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Fundamental Mass-Transport Equations for Zone Sedimentation Velocity*

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ABSTRACT: A theory is presented for the determination of sedimentation coefficients from observation of the migration of zones of sedimenting macromolecules. In developing this theory, it is assumed that mass transport is caused by sedimentation and diffusion alone. With these assumptions it may be shown that (1) for a centrifuge cell of uniform cross-sectional area in a gravitational field, the velocity of the center of gravity of the migrating zone is equal to the average sedimentation velocity of the individual particles; (2) for a radially shaped cell in a centrifugal field, the migration of the "center of sedimentation," defined as the mass averaged value of the logarithm of the radius, is determined by the average sedimentation coefficients of the individual particles; (3) for a uniform cell in a centrifugal field the movement of the center of gravity is equal to the average sedimentation velocity of the individual particles. The effects of concentration dependence of both the sedimentation and the diffusion coefficients are treated in detail as are the effects of a superimposed viscosity and density gradient. Both single-component and polydisperse systems are considered.

During the last decade a new technique has been developed which has been called "rate zonal sedimentation" (Brakke, 1956), or "gradient differential sedimentation" (Anderson, 1956). It has proved to be a tool of great power for the fractionation and characterization of nuclei, mitochondria, and microsomes (Anderson, 1956), polyribosomes (Warner *et al.*, 1963), ribosomes (Britten *et al.*, 1962), virus (Brakke, 1960), nucleic acids (Vinograd *et al.*, 1963), and proteins (Martin and Ames, 1961). In using this technique, a solution containing the particles to be studied is layered as a thin zone on top (or on the bottom for zone flotation) of a second solution which contains or develops a density gradient to stabilize the system against convection. During the subsequent centrifugation, the particles sediment as zones from the top to the bottom of the tube. During the course of this migration, separation of zones of particles possessing different sedimentation coefficients occurs, making possible characterization and fractionation of different types of particles according to *sedimentation coefficient*.

The technique of zone sedimentation velocity should be distinguished from isopycnic density gradient centrifugation (Anderson, 1956), and zone sedimentation equilibrium in a buoyant density gradient (Vinograd, 1963), where particles are characterized and fractionated according to their *densities*.

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The development of mass-transport equations for zone sedimentation velocity has been delayed by the very real possibility that some convection occurs in all such experiments. Indeed, De Duve *et al.* (1959) have derived a set of equations which attempt to take into account convective loss of material during centrifugation. Recent work has shown, however, that correct sedimentation coefficients can be determined from zone sedimentation velocity experiments when dilute solutions of macromolecules are used (Vinograd *et al.*, 1963; Martin and Ames, 1961). In these experiments, the migrating bands remained intact. It is probable, therefore, that convective disturbances did not cause a net transfer of mass in a radial direction within the centrifuge cell. The results of these experiments have prompted us to develop the mass transport equations for band sedimentation velocity for convection free systems.

In the subsequent treatment, the transport of mass is considered to be caused by sedimentation and diffusion alone. Equations are developed which allow the determination of sedimentation coefficients from an examination of the movement of the entire zone of macromolecules. For a cell of uniform cross-sectional area in either a uniform or a centrifugal field, the migration of the center of gravity of the distribution is shown to be simply related to the sedimentation velocity of the macromolecules. For a radially shaped cell in a centrifugal field, the migration of the center of sedimentation, defined as the mass average value of the logarithm of the radius, is shown to be a simple function of the sedimentation coefficient.

The effects of the concentration dependence, polydispersity, and the presence of a superimposed viscosity and density gradient are treated in detail.

Theory

Frequently, in the derivations to follow, mass averaged quantities are used. These will be indicated by brackets $\langle\ \rangle$ surrounding the quantity to be averaged. For example, the mass average value of a function, F, will be defined as $\langle F\rangle \equiv \int F\,dm / \int dm$ where m is mass of the sedimenting macromolecules, and where the integration is carried out across the migrating zone, usually from the meniscus to the cell bottom.

While most of the equations to follow are derived without approximation from the postulated fundamental equations, in certain instances approximations are made. These approximations are discussed in detail in the text, and the affected equations are indicated by placing an asterisk with the equation number, i.e., (25*).

