the top of the liquid instead of uniformly throughout (Fig. 1). In the new method all resolved macromolecular components are physically separated. Sedimentation coefficients and relative concentrations measured in such mixtures are free from the effects of interaction between components.\(^3\)

\textit{Stability Considerations.}—The negative density gradients associated with the distribution of macromolecules at the leading side of the band must be compensated by positive gradients in the binary solvent to avoid convection. If the positive gradients are inadequate, limited convection causes forward spreading.

In the experiments described in this communication, the lamellar solution contained macromolecules in dilute aqueous electrolyte. The denser bulk solution or solvent contained \(\text{D}_2\text{O}\) or more concentrated electrolyte, such as 0.5 \(M\) KCl or 1.0 \(M\) NaCl. In such solvents the sedimentation coefficient is only slightly affected by preferential interaction\(^4\) of the macromolecules with small molecules. With more concentrated binary solvents, significant preferential interactions may occur. In very concentrated CsCl the macromolecules may sediment through a solution of significantly variable density, and slow down or even stop\(^5\) during the experiment.

\textit{Noninteracting Systems.}—1. Homogeneous materials: A single macromolecular substance with constant sedimentation coefficient and diffusion coefficient \(D\) forms a Gaussian band which remains Gaussian during sedimentation (Fig. 2A). The band broadens with time and with the distance sedimented according to the relation

\[ \sigma^2 = \left( \frac{r_0}{r_0^0} \right)^2 \left\{ \frac{r_0}{r_0^0} \sigma^{02} + 2D(t - t^0) \right\}, \tag{1} \]

where \(\sigma\), \(r_0\), and \(t\) are the standard deviation, the radial distance to band center, and the time. The superscript zero refers to the properties of the band at time \(t^0\). The sedimentation coefficient is evaluated from the motion of band center or the maximum in the concentration distribution with the equation

\[ \ln r_0 = s\omega^2 t + \text{constant}, \tag{2} \]

where \(\omega\) is the angular velocity. The amplitudes and standard deviations of bands
of macromolecules having a variety of diffusion coefficients are given in Figure 3, a useful guide for selection of initial concentrations and speeds of centrifugation.

2. Heterogenous materials: A mixture of noninteracting substances forms multiple bands which resolve if the sedimentation coefficients are sufficiently different. Resolution is increased for components of different buoyant density if the density of the bulk solution is near that of the buoyant density of one of the species. The results of experiments in concentrated CsCl are shown for two mutant forms of lambda virus which differ in buoyant density by 0.024 g cm\(^{-3}\) (Fig. 2B, C). 

Contamination of a homogeneous material with unresolved polydisperse impurities leads to bands with forward and/or trailing elements which dilute out as sedimentation proceeds. Several preparations of bacterial viruses—T-4, \(\phi X-174\), and lambda—contained unresolved fast contaminants. That inadequate stabilizing density gradients, which also lead to forward spreading, were not responsible was shown by further experiments in concentrated CsCl and also by boundary experiments. In the latter the nonresolved fast material was evident in

Fig. 2.—(A) Band-centrifugation of Southern bean mosaic virus in 1 M NaCl, 0.04 M NaPO\(_4\), pH 6.9, 50 \(\mu\)l lamella, 1.60 \(\mu\)g SBMV, OD\(_{550}\) = 0.2, 1.80 ml bulk solution, 30 mm center-piece. Photographed at 16 min intervals, 12,590 rpm, 20.0\(^{\circ}\). Densitometer records of this film show the bands to be Gaussian over 90\% of the mass. (B, C) Band-centrifugation of a mixture of \(6 \times 10^5\) \(\phi 2\)-b5 lambda virus, buoyant density 1.508 g cm\(^{-3}\), and \(5 \times 10^6\) \(\phi 2\)-b5 lambda virus, 1.484 g cm\(^{-3}\), in two different CsCl solutions (0.01 M tris pH 7.0). Thirty mm Kel-F center-piece, 25 \(\mu\)l lamella, 1.40 ml bulk solution, 25,000, 4 min intervals. (B) \(\rho = 1.25\), 12,590 rpm. (C) \(\rho = 1.39\), 20,410 rpm. The first two exposures in (C) show the effect of excessive refractive index gradients. These were avoided in (B) by holding the rotor speed at approximately 5,000 rpm for 10 min during acceleration.

