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LIGHT-SCATTERING STUDIES ON RABBIT BRAIN MICROSOMES

I. EVIDENCE FOR OSMOTIC BEHAVIOR*

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SUMMARY

The osmotic behavior of microsomal vesicles isolated from rabbit brain was studied by light-scattering measurements. When suspended in sucrose solutions of various osmolar concentrations, angular scanning of the intensity of light scattered by microsomes was found to afford a means of estimating relative volume changes of microsomal vesicles on the basis of light-scattering theory. With this method, it was demonstrated that, as reported by TEDESCHI *et al.*[3] and TEDESCHI AND HARRIS[6,7], the Boyle–Van't Hoff's relationship was applicable to the osmotic behavior of microsomal vesicles in sucrose media. In NaCl or KCl solutions, microsomes also responded as osmometers to changes in the osmotic pressure.

INTRODUCTION

PALADE AND SIEKEVITZ[1] demonstrated by electron microscopy that liver microsomes fixed in hypotonic solutions appeared to swell, and they therefore proposed that these vesicles respond *in vitro* as osmometers to the osmotic pressure of the medium. Since then, several studies have been reported on the osmotic behavior of microsomes which employed photometric observations of the light-scattering properties of microsomal suspensions[2–5]. However, these observations have been entirely confined to studies of the opacity of the suspension. Moreover, some discrepancies were found among the results; in contrast to the findings of PALADE AND SIEKEVITZ[1], SHARE AND HANSROTE[2] failed to detect changes in absorbance among microsomal suspensions incubated in sucrose solutions of different concentrations, while TEDESCHI *et al.*[3] and TEDESCHI AND HARRIS[6,7] demonstrated experimentally an empirical relation between the size of microsomes as well as of mitochondria and the absorbance of their suspensions, which was based on Boyle–Van't Hoff's osmotic law.

In studies on size, shape and volume changes of isolated subcellular particles such as mitochondria and microsomes, the measurement of absorbance has been widely employed. Indeed, the use of such turbidimetric methods has been important in elucidating the mechanism of mitochondrial swelling. Similar studies have been also reported on microsomes: for instance, the effects of carcinogenic chemicals[8], lipid

* This work was done as part of a dissertation for the M.D. degree (K.K.), and the paper is dedicated to the late H. Murakami, an excellent student of physiology.

peroxide formation[9], and ATP and ions[10,11] on the swelling of microsomes. However, as discussed by KOCH[12] many factors must be taken into consideration to correlate the total amount of light scattered, *i.e.*, the turbidity, with the mass, size, shape and volume of the particles in the solution. It might indeed be true that no available light-scattering theory has yet been sufficiently developed to permit a satisfactory interpretation of data on objects having the size, shape and index of refraction of subcellular particles. But angular light-scattering measurements could afford, as pointed out by GOTTERER *et al.*[13], some advantages over simple absorbance measurements in the study on shape and volume changes of these subcellular particles.

Light-scattering measurements have already been applied to microsomal suspensions by PACKER AND RAHMAN[14]; they reported reversible structural changes in the microsomal system induced by ATP and related compounds, but neither the quantitative relationship between changes in the intensity of light scattered and volume changes of microsomes nor the angular light-scattering pattern was measured. The present study was therefore undertaken to consider the possible contribution of light scattering studies to knowledge on changes in the shape and volume of microsomes under various conditions. This article reports reversible change in the intensity of light scattered from the microsomal suspension induced by changes in osmolar concentration of their suspending media and their interpretation based on MIE'S[15] theory. A preliminary communication concerning part of this work has already been reported[16].

METHODS

With a Teflon–glass homogenizer, rabbit brains were homogenized in 10 vol. of 0.25 M sucrose solution containing 0.01 M Tris–HCl buffer (pH 7.3). The homogenate was centrifuged for 20 min at 12000 × *g*, the supernatant being separated carefully with a pipette without disturbing the loosely packed material above the pellet. To minimize the possibility of contamination by large particles which would have serious effects on light scattering, the supernatant was subjected to recentrifugation under the same conditions. The final supernatant fraction was carefully removed and centrifuged for 120 min at 78000 × *g*, the pellet being suspended in buffered 0.25 M sucrose (about 1 ml/g of brain) and stored at 0°. When salt solution was used as a suspension medium, the final pellet was suspended in 0.125 M solution (pH 7.3 with 0.01 M Tris–HCl buffer).

