PRELIMINARY NOTE

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Separation of mitochondria, peroxisomes and lysosomes by zonal centrifugation in a Ficoll gradient

Methods for the partial separation of mitochondria, peroxisomes and lysosomes, developed largely by DE DUVE and co-workers, have recently been reviewed[1]. The procedures most recently employed use differential centrifugation to obtain the so-called "L" fraction[2], followed by isopycnic banding in a special rotor designed by BEAUFAY[3]. To achieve the desired separations in these experiments, Triton WR-1339 was injected into the animals in order to alter the banding density of the lysosomes[4].

Using either the B-XV, B-XXIII or B-XXIX zonal rotors[5,6] we have been able to separate mitochondria, peroxisomes and lysosomes from livers of untreated rats using a sucrose–Ficoll gradient which is approximately iso-osmotic throughout its length. The gradient had a total volume of 1 l and contained 0.25 M sucrose throughout, but varied from 0 to 15% (w/v) Ficoll. In the region of the gradient used for these separations, Ficoll contributed less than a half of 1% of the total osmotic

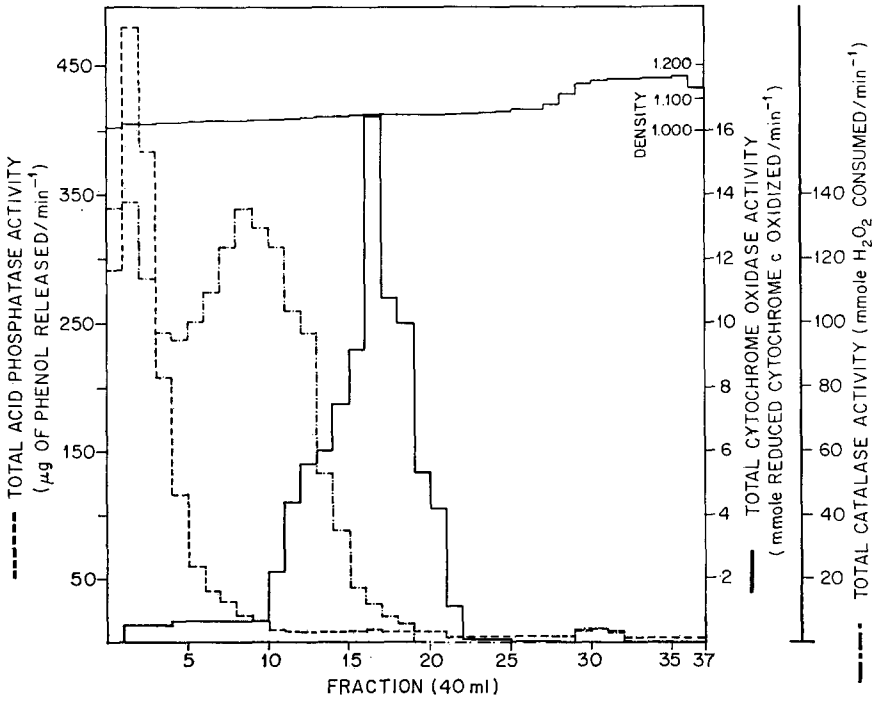

Fig. 1. Subcellular particle distribution from a 95-ml sample of a 5% rat liver homogenate centrifuged in a B-XXIII rotor. The rotor contained a 1-l linear gradient of Ficoll (Pharmacia) with a 250-ml cushion of 45% sucrose. 0.25 M sucrose was distributed throughout the gradient. The same was centrifuged at 10000 rev./min for about 10 min or total integral of $\omega^2 dt = 55 \cdot 10^7$. ———, total cytochrome oxidase activity (mitochondria); - - - - - - -, total acid phosphatase activity (lysosomes); –·—·–, total catalase activity (peroxisomes).

pressure. In the experiment illustrated in Fig. 1, a 100-ml sample of a 5 % rat liver brei in 6 % sucrose was introduced centripetally to the gradient while the rotor was spinning at 2000 rev./min.

An overlay of 100 ml of H_2O was used to move the sample layer away from the tapered core surfaces. The separation is achieved on the basis of sedimentation rate in one step using a total of 15-min centrifugation at approx. 10000 rev./min. The integrated centrifugal force was determined using a Beckman digital integrator, and the centrifuge was unloaded when the integral of $\omega^2 dt = 50 \cdot 10^7 - 55 \cdot 10^7$. To complete the separation of those fractions having acid phosphatase activity (lysosomes) but which still contain ribosomes, microsomes, glycogen and soluble proteins, an additional centrifugal step is required. In this experiment slightly more than 50 % of the acid phosphatase activity is sedimentable by centrifuging the fractions at 30000 rev./min for 30 min in a No. 30 Spinco rotor. The procedure thus far described may be effected in a B-XV rotor unloaded from the center. Using the recently designed B-XXIII (ref. 6) or B-XXIX rotors (ref. 7), which may be unloaded from the edge, the mitochondrial and peroxisomal bands may be unloaded and the centrifugal (or denser) portion of the gradient replaced. Centrifugation may then be continued until the lysosomes begin to band isopycnically. Both a rate-zonal and an isopycnic-zonal centrifugation may be completed in one rotor using the latter method. The separation requires less time than previous methods.

Enzyme activities were determined using Technicon autoanalyzers. A detailed biochemical and electron microscopic analysis of cell fractionation studies using Ficoll gradients in zonal rotors will be published elsewhere.

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1 C. DE DUVE, *Harvey Lectures Ser.*, 59 (1965).
2 E. E. BOWERS AND C. DE DUVE, *J. Cell Biol.*, 32 (1967) 339.
3 H. BEAUFAY, *La Centrifugation en Gradient de Densité*, Thesis, applications à l'étude des organites subcellularies, Louvain, 1966.
4 W. M. WATTIAUX AND J. P. BANDBURN, in A. V. S. DE RENCL AND M. P. CAMERON, *Ciba Found. Symp. Lysosomes*, (1963) 176.
5 N. G. ANDERSON, D. A. WATERS, W. D. FISHER, G. B. CLINE, C. E. NUNLEY, L. H. ELROD AND C. T. RANKIN, JR., *Anal. Biochem.*, 21 (1967) 235.
6 N. G. ANDERSON, C. T. RANKIN, JR., D. H. BROWN, C. E. NUNLEY AND H. W. HSU, *Anal. Biochem.*, in the press.
7 N. G. ANDERSON, C. E. NUNLEY AND C. T. RANKIN, JR., *Federation Proc.*, 27 (1968) 366.

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