Fig. 3.—(A) The standard deviation and (B) relative concentration at band center at various times for macromolecules with different diffusion coefficients. The calculations were performed for nonsettling distributions in a rectangular cell. The standard deviations were calculated with the relation \(\sigma^2 = 2D(t - \varphi)\). The amplitudes were calculated with the relation \(c = c_0(\varphi_0\Sigma^2/\varphi_0)\) where \(c_0\) is the concentration at band center.
the densitometer records, but not, as in the band experiments, upon visual inspection of the films. Viral RNA preparations always gave bands with rapidly diluting slow components, presumably due to partial hydrolysis by traces of ribonuclease. In these experiments the maximum in the distribution moved with the velocity of the intact viral RNA (Fig. 4). The weight-average sedimentation coefficient of

![Figure 4](image)

any distribution of materials which sediment away from the meniscus may be evaluated from the motion of the center of gravity of the mass (cf. equation (13)).

Sedimentation coefficients obtained with equation (2) from band-centrifugation experiments agree satisfactorily with the results by boundary-centrifugation with the same samples (Table 1). The data also show that the measured sedimentation coefficients may be satisfactorily corrected for the effects of the solvents.

### Table 1

**Sedimentation Coefficients by Band and Boundary Centrifugation**

<table>
<thead>
<tr>
<th>Material</th>
<th>Lamellaa</th>
<th>µl</th>
<th>µg</th>
<th>Solventb</th>
<th>Band</th>
<th>Boundary</th>
<th>lit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb(A)</td>
<td>10</td>
<td>17.0</td>
<td>0.5</td>
<td>M KCl</td>
<td>3.62</td>
<td>3.66</td>
<td>3.98</td>
</tr>
<tr>
<td>MS-2 RNA</td>
<td>10</td>
<td>0.50</td>
<td>0.5</td>
<td>M KCl</td>
<td>20.2</td>
<td>19.9</td>
<td>30.2</td>
</tr>
<tr>
<td>MS-2 RNA²</td>
<td>10</td>
<td>0.80</td>
<td></td>
<td>in 50% D₂O</td>
<td>17.2</td>
<td>17.6</td>
<td>30.8</td>
</tr>
<tr>
<td>T² DNA</td>
<td>10</td>
<td>0.05</td>
<td>0.5</td>
<td>M KCl</td>
<td>33.2</td>
<td>32.0ₜ</td>
<td>32.6₁</td>
</tr>
<tr>
<td>ÆX-174</td>
<td>10</td>
<td>1.60</td>
<td>98%</td>
<td>D₂O</td>
<td>83</td>
<td>84</td>
<td>117</td>
</tr>
<tr>
<td>SBMV</td>
<td>20</td>
<td>3.20</td>
<td></td>
<td>1.0 M NaCl</td>
<td>91</td>
<td>90</td>
<td>114</td>
</tr>
</tbody>
</table>

a The lamellar fluid contains buffer and water, the solvents contain the same buffer. b Data in this column have been reduced to standard conditions and are given as nondeuterated sodium salts. 1% deuterium is assumed. c Human hemoglobin, pH 7.0, 20.0ₜ, 56, 100 rpm. A slight front sharpening presumably caused by concentration-dependent dissociation was observed in these experiments. d 0.04 M KPO₄ pH 7.0 for the KCl solvents. e Rossi-Fanelli, A., E. Antonini, and A. Caputo, *J. Biol. Chem.*, 236, 391 (1961), value for 0.4% Hb in phosphate 0.5 M KCl, pH 7.0, 5.0ₜ, 31.410 rpm. f Private communication, J. Strauss and R. L. Sinsheimer. g pH 7.0, 5.0ₜ, 44,770 rpm. h 0.04 M KPO₄ pH 7.0. i Legend Fig. 5.4. p, pH 7.0, 20.0ₜ, 29,500 rpm. j The band experiments were performed in a 30 mm centerpiece. k These are limiting values at c → 0. l Davison, P. F., and D. Feilfeder, *J. Mol. Biol.*, 6, 543 (1962). m 0.04 M NaB₄O₇ pH 9.0, 23,150 rpm, 24.5ₜ. n 0.04 M NaB₄O₇ pH 9.0 (measured). o R. L. Sinsheimer, *J. Mol. Biol.*, 37 (1959). p Southern bean mosaic virus, pH 6.95, 20.0ₜ, 12,500 rpm. q 0.04 M NaPO₄ pH 6.9. r Miller, G. L., and W. C. Price, *Arch. Biochem.*, 10, 467 (1946).