The stock suspension was submitted to light-scattering measurements after dilution with a Tris-containing sucrose or saline of the appropriate concentration or distilled water to obtain the desired osmolarity and/or a suitable microsomal concentration. Since a linear relationship between microsomal concentration (expressed as μg protein by the method of LOWRY *et al.*[17]) and the intensity of light scattered at 45° or 90° from the incident light direction was observed up to 500 μg/ml, 20–100 μg/ml of microsomal concentration was usually employed.

The light-scattering measurements were made with a Brice–Phoenix type photoelectric light-scattering photometer (Shimadzu PG 2 type) at 20–24°. A semioctagonal cell with an optical path of 24 mm (usually for measurements of I_{45}, I_{90} and the dissymmetry coefficient) and a cylindrical cell, 15 mm in diameter (for angular scanning), were employed. The intensity of scattered light as a function of the angle was measured

in the horizontal plane, the angle of 0° being taken as the direction of the incident beam. No attempt was made to obtain an absolute value of the light intensity; it was referred to that at 0° (I_0), *i.e.*, the light intensity at $\theta°$,

$$I_\theta = \frac{\text{deflection of galvanometer at } \theta°}{\text{deflection of galvanometer at } 0°}$$

The wavelengths used were 436, 546 and 578 mμ, from a high-pressure mercury lamp (200 W).

To minimize contamination with dust particles, all the solutions used for the light-scattering measurements, for instance those for preparing the stock solutions or for diluting them, were centrifuged for 90 min at 12000 × *g* or passed through a Millipore filter (Gelman Instrument Co., pore size, 100 ± 8 mμ) under pressure before use. The differences between the refractive index of the suspensions and that of the suspending media ($\Delta\mu$) were measured with a differential refractometer (Shimadzu DR-4 type); the refractive index of the suspension media (μ_1) was measured with an Abbé refractometer.

The gravimetric estimation of the water content of microsomes was performed on an aliquot of the suspension extrapolated to a large volume by a procedure similar to that used by BENTZEL AND SOLOMON[18]; the wet and dry weights (drying at 75° for 30–40 h to attain a constant weight) of the pellet were determined, the percentage decrease in weight being used as a measure of the water content.

A portion of the microsomal pellet was fixed with 1% O_8O_4 in Dalton's buffer (pH 7.3), for 2 h at 0°, dehydrated in ethanol, embedded in Epon[19], and observed in an Hitachi electron microscope (HU-11) after thin-sectioning.

RESULTS

Electron microscopy of brain microsomes

Electron microscopy revealed that the microsomal pellets obtained from the stock suspension consisted mainly of spherical or ellipsoid vesicles ranging in diameter from about 100 to 500 mμ, while far smaller numbers of free particles (ribosomes) were observed. The distribution of vesicles (sample = 1272) according to diameter is shown in Fig. 1; vesicles 100–200 mμ in diameter predominate. Of course, some factors, such as the effects of fixation and the deposition of osmium, should be taken into account when estimating vesicle size from electron micrographs. As a first approximation, however, the microsomes used in the present experiments might be regarded as particles with a mean diameter ($\bar{d}$) of about 150 mμ.

The dependence of light scattering of microsomal suspension on the sucrose concentration

When the sucrose concentration of the microsomal suspension is raised from 0.25 to 0.5–2.0 M, the intensity of light scattered at 45°, 90° or 135° (I_{45}, I_{90} and I_{135}) rapidly decreases, within 1–2 min reaching a constant value which varies linearly, to some extent, with the reciprocal of the sucrose concentration, $1/c$ (Fig. 2B). Such a nearly linear relationship between I_θ and $1/c$ holds not only for any given microsomal concentration (20–150 μg/ml), but also for any wavelength used (436, 546 and 578 mμ) (Fig. 3).

To see whether such decreases in scattering are reversible, the hypertonic sucrose

concentration was readjusted to 0.25 M by diluting 8 times with a sucrose solution of the appropriate concentration, or distilled water, and the I_{45} values of diluted suspensions were then compared with that in 0.25 M sucrose. Such a dilution of the microsomal suspension caused a decrease in I_{45}. However, as illustrated in Fig. 2B, the I_{45} values of all the diluted suspensions were found to be identical. Furthermore, when the osmolarity of these diluted suspensions was again raised by adding sucrose, the relationship between I_{45} and $1/c$ reappeared (Fig. 2B). These facts indicate that the change in light scattering of the microsomal suspension is reversible and dependent only on the sucrose concentration.