A Cell of Uniform Cross-sectional Area in a Homogeneous Field. Let J be the total flux per unit area of macromolecules across a stationary plane at position x. The relation between J and mass transport owing to sedimentation and diffusion is given by equation (1):

$$AJ = +Avc - AD\left(\frac{\partial c}{\partial x}\right)_t \qquad (1)$$

The symbols, D, v, c, and A represent the diffusion coefficient, the sedimentation velocity, the concentration, and the cross-sectional area.

Equation (1) may be multiplied by dx and integrated across the cell from the meniscus, a, to the cell bottom, b:

$$\int_a^b AJ\,dx = +v\int_a^b Ac\,dx - AD\int_a^b \left(\frac{\partial c}{\partial x}\right)_t dx \qquad (2)$$

Here it has been assumed that v and D are constants. Concentration-dependent sedimentation and diffusion are discussed later.

For a sedimenting zone which has pulled away from the meniscus but not yet reached the cell bottom, we may assume that the concentration of macromolecules is effectively zero at a and b. Therefore the second term on the right vanishes, eliminating the diffusion coefficient from further consideration. Defining the mass increment as $dm \equiv Ac\,dx$, equation (2) becomes:

$$\int_a^b AJ\,dx = v\int_a^b dm \qquad (3)$$

Since there is no mass flow through the meniscus or cell bottom, $J_a = J_b = 0$. Therefore, the following mathematical identity may be evaluated:

$$0 = AJx\Big|_a^b = \int_a^b \left[\frac{\partial AJx}{\partial x}\right]_t dx$$

$$= \int_a^b x\left[\frac{\partial AJ}{\partial x}\right]_t dx + \int_a^b AJ\,dx$$

Substituting this expression into equation (3) yields:

$$-\int_a^b x\left[\frac{\partial AJ}{\partial x}\right]_t dx = +v\int_a^b dm \qquad (4)$$

The equation of continuity may be written (Tanford, 1961):

$$-\left(\frac{\partial AJ}{\partial x}\right)_t = A\left(\frac{\partial c}{\partial t}\right)_x \qquad (5)$$

Equations (5) and (4) may be combined to give:

$$+\int_a^b xA\left(\frac{\partial c}{\partial t}\right)_x dx = \frac{\partial}{\partial t}\left[\int_a^b xAc\,dx\right]_x$$

$$= \frac{d}{dt}\int_a^b xAc\,dx = v\int_a^b dm \qquad (6)$$

where the total derivative has been used since the integral in brackets is not a function of x. The x coordinate of the center of gravity of a distribution of macromolecules is defined as $\langle x\rangle = \int_a^b x\,dm \Big/ \int_a^b dm$. Since $\int_a^b dm$ is the total mass within the cell, which is not a

function of time, equation (6) may be written:

$$\frac{d\langle x\rangle}{dt} = v \tag{7}$$

Equation (7) may be expressed as the following law: *For a uniform cell in a homogeneous field, the sedimentation velocity of the individual molecules in a sedimenting zone is equal to the sedimentation velocity of the center of gravity of that zone.* This law is valid for concentration-independent sedimentation of a single species.

Multiple-Component Systems. If multiple sedimenting species, i, are present within one zone and have different sedimentation velocities, v_i, then, for the case in which the species do not interact, we may write equation (7) independently for each of the i species. If each of these equations is now multiplied by the total mass of component i within the cell, m_i, and the equations are summed, the following expression is obtained:

$$\frac{d}{dt}\left(\sum_i [m_i\langle x\rangle_i]\right) = \sum_i v_i m_i \tag{8}$$

The weight-average sedimentation velocity is defined as:

$$\langle v\rangle \equiv \frac{\sum_i v_i m_i}{\sum_i m_i} \tag{9}$$

The observed center of gravity of the sedimenting zone in a cell of uniform cross-sectional area may be expressed as the weighted average of the individual centers of gravity of the various species:

$$\langle x\rangle_{\text{obs}} = \frac{\sum_i m_i\langle x\rangle_i}{\sum_i m_i} \tag{10}$$

where $\langle x\rangle_{\text{obs}}$ is the observed center of gravity of the distribution. Dividing equation (8) by $\sum_i m_i$, and using relations (9) and (10), the equation for a multiple component system becomes:

$$\frac{d\langle x\rangle_{\text{obs}}}{dt} = \langle v\rangle \tag{11}$$

Hence, from the rate of migration of the observed center of gravity of a zone composed of several species, the weight-average sedimentation velocity is obtained.

