

References and Notes

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Polymeric Particles of Protein Insoluble at pH 5 from Rat Liver

Abstract. A procedure is given for approximating the volumes of small particles of protein insoluble at pH 5. Among particles smaller than 2.08×10^7 cubic angstroms the change in size was linear. The distribution of sizes indicated a polymeric relationship among the particles.

Approximately 25 percent (1) of the soluble protein fraction (2) from rat liver is insoluble at pH 5. When fixed in OsO_4 and viewed in an electron microscope, this insoluble protein has some of the dimensional characteristics of the endoplasmic reticulum (3). Because of the presumed role of the ergastoplasm (4) in the synthesis of cellular end products and the relations of the microsomal fraction of cells to this organelle, we examined the further possibility that the soluble proteins might serve as a source of α and γ cytomembranes (5). This study was primarily concerned with the distribution of the sizes of the smallest particles derived from the insoluble protein of the "soluble protein fraction" of liver cells.

Rats were killed by decapitation, and the livers were immediately removed and placed in cold pH 7.12 phosphate-buffered 0.25M sucrose solution (2.5 ml of sucrose solution per

gram of tissue). Homogenization was begun within 10 min post mortem. At 20 min post mortem the homogenate was spun in the centrifuge at 20,000g for 90 min to remove mitochondria, and 2 days after that it was spun at 102,000g for 70 min to remove the microsomal fraction. The pH of the resulting supernatant was adjusted to 5.07 with 0.10N HCl. The precipitate that formed promptly was spun down to give a pellet of protein insoluble at pH 5.

Only a slight amount of precipitate developed from the 20,000g (mitochondrial) supernatant in the 2 days preceding the final spin. It was assumed, therefore, that the Mg^{++} concentration was high enough (6) to prevent clumping of the cytomembranes and that a normal microsomal fraction could be removed from this sample by centrifugation. We have found that the precipitation of this insoluble protein from a mitochondrial supernatant also removes the microsomal fraction. However, because this insoluble protein from the soluble protein fraction, prepared as described, had none of the electron-dense characteristics (4) of the microsomal fraction, we considered the precipitate obtained to be essentially free of microsomes.

The pellet of protein was suspended in 10 ml of a 1-percent OsO_4 solution at pH 5.04. The fixed pellet was triturated by using a glass grinder and resuspended in a test tube. Those particles small enough to remain in suspension after 24 hours in the OsO_4 were examined in the electron microscope. Small drops of the suspension were dried on collodion-covered RCA stainless steel grids in a desiccator for 1 week. The grids were shadowed with chromium at an angle of 5 : 1 ($\tan \angle$

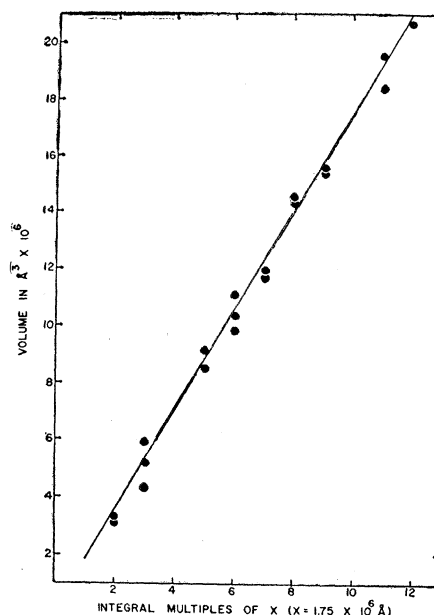


Fig. 2. Plot of the particle sizes of protein insoluble at pH 5, expressing the linearity of the volumes with respect to integral multiples of the smallest volume.

$= 0.2$). The particles on the grids were viewed and photographed in a Philips model EMU electron microscope. A magnification of 40,000 was the most useful for measuring the dimensions of the particles.