These findings on brain microsomes are in agreement with those reported by TEDESCHI *et al.*[3] on liver microsomes and suggest that changes in the osmotic volume

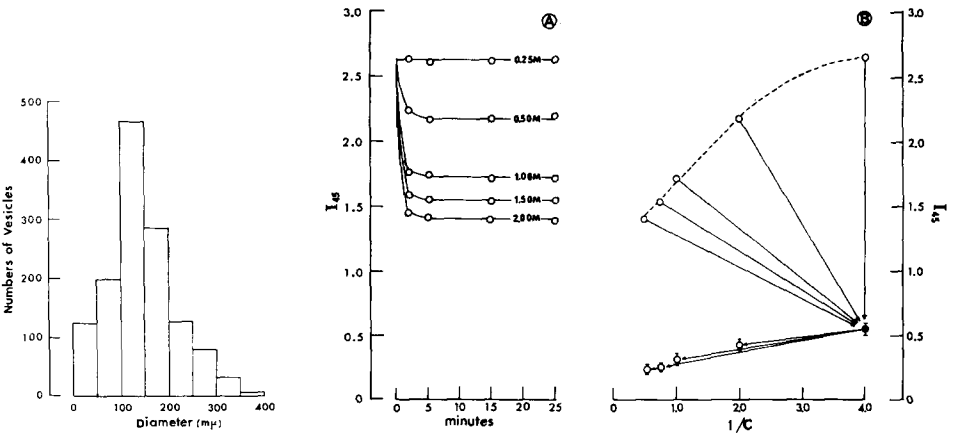

Fig. 1. The distribution of the diameter of 1272 brain microsomal vesicles observed in electron micrographs.

Fig. 2. Effect of osmolarity on the light scattering from brain microsomal suspension (187 μg microsomal protein per ml). (A) The time course of the change in light scattering at 45° for a wavelength of 436 mμ (I_{45}). (B) Reversibility of osmotically induced light-scattering changes. The arrows pointing downwards to the black symbol corresponding to 0.25 M sucrose represent the change of I_{45} occurring when the hypertonic sucrose concentrations are readjusted to 0.25 M by diluting 8 times. The arrows from the black symbol to the empty ones stand for changes in I_{45} induced by adding sucrose to the diluted suspension.

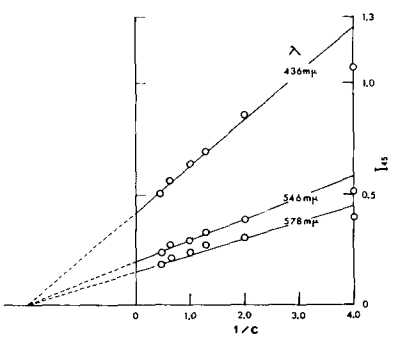

Fig. 3. The dependence of light scattering at 45° (I_{45}) of a microsomal suspension (132 μg protein per ml) on the reciprocal of sucrose concentration ($1/c$) and the wavelength of incident light (λ).

of suspended microsomes are reflected by changes in light scattering, with the decrease in $I_\theta(\Delta I_\theta)$ being approximated just as the reciprocal of absorbance in the formulation of these investigators by

$$\Delta I_\theta = \frac{L_\lambda}{G_n}\left(\frac{\kappa}{c} + \beta\right) \quad (1)$$

where c is the sucrose concentration and other symbols are constants corresponding to those used by TEDESCHI *et al.*[3], *i.e.*, κ and β are empirical constants independent of λ and microsomal concentration (n), while L_λ depends solely on λ, and G_n on n. I_θ would provide a measure of the relative changes in volume of microsomal vesicles in response to hypertonicity (see Fig. 7B): the smaller the θ and λ, the higher the value of ΔI_θ. However, the effect of stray light becomes larger for $\theta < 45°$. In the present study, therefore, I_{45} with a λ of 436 mμ was routinely employed.