Concentration Dependence of the Diffusion Coefficient. Equation (11) remains unchanged even if the diffusion coefficient is a function of concentration. To show this, it is necessary to show that the second term on the right-hand side of equation (2) vanishes if D is included within the integral and is a function of concentration. The diffusion coefficient may be expressed as a power series in c:

$$D = D^\circ(1 + K_1c + K_2c^2 + \cdots + K_nc^n + \cdots) \tag{12}$$

In this case, the second term on the right-hand side of equation (2) becomes:

$$\int_a^b D\left(\frac{\partial c}{\partial x}\right)_t dx = D^\circ \int_a^b \left[\frac{\partial c}{\partial x} + \frac{K_1}{2}\frac{\partial c^2}{\partial x} + \frac{K_2}{3}\frac{\partial c^3}{\partial x} + \cdots + \frac{K_n}{(n+1)}\frac{\partial c^{n+1}}{\partial x} + \cdots\right]_t dx = 0 \tag{13}$$

Since the term on the right-hand side of the equation (13) vanishes when integrated, the concentration dependence of the diffusion coefficient has no effect on the velocity of sedimentation of the center of gravity of the zone and equation (11) remains valid.

Concentration Dependence of the Sedimentation Coefficient. If:

$$v = v^\circ(1 - k_1c + k_2c^2 + \cdots + k_nc^n + \cdots) \tag{14}$$

equation (7) becomes:

$$\frac{d\langle x\rangle}{dt} = v^\circ(1 - k_1\langle c\rangle + k_2\langle c^2\rangle + \cdots + k_n\langle c^n\rangle + \cdots) \tag{15}$$

where

$$\langle c^n\rangle \equiv \int_a^b c^n\, dm \Big/ \int_a^b dm$$

For a sedimenting zone which is Gaussian in shape, $\langle c\rangle = c_{\max}/\sqrt{2}$, where $c_{\max}$ is the concentration at the center of the sedimenting zone.

Radially Shaped Cell in a Centrifugal Field. The flow of mass across a radial plane is given by:

$$(\theta br)J = +\omega^2 scr(\theta br) - D\left(\frac{\partial c}{\partial r}\right)_t \theta br \tag{16}$$

and the equation of continuity may be written

$$(\theta br)\left(\frac{\partial c}{\partial t}\right)_r = -\left(\frac{\partial(J\theta br)}{\partial r}\right)_t$$

where θbr is the cross-sectional area of the radially shaped cell, and where the symbol r is used for the distance from the axis of rotation, ω is the angular velocity of the centrifuge, and s is the sedimentation coefficient of the macromolecules within the migrating zone.

Equation (16) may be multiplied by dr/r and then integrated across the zone from the meniscus at a to the cell bottom at b. Since $c(a) = c(b) = 0$, the last term on the right-hand side, which contains the diffusion coefficient, vanishes, leaving:

$$\left[\int_a^b \theta brJ(d\ln r)\right] = +\omega^2 s\int_a^b dm \tag{17}$$

where the substitution $dm = c(\theta br)\, dr$ has been used.