Our measurements indicated that the particles were not spherical, but ellipsoid. By assuming that they are probably flattened ellipsoids, useful presumptive calculations of the volumes of the particles are possible. A further assumption, obviously inaccurate but reasonable for the purposes of such determinations, was that the beam of chromium was perpendicular to the major axis of the ellipsoids. A fair approximation of the volume of the particles was calculated in the following manner (Fig. 1):

Given that $\tan \alpha = 0.2$, and by measuring the length of the shadow, X , the apparent or measured height of the particle, M , can be calculated as

$$M = X \tan \alpha$$

By construction, $\triangle ADE \sim \triangle ABC$, therefore, $\angle DEA = \angle ABC = \alpha$. From this, it is apparent that

$$M = R + (R \cos \alpha)$$

where R is the radius of the hypothetical sphere and

$$R = M / (1 + \cos \alpha)$$

The formula for the volume of an ellipsoid is $V = 4/3 \pi AB^2$ where A is the length of the semimajor axis and B is

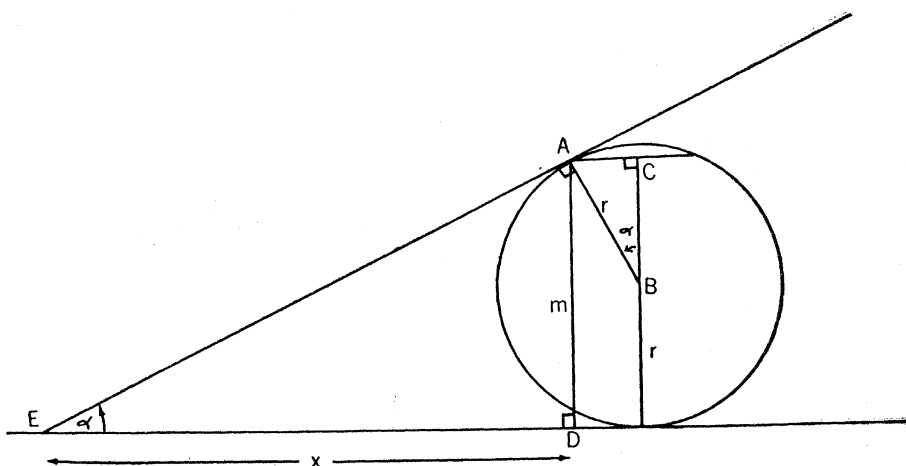


Fig. 1. Diagram of hypothetical particle upon which were based calculations of the volumes of the particles of protein insoluble at pH 5.

the length of the semiminor axis which is equal, in these calculations, to R .

Since we can measure A directly, the expression for the volume of the particles is

$$V = 4/3\pi AR^2$$

A population of 34 particles was measured from a typical field, and the volumes of each of the particles were calculated. Five arbitrary classes of particle size were designated. The minimum, maximum, and mean values for the length ($=2A$), height ($=2R$), and volume were tabulated for each of the particle classes (Table 1).

A further analysis was performed as follows. It was postulated that the three smallest particles might be "monomers" or, more correctly, small integral polymers of a monomer of protein insoluble at pH 5. The average volume of these three particles was $1.75 \times 10^6 \text{ A}^3$ and was designated as X . The hypothetical polymers of this assumed "monomer" were then calculated and are represented by the straight line in Fig. 2.

Figure 2 shows the linearity of the distribution of the particles of protein at pH 5 whose volumes were experimentally determined. Despite the errors inherently involved in the measurements and calculations of the volumes of the particles, the suggestion that they are small integral values of some "monomer" is correct. Class V particles (Table 1) were not included in Fig. 2 because the probability for linearity increases with larger multiples of X (7). Figure 2 also indicates that errors remaining in the calculation of volume are linear with respect to the actual volumes. The polymeric nature of the small particles of the protein strength-

ens the possibility that this material could have the dimensions of intracellular membranes and could contribute to their formation. We reason, too, that localized intracellular changes in pH likely exert a profound influence on the formation of membranes from the "soluble protein" fraction. The size classes of the particles, as presented in this report, have no definable biological reality, other than those implied above.