**Interacting Systems.**—A material with small D and a large s which decreases with increasing concentration forms skewed bands which are sharp on the trailing side and spread on the leading side. Such bands have been observed with several native viral DNA's: T-4, T-7, lambda, and polyoma. A single run contains information adequate to determine the constant which characterizes the concentration dependence of s and the sedimentation coefficient at infinite dilution, s₀. With the present approximate theory the value of s₀ obtained for T-7 DNA is in agreement with the result obtained by extrapolation of data from a set of boundary experiments.
Fig. 5.—Band-centrifugation of T-7 DNA in 0.5 M KCl 0.04 M KPO₄ pH 7.3, 20.0°, 29,500 rpm. (A) 10 μl, 50 μg/ml in 12 mm centerpiece. Densitometer records are from photographs taken at 8 min intervals. The graph shows the data from this experiment plotted according to equation (18). The concentration at the maximum expressed in OD₂₆₀ was obtained from linear densitometer records. The relation of the pen excursion to the concentration in the cell was evaluated by numerical integration. (B) 25 μl, 2 μg/ml in a 30 mm centerpiece. Upper densitometer record is from a photograph 28 min after reaching full speed. The second set of records are smoothed tracings of exposures at 8 min intervals. The graph contains the results from 2 separate experiments. The T-7 DNA was prepared by F. W. Studier, by the method of Mandell, J. D., and A. D. Hershey, Anal. Biochem., 1, 66 (1960). The results obtained in these experiments are compared with the results obtained by others in Table 1.

(Fig. 5A and Table 1). Correct values for s₀ were also obtained with very low lamellar concentrations of DNA, 2 μg/ml in 30 mm cells (Fig. 5B) or 5 μg/ml in 12 mm cells. In these experiments convective disturbances do not occur at low DNA concentrations as they may in boundary-centrifugation. At an intermediate lamellar concentration, 20 μg/ml, plots of the logarithm of the position of the maximum versus time are linear with a slope corresponding to 0.93 s₀.

Apparatus.—Equipment needed in addition to the Model E analytical ultracentrifuge with the ultraviolet optical system consists of special centerpieces and microsyringes. A commercially available boundary-forming centerpiece was tested and was satisfactory for macromolecules stable to shear. Most of the experiments were performed with band-forming centerpieces (Fig. 6).

Fig. 6.—Band-forming centerpieces are fabricated from 2° and 4°, 12 and 30 mm Kel-F centerpieces. The sample hole is 2.2 × 8 mm in the 12 mm, and 2.2 × 20 mm in the 30 mm centerpiece. The center of the hole is 4.5 mm above and 4.4 mm to the side of the axis of the centerpiece. The inner circle indicates the position of the inner edge of the raised bead. The dashed circle indicates the minimum diameter of the Kel-F cylinder. The liquid transfers through the 0.07 mm gap between the quartz window and the Kel-F surface. Layering occurs by direct flow with 0.55 ml or less in a 12/4° sector and by displacement when the normal 0.72 ml volume is used. After continued use the bead flattens and transfer is impeded. The bead may then be removed and replaced with a 0.004° polyethylene gasket (Beckman Instruments, Inc.).

fabricated from standard Kel-F centerpieces. The shear stresses with this type of centerpiece, which transfers at approximately 500 rpm (11 × g), are low enough to avoid shear degradation of T-7 DNA. The blind sample hole is filled (avoiding air pockets) while the cell is partially assembled with a 0.010 or 0.050 ml vaselined Hamilton syringe fitted with drawn-out no. 20 (0.032° ID) Kel-F tubing for shear sensitive materials or with the same type of syringes used normally. Dilution of the sample is sometimes performed in the sample hole. Bands that are sharp on at least one side may often be seen with the schlieren optical system.