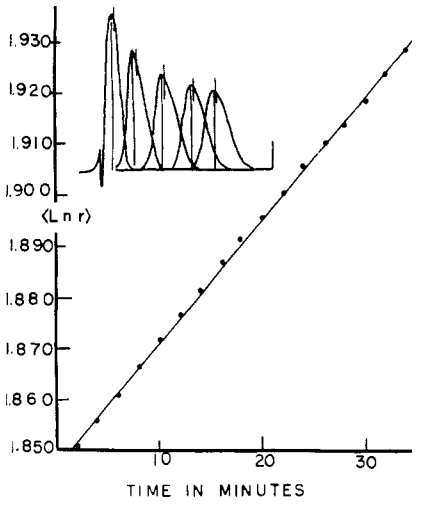

FIGURE 1: A plot of $\langle \ln r \rangle$ as a function of time for a preparation of catalase. A preformed density gradient was placed in an analytical ultracentrifuge cell using a gradient engine described by Rosenbloom and Schumaker (1963). Using a synthetic-boundary cell, a 1 mg/ml solution of catalase was layered on top of the gradient during acceleration of the rotor. Using an absorption optical system, pictures were taken at 2-minute intervals, using light of 410 mμ wavelength. The photographs were analyzed by means of a photodensitometer, and representative tracings are shown in the insert at the top of Figure 1. In the insert, the short lines lying just beyond the crest of the peaks are positions of $\langle \ln r \rangle$. The plot of $\langle \ln r \rangle$ against time is straight, and from this plot the value obtained for $s_{20,w}$ is 11.3 S. This value is identical with that reported by Sumner and Gralen (1938).

If the diffusion coefficient is concentration dependent, the term involving D still vanishes. This can be shown in a manner identical to that described above for the case of a uniform cell and homogeneous field.

Integrating the left-hand side of equation (17) by parts, taking the time derivative, and combining with the equation of continuity yields

$$\frac{d}{dt}\left[\int_a^b (\ln r)\, dm\right] = \omega^2 s \int_a^b dm \qquad (18)$$

where the time derivatives of the terms evaluated at the limits have been set equal to zero, in a manner similar to that used in deriving equation (6) from equation (3). The partial derivative has been replaced by a total derivative because the term in brackets is not a function of r.

A symbol $\langle \ln r \rangle$ is now defined as:

$$\langle \ln r \rangle \equiv \frac{\int_a^b (\ln r)\, dm}{\int_a^b dm} \qquad (19)$$

Using this definition, equation (18) may be written:

$$\frac{d\langle \ln r \rangle}{dt} = \omega^2 s \qquad (20)$$

Therefore, for the case of a radially shaped cell in a centrifugal field, *the sedimentation behavior of a moving zone is described by the migration of a point within that zone corresponding to the mass averaged value of* $\ln r$. We suggest that this point be called the center of sedimentation.

In Figure 1 is shown a plot of the center of sedimentation, $\langle \ln r \rangle$, as a function of time for a zone of catalase layered on top of a sucrose density gradient in an analytical ultracentrifuge cell. The experimental conditions for this run and the results are described in detail in the legend. The value for $s_{20,w}$ obtained in this experiment agrees closely with the literature values for this enzyme.

Multiple-Component Systems. If equation (18) is written independently for each component, i, and then summed over the several species present, the following equation is obtained:

$$\frac{d}{dt}\int_a^b (\ln r)\, d\left(\sum_i m_i\right) = \omega^2 \sum_i s_i m_i = \omega^2 \langle s \rangle \sum_i m_i$$

$$= \omega^2 \langle s \rangle \int_a^b d \sum_i m_i \qquad (21)$$

where m_i is the total mass of component i within the cell.

Since $d\Sigma m_i = dm_{\text{obs}}$, where m_{obs} is the total observed mass behind a particular plane in the cell, equation (21) becomes:

$$\frac{d\langle \ln r \rangle}{dt} = \omega^2 \langle s \rangle \qquad (22)$$

Therefore, from the movement of the center of sedimentation of a migrating zone containing several species with different sedimentation coefficients, the weight-average sedimentation coefficient of the macromolecules within the zone may be obtained.

Concentration Dependence. If the sedimentation coefficient of the macromolecules is concentration dependent, equation (20) becomes:

$$\frac{d\langle \ln r \rangle}{dt} = \omega^2 s^\circ (1 - k_1\langle c \rangle + k_2\langle c^2 \rangle$$

$$+ \cdots + k_n\langle c^n \rangle + \cdots) \qquad (23)$$

Effect of the Superimposed Density Gradient. For hydrodynamic stability, all zone centrifuge runs must be performed in the presence of a superimposed density gradient. Therefore, both the viscosity, η, and the density, ρ, will vary along the length of the cell. It will be assumed that these quantities are known as a function of r at each time the value of $\langle \ln r \rangle$ is deter-

mined. Then:

$$s = s_{20,w} \frac{(1 - \bar{v}\rho)}{(1 - \bar{v}\rho_{20,w})} \frac{\eta_{20,w}}{\eta}$$