The angular-scattering characteristics

Some examples of the angular-scattering pattern of microsomal suspensions are presented in Fig. 4. It is evident that the light scattering in the forward direction is far greater than that in the backward direction, with a minimum between 100 and 110°. These characteristics of the scattering pattern of dilute suspensions remain unaltered at different wavelengths (λ) and with different sucrose concentrations (c). Indeed, as seen in the figure, the angular variation curves for different concentrations entirely overlap when a suitable set of λ's is employed. The dissymmetry, the ratio of the intensity of light scattered at 45° to that at 135° ($Z = I_{45}/I_{135}$), computed from the results of angular scanning of light scattering, as well as being determined with a semioctagonal cell, remains fairly constant (4.5–6.0), its magnitude being similar to that of dilute mitochondrial suspensions[13]. It might be said, therefore, that the general

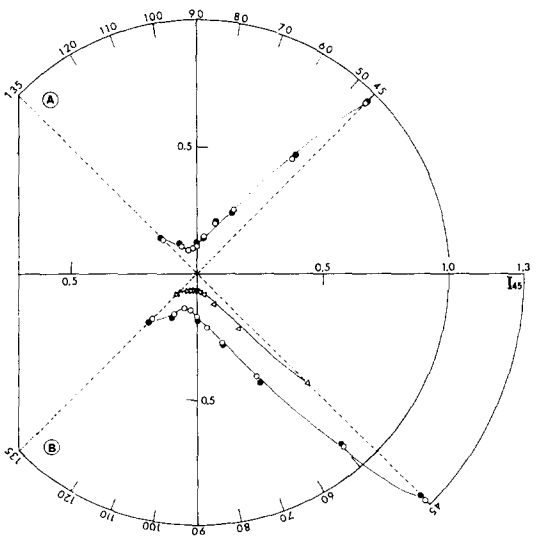

Fig. 4. Angular variation curves of microsomal suspensions (136 μg protein per ml) in sucrose media. (A) ○, c = 0.36 M, λ = 578 mμ; ●, c = 0.52 M, λ = 546 mμ. (B) ○, c = 0.25 M, λ = 546 mμ; ●, c = 2.0 M, λ = 436 mμ; △, c = 2.0 M, λ = 546 mμ.

shape of the scatter envelope of the microsomal suspension is similar, at least qualitatively, to that reported for mitochondrial suspensions and to that expected from MIE's[15] theory on spherical particles of such a large size. For theoretical considerations of particle size and shape, however, the value of Z extrapolated to zero particle concentration, $[Z]$, is needed. An example of such a measurement is given in Fig. 6, which clearly shows that the higher the sucrose concentration, the lower the value of $[Z]$.

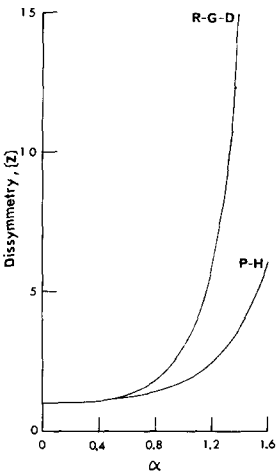

Fig. 5. Relationship between the dissymmetry ($[Z]$) and sucrose concentration as computed from PANGONIS AND HELLER's table (P–H) and Rayleigh–Gans–Debye's approximation (R–G–D).

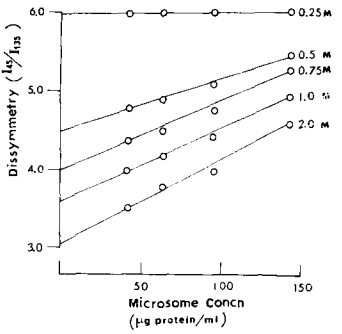

Fig. 6. The dissymmetry coefficient (Z) at a wavelength of 436 mμ of a microsomal suspension in sucrose media of various concentrations.

Some theoretical considerations

When the size of particles is not extremely small with respect to λ and the refractive index of the particles (μ_2) is not approximately equal to that of the medium (μ_1), there is no satisfactory theory available for interpreting the light-scattering data in detail except for MIE's[15] theory on solid spheres. Tables of the necessary function for his solution have been prepared by PANGONIS AND HELLER[20,21], but the solution does not usefully apply to particle shapes other than spherical. However, since microsomes may be regarded, as an approximation, as spherical shells, changes in the size of the microsomes could be estimated, at least semiquantitatively, from the light-scattering theory. This states that the angular scattering pattern of spherical particles is chiefly determined by two quantities, m and α, where $m = \mu_2/\mu_1$ and $\alpha = \pi d/\lambda'$ ($\lambda' = \lambda/\mu_1$).