Density Gradients in Band-Centrifugation. —The transient density gradient generated by diffusion of small molecules between the lamella and the bulk solution is given by

$$dρ = \frac{8Δρ(r - r_o)}{2\sqrt{4D}^3} e^{-(r - r_o)^2/4Dt}$$

(3)
where $D$ is the diffusion coefficient of the diffusible substance, $\delta$ the thickness of the lamella, $(r - r_2)$ the distance from the meniscus, and $\Delta \rho$ the difference in density between the lamella and the solvent. It is found by sample calculations that this gradient is in general more than sufficient to provide convective stability against the negative density gradient on the leading side of a Gaussian distribution of macromolecules with typical diffusion and light absorption coefficients. The "diffusion" gradients are proportional to $\Delta \rho$.

The density gradients generated by the sedimentation of the small molecules were calculated from equation (23) of Fujita and MacCoaam.\textsuperscript{13} They progress through the cell more slowly than the diffusion gradients even at high speed (35,000 rpm) and high salt concentrations (CsCl, $\rho = 1.50$ g cm$^{-3}$). Unlike the diffusion gradients, these "field" gradients increase regularly with time and, in longer experiments, approach equilibrium. A second set of field gradients advances from the bottom of the cell to the center at approximately the same rate. At low speeds and at low salt concentration the field gradients are inadequate to support the bands.

**Band-Centrifugation in Preparative Rotors.**—The method described here can be used in preparative ultracentrifuge rotors. The longer liquid columns require that greater density stabilization (larger values of $\Delta \rho$, equation (3)) be introduced. There is an additional requirement that convection be prevented during deceleration. In this laboratory viral DNA is routinely centrifuged in 3 cm liquid columns of CsCl, $\rho = 1.50$ g cm$^{-3}$, which are overlaid with a 1 cm layer of mineral oil. A 0.1 ml sample is introduced onto the surface of the CsCl to form a 0.1 cm lamella. The tubes are centrifuged in the SW-39 rotor at 35,000 rpm 3–5 hr. At the conclusion of the run analyses (radioactivity, biological activity, optical density) are performed on drops collected after piercing the tube.

**Theory of Band-Centrifugation.**—1. *Moment relations.*\textsuperscript{14} We consider a single component with constant $s$ and $D$, and ignore any effects arising from the constituents of the binary solvent. The time derivative of the expression for the $n$th moment of the mass,

$$m_n = \alpha \int_{r_2}^r cr \, dr,$$

where $\alpha$ is a constant, is

$$\frac{dm_n}{dt} = \alpha \int_{r_2}^r r \frac{\partial (cr)}{\partial t} \, dr.$$

We combine equation (5) with the continuity equation for a cylindrical sector

$$\frac{\partial c}{\partial t} = - \frac{1}{r} \left( \frac{\partial J}{\partial r} \right)_{t},$$

where the flow, $J$, through a unit cylindrical surface is

$$J = -D \left( \frac{\partial c}{\partial r} \right)_{t} + \omega \Delta crc,$$

and integrate by parts, remembering that $J_{r_2} = J_{r_1} = 0$. We introduce equation (7) into the result and integrate a second time by parts with $cr_2 = c_{r_1} = 0$ to obtain the recursion relation for the $n$th reduced moment,

$$d\mu_n/dt = n^2 D \mu_{n-2} + n s \omega^2 \mu_n,$$

where $\mu_n = m_n/m_.$. The differential equations for $n = 2$ and $n = 4$ are readily integrated,

$$(\mu_2 + 2\gamma)/[(\mu_2^0 + 2\gamma) = e^{s\omega^2(t - \phi)/D},$$

$$(\mu_4 - 2\mu_2^2)/[(\mu_4^0 - 2\mu_2^0) = e^{s\omega^2(t - \phi)/D},$$

where $\gamma = D/s\omega^2$. The superscript zero refers to a reference band at time $\phi$.

