A REMARK ABOUT THE DETERMINATION OF THE WATER CONTENT OF MITOCHONDRIA

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Glas and Bahr (1) have proposed a method for the determination of the water content of subcellular particles which can be schematically described as follows: A suspension of particles in 0.44 M sucrose is layered in a centrifuge tube over a silicone oil denser than the sucrose solution but less dense than the particles. The particles are sedimented through the silicone oil by high speed centrifugation, and they form a pellet which is supposedly free of extraparticulate aqueous phase. The water content of this pellet is measured directly by distillation.

The method is based on the implicit assumption that gravitation and buoyancy are the only factors at play. No account is taken of the interfacial tension which opposes the penetration of hydrophilic particles into the oil phase. We shall show in the present paper that the centrifugal field is far from being strong enough to force individual particles through the interface between the two phases. The pellet is actually formed by aggregates of particles which carry down a substantial amount of extraparticulate aqueous phase.

Since the subcellular particles are obviously very hydrophilic, we may assume as a first approximation that the surface tension between the particles and the sucrose solution in which they are suspended is small. When the particles are in contact with silicone oil, the interfacial tension may thus be assumed to be about the same as the surface tension between silicone oil and sucrose solutions. Let γ(dynes. cm^{-1}) be this interfacial tension. A spherical particle of radius r cm making a depression in the interface is repelled towards the aqueous phase with a force which depends on the depth of the depression and may reach $2\pi\gamma r$ dynes. Let g(cm.sec^{-2}) be the acceleration due to the centrifugal field. Let ρ_p and ρ_m be the density of the particle and of the medium, respectively. The force pushing the particles toward the silicone oil is $4\pi r^3 g(\rho_p - \rho_m)/3$ dynes. If we set ρ_m equal to the density of the sucrose solution, we obtain the maximum value of the force. The minimum acceleration g required to push the particle completely into the silicone oil is such that

$$2\pi\gamma r = 4\pi g r^3(\rho_p - \rho_m)/3,$$

or

$$g = 3\gamma/2r^2(\rho_p - \rho_m)\text{cm. sec}^{-2}.$$

This formula is easily applied to the conditions used by Glas and Bahr to measure the water content of rat liver mitochondria. The density of 0.44 M sucrose is 1.060 g. cm^{-3}. We made crude measurements of the interfacial tension between silicone oil (Dow Corning 702 fluid) and 0.44 M sucrose and found it to be at least 30 dynes. cm^{-1}. According to previous determinations (2), the modal density of rat liver mitochondria in 0.44 M sucrose is 1.122. We may assume that the average

radius of isolated mitochondria is about 4×10^{-5} cm (3). The minimum acceleration required in Glas and Bahr's procedure is computed from these data to be about 5×10^{11} cm. sec^{-2} or 5×10^{8} times gravity.

One may conclude that single mitochondria cannot cross the interface between the two phases at the centrifugal accelerations attainable with the presently available centrifuges. Even if our estimate of the interfacial tension is erroneous by three orders of magnitude, our conclusion still remains correct.

It is easy to imagine what happens when Glas and Bahr's procedure is used. The particles accumulate at the interface between the two phases. This layer breaks into pieces when it can form fragments large enough to cross the interface. This was easily confirmed by a very simple experiment. Tubes were prepared by layering over silicone oil small volumes of suspensions of mitochondria at various concentrations. The tubes were centrifuged for 10–20 min at low speed (about 2,000 times gravity) in swing-out buckets. In the tubes which contained dilute suspensions, the particles were packed as a thin cake above the silicone oil. A pellet in the bottom of the tube appeared only in tubes in which the concentration of particles was large, and, even then, a cake of particles remained at the interface. Centrifuging for a longer time did not change the results. Under the conditions used by Glas and Bahr (acceleration 5×10^4 *g*), the artifact is less obvious, because the particles remaining at the interface form a very thin layer. Nevertheless, there is no reason to believe that a similar type of drop sedimentation does not occur. According to the formula derived above, the drops of concentrated mitochondria suspension would have a diameter of 40 μ or more. It is clear that the amount of extraparticulate water included in the drops is not smaller than that in pellets isolated and packed by the usual centrifugation.

The technique of centrifuging subcellular particles through a layer of silicone oil has been described previously by Werkheiser and Bartley (4), but these authors used it for a different purpose and did not claim that it eliminates the extraparticulate solution completely. Indeed, their experimental data show that the water or sucrose content of the pellets is not appreciably decreased by centrifugation through silicone oil.

It was suggested a few years ago that some physical properties of cell organelles could be accurately determined by investigating their behavior in density gradients of various types (5). It was shown that the properties of mitochondria could be accounted for by a model of mitochondrial structure which involved an osmotic space, a sucrose space, and an hydrated matrix. The parameters describing the model were estimated from the experimental results (5, 6), and it was shown that these estimates were consistent with the observations made by other authors (4, 7–9). The water content of mitochondria can be computed from these parameters. It amounts to 1.88 ml per g dry weight for mitochondria suspended in 0.25 M sucrose (the weight of sucrose contained in the sucrose space is not included in the dry weight). A similar computation furnishes the value of 1.57 ml per g dry weight in 0.44 M sucrose, as against the value of 2.80 ml arrived at by Glas and Bahr (1). The excess water found by these authors corresponds to 1.40 ml of sucrose solution per g dry weight. According to the data of Beaufay and Berthet (6), the volume occupied by the mitochondria themselves is 2.42 ml per g dry weight. It thus appears, on the basis of these calculations, that 37% of the volume of a pellet isolated by the method of Glas and Bahr is extramitochondrial. This is a higher percentage than that observed when the particles are isolated without the use of silicone oil (4, 7, 8).

It thus seems clear, both from theoretical considerations and the experimental evidence, that centrifuging mitochondria through a layer of silicone oil is not an effective method for removing extraparticulate medium.

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