(1) α *value of microsomes.* PANGONIS AND HELLER's tabulation showed that the minimum light scattering at 100–105° occurs at an α of 1.0–1.2 for all values of m presented (1.05–1.30). Indeed, the mean diameter ($\bar{d}$) of brain microsomes estimated from electron microscopy (Fig. 1) gives an α of about 1.4 for $\lambda = 436$ mμ (*i.e.*, $\lambda' = 325$ mμ). The value of $[Z]$ for $\alpha = 1.0$–1.2, obtained from PANGONIS AND HELLER's tabulation, is in the same order of magnitude as those observed (Fig. 5), but somewhat lower (see Fig. 6). Microsomes are heterogeneous in size and shape, and moreover, cannot be assumed to be solid spheres to fit MIE's theory, so that such a minor dis-

crepancy is to be expected. The tendency toward qualitative similarity, however, seems worthy of note. As will be discussed later, the Rayleigh-Gans-Debye approximation also proved that the α value of brain microsomes is about 1.0–1.2, a value close to those estimated above.

(2) *Refractive index.* The effective refractive index (m) of microsomes is difficult to estimate. The difference between the refractive index of a suspension (μ_{12}) and that of its suspension medium (μ_1), is $\Delta\mu = \mu_{12} - \mu_1$; $\Delta m = \Delta\mu/\mu_1$ could be determined experimentally with a differential refractometer. According to the rule for mixtures, Δm of dilute suspensions would be approximated by

$$\Delta m = \phi(m - 1) \tag{2}$$

where ϕ is the volume fraction of microsomes and $m = \mu_2/\mu_1$. On the other hand, m can be approximated, as was done by WALLACH *et al.*[22], by

$$m = \frac{\mu_s}{\mu_i}\beta + \frac{\mu_i}{\mu_i}(1 - \beta) = m_s\beta + m_i(1 - \beta) \tag{3}$$

or

$$\beta = \frac{m - m_i}{m_s - m_i}, \quad \left(m_s = \frac{\mu_s}{\mu_1},\ m_i = \frac{\mu_i}{\mu_1}\right) \tag{4}$$

where β is the volume fraction of the shell[22], μ_s is the refractive index of the shell, and μ_i is that of the fluid within the shell.

As c, the sucrose concentration of the suspension medium, and therefore μ_1 increase, osmotic water loss and shrinkage of vesicles occur, resulting in an increase in β and μ_i. If the increase in μ_i roughly parallels that of μ_1, m_i would remain fairly constant, its value being nearly 1, as assumed by WALLACH *et al.*[22]. As discussed by these investigators, a large value such as 1.60–1.65 is the most likely one for μ_s because of the tight packing of lipid in the vesicle membranes, which might be assumed to remain constant irrespective of c. It follows a decrease in m_s for higher c and so for higher β. It is therefore obvious from Eqn. 3 that m remains constant if a change in $(m_s - 1)$ is inversely proportional to a change in β. Assuming $m_i = 1$, Eqns. 2 and 4 give

$$\frac{\Delta m}{m_s - 1} \equiv \frac{\Delta\mu}{\mu_s - \mu_1} = \phi\cdot\beta \tag{5}$$

where Δm can be ascertain edexperimentally and $m_s - 1$ is estimated by putting $\mu_s = 1.65$. The product, $\phi\cdot\beta$, is equivalent to the total mass of vesicular membranes per unit volume of suspension, so that it is constant for a given microsomal concentration. Hence the left hand expression in Eqn. 5 should be constant. As seen in Table I, the variation of $\Delta\mu/(\mu_s - \mu_1)$ was found to be within $\pm 12\%$, suggesting that the above-stated reasoning is not greatly in error. Thus, it might be said that under the present experimental conditions, m remains fairly constant irrespective of c, its probable value being around 1.05, as estimated by WALLACH *et al.*[22]. Since microsomal vesicles in 1-2 M sucrose solution shrink to about half of their volume in 0.25 M sucrose (see below), it follows that β incresases from about 0.3 in 0.25 M sucrose[22] to 0.6–0.7 in hypertonic conditions. Putting $\mu_s = 1.65$, however, Eqn. 3 shows that m increases from 1.06 in 0.25 M to at most 1.07 in hypertonic conditions.