The equation for the experimental determination of $D$ is obtained by introducing $\mu_1 = R$, $\mu_2 = R^2 + \Sigma \nu$, and the definition of the $n$th moment of mass about the center of gravity, $R; \Sigma \nu = \int_{r_2}^r (r - R) \omega \, cr \, dr/\int_{r_2}^r \omega \, cr \, dr$, into equation (10), taking the square root and inverting.

$$(R^2 + \Sigma \nu)/(R^2 - \Sigma \nu) \approx e^{s\omega^2(t - \phi)/D},$$

where $n$ is the number of equations.
Terms in $\Sigma u^{p}/R^{p}$, $\Sigma u^{q}/R^{q}$ and $\Sigma u^{s}/R^{s}$ in the numerator and corresponding terms in the denominator are omitted in equation (11). This approximation for equation (10) is valid for $2\gamma \geq \Sigma u$, a necessary condition for the experimental determination of the diffusion coefficient. Equations (9), (11), and $\mu_{s} = R^{3} + \Sigma u$ are combined to form a relation free of differences between large numbers for the diffusion coefficient,

$$\Sigma a\left(\frac{R^{p}}{R}\right)^{s} - \Sigma a^{p} = \frac{D}{\sigma^{3}} \left[1 - e^{-2\sigma\tau(t - \tau_{0})}\right] = 2D(t - \tau)[1 - \sigma\tau(t - \tau) + \ldots] \quad (12)$$

$$\Sigma c\left(\frac{R^{p}}{R}\right)^{s} - \Sigma c^{p} (\frac{R}{R^{p}}) = 2D(t - \tau). \quad (12a)$$

The effect of the inhomogeneous field, seen in equations (12), is to increase the width of the band by about 15% during the run. This broadening is normally small compared with the effect of diffusion (Fig. 3). With equation (1) or (12a) diffusion coefficients for homogeneous materials with constant $s$ and $D$ may be calculated. It is anticipated that the effects of concentration-dependent sedimentation should be less troublesome than in boundary-sedimentation because of the compensatory front spreading which occurs along with the rear-sharpening.

Equation (9) is simplified with an error of less than 0.1% in $s$ for rapidly diffusing macromolecules, $\gamma \leq 0.3$ cm$^{3}$ and $\Sigma u = 0.01$ cm$^{3}$ to give a relation between the motion of the center of gravity of the distribution and the sedimentation coefficient.

$$\ln \frac{R}{R^{p}} = \sigma\tau(t - \tau) \quad (13)$$

In most experiments the motion of band center or of the maximum in the distribution, equation (2), may be used with adequate accuracy to evaluate $s$.

2. The Gaussian approximation: The differential equation for spreading of an infinitely thin band in a sector has been solved by Carslaw and Jaeger in their consideration of the conduction of heat from an instantaneous annular source at a distance $r_{1}$ from the axis. In our variables the solution in Bessel functions for the large values of $a_{1}$ of $2D(t - \tau)$ in the present problem becomes

$$C = \frac{Ae^{(a - r_{1})^{2}2D}}{\sigma \sqrt{r_{1}}}, \quad (14)$$

The cylindrical geometry thus causes a negligible distortion of the Gaussian distribution. We anticipate that the distorting effect of the inhomogeneous field will also be small, and examine next the behavior of concentration distributions which remain Gaussian during band-centrifugation.

The relations for a single component in a sector

$$R^{2} = r^{2} + 2\sigma\tau(1 + \sigma^{2}/2r_{1}^{2}); \quad \Sigma u = \sigma\tau(1 - \sigma^{2}/r_{1}^{2}) \quad (15)$$

are combined with equation (11) to give equation (2) for $s$. Equation (1) for $D$ is obtained by combining equations (12a) and (15). Higher-order terms in equation (15) are neglected in the development of equations (1) and (2). Terms in $\sigma^{4}$ are also neglected in equation (2).

3. Concentration-dependent sedimentation in a constant field and in a rectangular cell: The centrifuge differential equation for a substance with $D$ constant and $s = s(1 - \kappa c)$ becomes

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial z} \left[ D \frac{\partial c}{\partial z} - \sigma\tau(1 - \kappa c)^{2} \right] \quad (16)$$

where the velocity at infinite dilution, $\nu^{0}$, is $e\sigma\tau^{2}$. A solution of equation (16) for the boundary conditions $c = 0$ at $z_{a}$ and $z_{b}$ has been obtained with a substitution suggested by Feynman.