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Transfer of Allergic Encephalomyelitis by Lymph Node Cells in Inbred Guinea Pigs

Abstract. Severe or lethal allergic encephalomyelitis was transferred between histocompatible guinea pigs by lymphoid cells capable of persistence in a viable state in the recipient. For optimum induction of passive disease, cells must be transferred before the day on which the disease becomes manifest in the donor.

Experimental allergic encephalomyelitis, induced in animals sensitized with brain or spinal cord preparations (1), was first passively transferred between parabiotic rats by Lipton and Freund (2). The disease has not been passively induced by transfer of anti-central nervous system serum or sensitized cells to normal random-bred animals, but has been transferred with lymphoid cells in "tolerant" rats by Paterson (3). Koprowski *et al.* (4) found histological evidence of passive sensitization in a small percentage of transfers between inbred rats (4 of 48 recipients showed damage in the central nervous system). The percentage of successful transfers was higher when the donors were splenectomized before lymph node cells were transferred (5). Since this disease is widely considered to be associated with hypersensitivity of the delayed type, and since the guinea pig is the animal of choice for studies in this

area, in the experiments described in this report guinea pigs of the Wright (6) histocompatible (7) strain 13 were used for passive transfer of allergic encephalomyelitis. The facility with which transfers of this type of hypersensitivity are accomplished within this strain of guinea pig [Chase (8); Bauer and Stone (9)] is attributable to the viability of the lymphoid transplant in the recipient animal.

Except for cases otherwise recorded in Table 1, adult male strain 13 guinea pigs were sensitized by a single dose of strain 13 brain or spinal cord emulsified in complete Freund's adjuvant (10) injected intracutaneously into multiple sites in the nuchal region (0.25 ml of a 50 percent suspension of spinal cord or brain in 0.25 ml of Arlacel-Bayol containing 2.5 mg killed *Mycobacterium tuberculosis*). These donor animals were killed 5, 8, 11, or 12 days after injection, and the lymph nodes draining the nuchal region were removed; cell suspensions were then prepared and transferred by injection into the peritoneal cavity of normal recipients, as previously described (9). The donor:recipient ratio was roughly $2\frac{1}{2} : 1$. Some of the recipients were skin-tested with purified protein derivative (PPD) of tuberculin 14 to 23 days after transfer. Random-bred guinea pigs of the Hartley strain were used as control recipients. Donor and recipient animals were weighed each day to determine the onset of disease.

Table 1 shows that the allergic encephalomyelitis induced by isologous brain or spinal cord can be transferred between strain 13 guinea pigs and that the transfer results in severe or lethal disease in a large percentage of the recipients. In groups receiving cells 5, 8, or 11 days after active sensitization of donors, 17 of 20 strain 13 and none of 10 Hartley guinea pigs had the disease. In confirmation of Chase's prediction (11) and of Koprowski's results with inbred rats (4), the transfer was more likely to result in passive disease when the cells were taken before the symptoms were apparent in the donor (transfer at 5 and 8 days after active sensitization). Eleven days could elapse between sensitization and the successful transplantation of lymphoid cells from guinea pigs actively sensitized with brain preparations; but guinea pigs sensitized with the more potent spinal cord preparations were frequently manifestly ill by this time, and transfers at 11 or 12 days from these animals were not made under

Table 1. Particle sizes of insoluble protein from soluble fraction of rat liver.

Particle class	Min.	Max.	Mean
<i>Height (A)</i>			
I	68	145	105
II	148	194	164
III	171	194	184
IV	201	223	212
V	240	308	281
<i>Length (A)</i>			
I	424	791	576
II	622	791	740
III	734	961	833
IV	828	993	883
V	960	1545	1184
<i>Volume (10^6 A^3)</i>			
I	1.642	5.994	3.393
II	8.419	11.95	10.26
III	14.28	15.03	14.73
IV	18.47	22.76	20.58
V	28.95	72.75	50.02