TABLE I

REFRACTIVE INDEX (μ_1) OF SUCROSE SOLUTION AND REFRACTIVE INDEX INCREMENT (μ) OF MICROSOMES (113 μg PROTEIN PER ml)

Sucrose concn. (M)	μ_1	$\Delta\mu$ ($\times 10^6$)	$\frac{\Delta\mu^*}{\mu_s - \mu_1}$ ($\times 10^6$)	*Relative volume change***		
				(1)	(2)	(3)
0.25	1.344	292	95	100	100	100
0.50	1.362	276	96	93	70 ± 5	(65)
1.00	1.386	242	92	80	57 ± 7	(48)
2.00	1.470	203	113	70	48 ± 10	40

* Calculated assuming that $\mu_s = 1.65$.

** (1) Estimated by $(\Delta m)_c/(\Delta m)_{0.25}$; (2) estimated from $[Z]$; (3) estimated from the overlapping of angular-scattering patterns. The values given in parentheses are those obtained by interpolation of Fig. 7.

(3) *Dissymmetry.* KOCH[12] has already discussed the applicability of the Rayleigh–Gans–Debye approximation to bacteria and mitochondria. The usual criterion given for applicability of the Rayleigh–Gans–Debye method is that $2\alpha(m-1) \ll 1$. The above considerations show that this criterion seems to be met fairly well by the microsomal vesicles used. As the distribution of particle size becomes broader, and as the particles become asymmetric, the phase shift in the light traversing the particle becomes less significant, so that the application of an average interference factor, $P(\theta)$, to the computation of the intensity at an angle θ would also be justified.

For a shell of diameter d, $P(\theta)$ can be written in analytical form:

$$P(\theta) = \left(\frac{\sin x}{x}\right)^2; \quad x = \frac{2\pi d}{\lambda'} \sin\frac{\theta}{2} = 2\alpha \sin\frac{\theta}{2}. \tag{6}$$

It follows that

$$[Z] = 5.82 \left\{\frac{\sin(2 \sin 22.5^\circ)}{\sin(2 \sin 67.5^\circ)}\right\}^2 \tag{6'}$$

Using Eqn. 6', α can be plotted against $[Z]$ as shown in Fig. 5, in which the relationship α–$[Z]$ for a solid sphere, computed from PANGONIS AND HELLER's table ($m = 1.05$), was included for comparison. Since $[Z]$ of microsomal suspensions extrapolated to zero concentration ranged from 3.0 to 6.0 (Fig. 6), their α value as expected from the Rayleigh–Gans–Debye method is about 1.0–1.2, in very good agreement with the values derived from the reasoning given above based on MIE's theory, and from electron-microscopic observations.

Such an agreement may be only fortuitous; as expected from Eqns. 6 and 6', $P(\theta)$ becomes inversely proportional to x^2, with $[Z]$ being about 5.8, independently of α, if a quite broad distribution of shells occur. Indeed, mitochondria show a $[Z]$ of about 6 (as reported by GOTTERER *et al.*[13]) in spite of their being far larger than microsomes. The finding that $[Z]$ decreases significantly when the expected osmotic shrinkage of vesicles occurs in hypertonic media (Fig. 6), however, strongly suggests that this is not the case.

(4) *Estimation of volume change.* Suppose that the mean diameter of vesicles in

0.25 M sucrose, $\bar{d}$ shrinks to $\bar{d}^*$ in a hypertonic medium (sucrose concentration of c), and the dissymmetry of both suspensions ($[Z]$ and $[Z]^*$) is measured with the same wavelength of light, λ. Since the mean volume of microsomal vesicles, $\bar{V}$, is proportional to $\bar{d}^3$, it is easily shown that

$$\frac{\bar{V}^*}{\bar{V}} = \left(\frac{a^*}{a}\right)^3 \cdot \left(\frac{\mu_1}{\mu_1^*}\right)^3 \tag{7}$$

where the asterisk denotes quantities under hypertonic conditions, corresponding to d^* and $[Z]^*$. The first term on the right-hand side of Eqn. 7 can be computed from Fig. 5 from the observed $[Z]$ and $[Z]^*$, while the second term can be determined experimentally. Thus osmotic volume changes of vesicles can be estimated approximately if the α–$[Z]$ relation based on the Rayleigh–Gans–Debye method provides a fairly good approximation. The results obtained by such a procedure are presented in Table I and Fig. 7A, in which v ($\bar{V}^*/\bar{V}$) is plotted against $1/P$, P being the osmotic pressure of the suspension medium.