$$D = D_{20,w} \frac{\eta_{20,w}}{\eta}$$

If these expressions are substituted into equation (16), the term involving the diffusion coefficient no longer vanishes. The equation for the migration of the center of sedimentation becomes:

$$\frac{d\langle \ln r \rangle}{dt} = \omega^2 s_{20,w} \left\langle \frac{(1 - \bar{v}\rho)}{(1 - \bar{v}\rho_{20,w})} \frac{\eta_{20,w}}{\eta} \right\rangle - D_{20,w} \left\langle \frac{1}{r} \frac{\eta_{20,w}}{\eta^2} \left(\frac{\partial \eta}{\partial r}\right)_t \right\rangle \quad (24)$$

The brackets $\langle\ \rangle$ denote mass averaged values of the quantities included within the brackets. In deriving equation (24), the coefficient of $D_{20,w}$ has been integrated by parts and evaluated at the limits to give the term included in equation (24). No mathematical approximations have been made. But computations of the mass average quantities of the right-hand side would be laborious. In any practical case little error should be introduced by evaluating these terms at the center of sedimentation and using the resulting values in place of the mass averaged quantities. Letting the subscript (c) denote values determined at the center of sedimentation, equation (24) becomes:

$$\frac{d\langle \ln r \rangle}{dt} = \omega^2 s_{20,w} \frac{(1 - \bar{v}\rho_c)}{(1 - \bar{v}\rho_{20,w})} \frac{\eta_{20,w}}{\eta_c} - D_{20,w} \frac{1}{r_c} \frac{\eta_{20,w}}{\eta_c^2} \left(\frac{\partial \eta}{\partial r}\right)_{t,c} \quad (25^*)$$

It can be seen from equation (25*) that the diffusion coefficient is now included in a term which has a finite value when the viscosity gradient is different from zero. However, this term is usually very small. To determine $s_{20,w}$, values of $\langle \ln r \rangle$ should be plotted as a function of time and the slope measured. The small term involving $D_{20,w}$ is then added to correct for diffusion, and finally $s_{20,w}$ is calculated.

Uniform Cell and Centrifugal Field. The equations developed in this section should be applicable to data obtained from the swinging-bucket preparatory centrifuge. Since sedimentation is radial, material tends to accumulate at the sides of the tube and convect. If there exists a large, superimposed density gradient along the length of the tube, radial convection is prevented. Convection would then occur in a tangential direction, causing uniform mixing at any given level. This is assumed to be the case for the following derivation.

The flow of mass across a given level in the cell is:

$$AJ = +A\omega^2 scr - AD\left(\frac{\partial c}{\partial r}\right)_t \quad (26)$$

Equation (26) is multiplied by dr, and integrated across the zone from the meniscus at a to the cell bottom b. Since $c(a) = c(b) = 0$, the term involving the diffusion coefficient vanishes. As before, this is true even if the diffusion coefficient is a function of concentration. Integrating by parts, taking the time derivative of the left-hand side, and combining with equation (5) yields:

$$\frac{d}{dt}\left[\int_a^t r\, dm\right] = \omega^2 s \int_a^b r\, dm \quad (27)$$

where the total derivative has been used, since the quantity in brackets is not a function of r.

Dividing (27) by $\int_a^b dm$, and letting the symbol $\langle r \rangle$ represent the center of gravity of the sedimenting zone, gives:

$$\frac{d\langle r \rangle}{dt} = \omega^2 s \langle r \rangle \quad (28a)$$

or

$$\frac{d \ln \langle r \rangle}{dt} = \omega^2 s \quad (28b)$$

If the sedimentation coefficient is concentration dependent, equation (28) becomes:

$$\frac{d\langle r \rangle}{dt} = \omega^2 s_0(\langle r \rangle - k_1\langle cr \rangle + k_2\langle c^2 r \rangle + \cdots) \quad (29)$$

Multiple-Component Systems. If several sedimenting species are present within a single migrating zone, the sedimentation property of the center of gravity is not equal to the weight-average sedimentation coefficient but instead to the weight-average sedimentation velocity:

$$\frac{d\langle r \rangle_{obs}}{dt} = \langle v \rangle \quad (30)$$

where

$$\langle v \rangle \equiv \frac{\sum_i \langle v_i \rangle m_i}{\Sigma m_i} = \frac{\sum_i \omega^2 s_i \langle r \rangle_i m_i}{\sum_i m_i}$$

where m_i is the total mass of component i within the cell.