$$c(z,t) = \frac{D}{e^{\nu\nu^{0}} 2\sqrt{D(t - \tau)}} e^{2\nu^{0}z} \quad (17)$$

where

$$\nu^{0} = \frac{(z - z) - \sigma\tau(t - \tau)}{2\sqrt{D(t - \tau)}} \quad a = \frac{\pi}{2} \frac{\nu\nu^{0}}{2\tan(\nu\nu^{0})},$$

and

$$\Gamma = \int_{z_{a}}^{z_{b}} c \, dz.$$
We differentiate the solution with respect to distance and equate the derivative to zero to observe the behavior of the position $z_m$ and the concentration $c_m$ at the maximum in the distribution.

$$z_m - z_m^0 = \frac{\varphi(t - \varphi)}{(1 - 2kcm)}$$

(18)

**Comparison of Band- and Boundary-Centrifugation.**—Band-centrifugation offers these advantages: (1) All resolved components are examined in a physically separated state. Slow contaminants and degradation products usually do not interfere. Fast components are detectable with high sensitivity. The effects of interaction between resolved components are avoided. (2) Valuable information is available from a comparison of the two sides of the band. (3) Differential instead of cumulative concentration distributions are obtained. (4) Less material, $1/8$ to $1/40$, is required. (5) Dialysis is usually not necessary. (6) Band centrifuge runs are more appropriate as pilot experiments for zone centrifugation runs in preparative rotors.

Band-centrifugation has the following disadvantages: (1) The initial distribution is less well defined. (2) Bands widen and dilute faster than boundaries. (3) The effects of concentration-dependent sedimentation and the admixture of non-resolved fast materials are more difficult to separate. (4) There is less latitude in the choice of solvents and in the upper limit of the concentration of macromolecules. (5) Refractometric recording systems are less readily employed.

**Summary.**—A new method for carrying out sedimentation and diffusion studies in the ultracentrifuge is described. Examples are given of sedimentation results obtained with some typical RNA, DNA, protein, and virus preparations. Band-centrifugation and boundary-centrifugation are compared.


* This work was supported in part by grant HE 03394 from the U.S. Public Health Service and a grant from the National Foundation.

† Contribution No. 2955.

‡ Statements marked with a double dagger are elaborated in sections on theory.

1 The method is distinguished from zone-sedimentation in that the stabilizing density gradient is generated during the experiment. Attention is called to an experiment by R. T. Hersh and H. K. Schachman, *J. Phys. Chem.*, 62, 170 (1958) in which, incidental to other studies, a wide band of virus was observed to sediment through an H$_2$O-D$_2$O gradient.


6 It will be recommended elsewhere that equilibrium experiments in a buoyant density gradient be set up as band-centrifugation experiments with buoyant solvents.


8 The theory for the behavior of multicomponent systems will be given elsewhere. The result in the text is accurate to within 1% in $s$ for most distributions.

INFORMATION CONTENTS OF DISTRIBUTIONS

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Communicated April 12, 1963

1. Introduction.—The entropy, as usually defined, is a measure of our ignorance and, if multiplied by $-1$, can be considered as a measure of our knowledge of the state of a system. It is a measure of our total knowledge into which the knowledge of the value of any observable enters in the same way (cf. section 3). It is this last circumstance which prompted the considerations leading to the present note. According to quantum mechanical theory, some observables can be measured much more easily than others: the observables which commute with the additive conserved quantities (energy, components of the linear and angular momenta, electric charge) can be measured with microscopic apparatuses; those which do not commute with these quantities need for their measurement macroscopic systems. Hence, the problem of defining a measure of our knowledge with respect to the latter quantities arises. The present note will be restricted to the case in which there is only one conserved additive quantity; this will be denoted by $k$. The name "skew information" has been proposed\(^1\) for the amount of information which an ensemble described by a state vector or a statistical matrix contains with respect to the not easily measured quantities. This information relates to the transition probabilities into states which lie askew to the characteristic vectors of the additive conserved quantities.

2. Postulates on the Information Content.—The requirements which an expression for the information content should satisfy are the following:

(a) If two different ensembles are united, the information content of the resulting ensemble should be smaller than the average information content of the component ensembles. By uniting two ensembles, one "forgets" from which of these a particular sample stems. Hence, the information content should decrease. Even