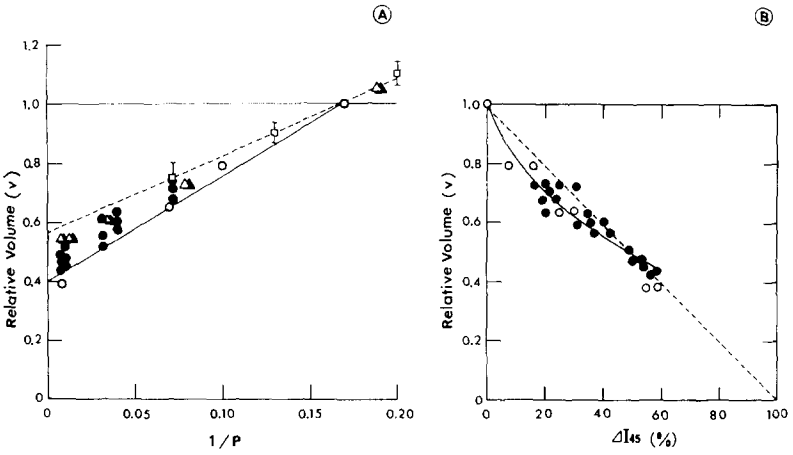

Fig. 7. (A) Reciprocal of osmotic pressure in Torr of suspension media ($1/P$) and relative volume of microsomal vesicles (v) referred to that in 0.25 M sucrose. In sucrose media: ○, overlapping of angular scattering; ●, determination of dissymmetry coefficient; □, measurement of the water content. In salt media: v was estimated from ΔI_{45}–v relation in (B); △, in NaCl solution; ▲, in KCl solution. (B) Correlation between the decrease in light scattering at 45° (ΔI_{45}) and v in sucrose media. Open and solid symbols are the same as in (A).

Alternatively, we could estimate v in the following way. Suppose that we can choose a set of λ and λ^* values to satisfy the equation $\alpha = \alpha^*$. When the angular scanning is performed with microsomal suspensions of the same concentration in sucrose (concn. 0.25 M and c), with λ and λ^*, respectively, both angular variation curves should be identical because m is constant irrespective of c (*i.e.*, $m = m^*$), as discussed above. Indeed, the examples presented in Fig. 4 provide experimental proof of the existence of such a condition. Now, under these conditions it is obvious that

$$v = \left(\frac{\lambda^*}{\lambda}\right)^3 \cdot \left(\frac{\mu_1}{\mu_1^*}\right)^3 \tag{8}$$

and v can be easily estimated. The results obtained by this procedure are also included

in Table I and Fig. 7, and show a fairly good agreement with those estimated by Eqn. 7.

If m is constant, independently of c, and the Eqn. 2 is valid, v would be evaluated from $\Delta m{:}\Delta m$ at 0.25 M. The values of v thus computed are presented in Table I, and show a rather poor agreement with those obtained from $[Z]$ and the overlapping of angular scattering patterns. Since Δm is determined by $m-1$, an increase of 0.01 in m results in about a 20% increase in v as estimated from Δm. Moreover, the accuracy with which $\Delta\mu$ can be determined is restricted. Therefore a discrepancy such as that seen in Table I is not unexpected.

Applicability of the Boyle–van 't Hoff relation

Employing $1/c$ instead of $1/P$, TEDESCHI *et al.*[3] proved a linear relation between $1/c$ and the reciprocal of absorbance of a microsomal suspension, a finding which suggests that the Boyle–van 't Hoff relation is applicable to the osmotic behavior of liver microsomes. It is well-known, however, that the observed P of concentrated sucrose solutions (about 0.4 M) showed a considerable deviation from that calculated according to the theory of van 't Hoff[23]. Therefore the interpolated values obtained from FINDLEY's[23] monograph have been used to plot v against $1/P$ in Fig. 7A. Using Eqn. 8 to estimate v was more or less a method of trial and error. In Fig. 7A were included data obtained from measurements of the water content of microsomes. These measurements were only made at sucrose concentrations below 0.5 M because of inaccuracies caused by sucrose remaining attached to the pellet in media of higher concentrations. As seen in Fig. 7A, v varies linearly with $1/P$, irrespective of the method of evaluation;

$$P(v-b) = K \tag{9}$$

It might be concluded, therefore, that the Boyle–van 't Hoff relation is applicable to the osmotic behavior of brain microsomes. Using Eqn. 1, ΔI_{45} can be related to v as shown in Fig. 7B, where the former varies linearly with v only for high osmolar concentrations (see Figs. 2 and 3). Roughly speaking, however, these results might be said to support the results reported by TEDESCHI *et al.* for liver microsomes.