An apparent sedimentation coefficient may now be defined as:

$$s_{app} \equiv \frac{\langle v \rangle}{\omega^2 \langle r \rangle_{obs}} = \frac{\sum_i s_i e^{+\omega^2 s i t} m_i}{\sum_i e^{+\omega^2 s i t} m_i} \quad (31)$$

This quantity varies with time during the course of a single run. To estimate the size of the error which would be caused by using the migration of the center of gravity of the distribution to estimate the weight-average sedimentation coefficient, an extreme case may be considered. If a 50:50 mixture of a 10 S and a 20 S component is layered on a density gradient at a distance of 4 cm from the axis of rotation, then, by the time the faster component has migrated to a depth of 8 cm from axis rotation, the slower component is located at a depth of 5.657 cm. The center of gravity is the average of these two values, 6.829 cm. The apparent sedimentation coefficient of the center of gravity is then 15.434 S. Since the weight-average sedimentation coefficient is 15 S, an error of 2.89% is found in this exceptional case. For more compact zones the errors are correspondingly less; in all probability they would be negligible in any practical situation.

Effect of a Superimposed Density Gradient. An exact solution to the problem of the boundary location in the presence of a superimposed density gradient would require complete knowledge of the boundary shapes and the viscosities and densities of the solvent as a function of time and position within the centrifuge cell. With present techniques it is not practical to obtain all of this information during the course of a preparatory run, and some simplifying assumptions seem to be required. At the conclusion of the run, the viscosity and the density of the solvent may be determined point by point throughout the cell. It will be assumed that these quantities remain constant during the course of the run, and that the sedimentation behavior of the boundary is adequately described by the position of the center of gravity of the boundary. (In doing this, a correction term involving $D_{20,w}$ and the viscosity gradient, similar to that included in equation (25*), is assumed to be very small and is not included in the treatment.)

Equation (28) may now be written for the case of a superimposed viscosity-density gradient as:

$$\frac{d \ln \langle r \rangle}{dt} = \omega^2 s_{20,w} \frac{(1 - \bar{v}\rho_c)}{(1 - \bar{v}\rho_{20,w})} \frac{\eta_{20,w}}{\eta_c} \qquad (32^*)$$

where the subscript (c) refers to values of η and ρ calculated at the center of gravity of the migrating zone. Equation (32*) may be integrated from the initial position of the center of gravity, $\langle r \rangle_0$, to the final position of the center of gravity, $\langle r \rangle_f$, to yield:

$$\frac{1}{\omega^2 t_{eff}} \int_{\langle r \rangle_0}^{\langle r \rangle_f} \left[\frac{(1 - \bar{v}\rho_{20,w})}{(1 - \bar{v}\rho_c)} \frac{\eta_c}{\eta_{20,w}} \right] d \ln \langle r \rangle = s_{20,w} \qquad (33^*)$$

where t_{eff} is the effective time of centrifugation.

A simple and rapid method of evaluating the integral on the left-hand side of equation (33*) is to plot

$$(1 - \bar{v}\rho_{20,w})\eta_c/(1 - \bar{v}\rho_c)\eta_{20,w}$$

as a function of ln r between $\langle r \rangle_0$ and $\langle r \rangle_f$, then to determine the area under the curve, and hence the value of the integral, by planimetry. Then if the effective time of sedimentation is known, $s_{20,w}$ may be calculated from equation (33*).

Discussion

The foregoing equations are restricted to cases where there exists no convective transport of mass in a radial direction because the movement of the total mass in the cell is considered in the determination of sedimentation coefficients. Therefore there is an important corollary to the application of these equations: *If the sedimentation coefficients obtained by zonal centrifugation and the use of these equations agree with the values obtained in the analytical ultracentrifuge by conventional boundary centrifugation, then this agreement implies that no net convective transport of mass has occurred in a radial direction during the zone centrifuge run.* However, a convective rift which occurred in a tangential direction, for example, from the walls toward the central axis of the tube, would not be detected by this method unless it also caused a net transport of mass in a radial direction.