Osmotic behavior in salt media

With exactly the same procedures as those applied to suspensions in sucrose solutions, the osmotic behavior of microsomal vesicles in NaCl or KCl solutions (0.125–1.0 M) was examined. Hardly any significant difference could be found between the responses of the vesicles in NaCl and KCl media.

Using the v-ΔI_{45} relationship given in Fig. 7B, v in various salt concentrations is plotted against $1/P$, which was computed according to van 't Hoff's law by applying the osmotic coefficient given in Lewis and Randall's textbook[24] (Fig. 7A). The results presented demonstrated that the Boyle–van 't Hoff relation applies to vesicles in the salt media just as to those in the sucrose solutions.

When a very small amount of divalent cations such as Ca^{2+} and Mg^{2+} (2–20 mM) was added to microsomes suspended in sucrose or KCl, a significant increase in I_θ was observed, a fact already noted by ROBINSON[5], who suggested that aggregation or large structural changes of the vesicles had occurred. In their light-scattering study on spinach chloroplast grana, which showed an angular-scattering pattern quite sim-

ilar to that of microsomes, GROSS AND PACKER[25,26] also reported that the increase in light scattering observed upon adding divalent ions to the suspension of grana was a true change in volume but not osmotic in nature. It might be said, therefore, that divalent cations have a specific action on microsomal membranes, which is not correlated with their osmotic effect.

DISCUSSION

The results presented clearly demonstrate that when brain microsomes are suspended in hypertonic solutions, scattering changes occur in a manner quite similar to that observed for absorbance changes of liver microsomes[3]. Decreases in scattering follow increases in the absorbance, so that the reciprocal of the absorbance varies, at least qualitatively, with the scattering changes. It is quite natural, therefore, that Eqn. 1 is approximately applicable to the decreases in scattering.

To correlate such scattering changes of microsomes with changes in their volume, two methods are presented: one is based on dissymmetry, and the other on the overlapping of angular-scattering patterns obtained at different wavelengths. The former employs the approximation of Rayleigh–Gans–Debye, the applicability of which is assured by satisfying the usual criterion, $2\alpha(m-1) \ll 1$. In this respect, the heterogeneity of microsomal vesicles which has been revealed by electron microscopy should be taken into consideration. During the preparation of this manuscript, however, a paper by KOCH[27] appeared, in which he demonstrated that the heterogeneity of shells does not seriously affect the applicability of this approximation method. The theory behind the latter method is sound in so far as microsomal particles are assumed to be spherical. However, it does have a drawback in that it involves a trial-and-error procedure. As presented above, the results obtained from the two methods showed a fairly good agreement not only with each other, but also with changes in volume as estimated from the water content of microsomes. Thus, it might be said that estimation of the osmotic volume changes of microsomal vesicles from light-scattering data is, semi-quantitatively at least, justified. The results of the present light-scattering study of microsomes also appear to be more or less consistent with those on spinach chloroplast grana reported by GROSS AND PACKER[25,26].

Using the volume changes thus estimated, it was demonstrated (Fig. 7) that the Boyle–van 't Hoff relation is applicable to the osmotic behavior of brain microsomes, a fact which provides convincing support for the results reported by PALADE AND SIEKEWITZ[1] and TEDESCHI *et al.*[3].

Our results do not necessarily mean, however, that microsomal vesicles are entirely impermeable to sucrose or monovalent cations. Over a relatively long experimental period microsomes were reported to equilibrate almost completely with added [^{14}C]sucrose[2]. According to ROBINSON[10], the sucrose space of brain microsomes during incubations at pH 7.3 is about 30% of that for glycerol. The constant b in Eqn. 8 is usually interpreted as osmotic dead space, *e.g.*, the volume occupied by solid structures in vesicles such as vesicular membrane. However, the value of b obtained from Fig. 7A is somewhat larger than the volume fraction of the microsome shell, β, as estimated by WALLACH *et al.*[22]. Therefore, in view of the penetrability of sucrose or ions across microsomal membranes, Eqn. 8 should apply only to short experimental periods, b being interpreted as an empirical constant, instead.

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