Using a swinging-bucket rotor and cylindrical cells, Martin and Ames (1961) have measured the sedimentation coefficients of a number of proteins by zonal centrifugation in a density gradient. The values of $s_{20,w}$ which they have obtained agree closely with the values reported by other workers using the analytical ultracentrifuge. Martin and Ames (1961) used the positions of the centers of the enzyme peaks in their calculations of $s_{20,w}$ instead of the positions of the centers of gravity, $\langle r \rangle$, as is suggested in this communication. However, the published illustrations show that their migrating zones are nearly symmetrical in shape; therefore the peak values of position are almost identical with the centers of gravity. In this case, the results of Martin and Ames (1961) may be used as evidence that little or no convective transport of mass occurred in a radial direction in cells of uniform cross-sectional area.

Vinograd *et al.* (1963) have described an elegant method for the formation and sedimentation of zones of macromolecules in the analytical ultracentrifuge. Using a synthetic-boundary cell of their own design, a thin lamina of a solution of macromolecules is layered over a denser bulk solution. The necessary density gradient for the prevention of convection is then generated, during the course of the centrifuge run, by the diffusion of small molecules between the lamina and the bulk solution. The sedimentation coefficients obtained from band centrifugation agree well with those obtained by conventional boundary centrifugation for protein, RNA, DNA, and virus. In developing a theory for band centrifugation, these authors have considered the time derivatives of the various reduced moments of mass, $d\langle r^n \rangle/dt$. Neglecting small terms, they write (equation 13, Vinograd *et al.*, 1963): $\ln (R/R^\circ) = s\omega^2(t - t^\circ)$, where their symbol R is identical to our symbol $\langle r \rangle$. Taking the time derivative of this expression yields:

$$\frac{d \ln \langle r \rangle}{dt} = \omega^2 s \qquad (34^*)$$

This approximate equation has been developed by Vinograd *et al.* for the radially shaped cell in a centrifugal field. It can be seen to be identical to our equation (28), which is derived without approximation for a cell of uniform cross-sectional area in a centrifugal field. An exact solution for the case of the radially shaped cell is given by our equation (20). However, errors involved in using equation (34*) are insignificant when narrow bands are studied.

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Purification of Staphylococcal Enterotoxin B*

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ABSTRACT: A method has been developed for the isolation of enterotoxin B in highly purified form and in yields of 50–60% from cultures of *Staphylococcus aureus*. The method involves removal of the toxin from the culture and from the bulk of the impurities with CG-50 resin and purifying it to a high degree by chromatography on carboxymethyl-cellulose. Physical, chemical, and biological studies on the purified preparation show that it is a simple protein, molecular weight 35,300. The dose of purified protein required to produce emesis or diarrhea in monkeys is 0.1 μg by intravenous injection and 0.9 μg by oral feeding per kg of animal weight.

Staphylococcus aureus produces a variety of toxic substances. One of these is enterotoxin B, which causes emesis and diarrhea in experimental animals very similar to that caused by enterotoxin A, which is usually found in cases of food poisoning in humans (Casman *et al.*, 1963). The enterotoxins apparently are metabolic products of *S. aureus* formed at the surface of the cell and released into the medium (Friedman and White, 1965), and are chemically, physically, and biologically distinct from the endotoxins (Martin and Marcus, 1964). Bergdoll *et al.* (1959a, 1961) reported the first significant purification of this toxin by a combination of acid precipitation, adsorption on Amberlite IRC-50 (XE-64), ethanol precipitation, and starch-bed electrophoresis. Recently Frea *et al.* (1963) effected a partial purification of this toxin by a combination of ethanol precipitation, filtration on Sephadex, and electrophoresis on Sephadex. Only milligram quantities of toxin were obtained by these methods. This paper describes a method of purification on a larger scale based on chromatographic procedures employing carboxylic acid resins that has resulted in preparation of enterotoxin B of higher purity and in higher yields than that obtained by the cited methods.

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Methods

The toxin was produced by culturing *S. aureus* strain S-6 for 18 hours with aeration in a medium adjusted to