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Citation Classics

De Duve C, Pressman B C, Gianetto R, Wattiaux R & Appelmans F. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochemical Journal* **60**:604-17, 1955.

The finding that the acid phosphatase of rat liver is enclosed within a special type of cytoplasmic granule, with sedimentation properties intermediate between those of mitochondria and microsomes, has led to the development of a new scheme of fractionation, whereby enzymes attached to these granules can be readily identified. This paper describes the method for determining enzyme distribution patterns. The authors contend that specific enzymic species have single intracellular locations and that granules of a given class are enzymically homogeneous. [The *SCI*® indicates that this paper was cited 1,402 times in the period 1961-1975.]

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"This paper is part of a series which eventually ran into 18 installments, published between the years 1951 and 1964. It is clearly not a 'classic' in the sense of the Watson and Crick or Jacob and Monod papers But it is the most important paper of the series, and probably the most significant of my own publications. In this paper we were committing ourselves to a new approach and trusting it to the point of affirming the existence of a new cell particle on the basis of purely biochemical results.

"My collaborators were two postdoctoral fellows and two medical students. Berton Pressman had come from Henry Lardy's laboratory at the University of Wisconsin. He has since made a distinguished career for himself, and is best known for his work on ionophores and mitochondrial ion transport. He is now at the University of Miami Robert Gianetto had come to us from the Universite de Montreal, where he is now a professor in the department of biochemistry and heads a clinical laboratory. Robert Wattiaux, who became professor of biochemistry in Namur after spending a number of years with us, has recently made important observations on the damage subcellular particles may sustain under the influence of the strong hydrostatic pressures generated during high-speed centrifugation Francoise Appelmans left the laboratory after she graduated, to go into clinical practice....

"The experiments were largely completed by the spring of 1954. But it took me almost a year to write the paper. I remember mulling over the results and searching for various ways of presenting them, finally hitting upon the relative specific activity vs. protein histograms, now known in some laboratories as 'duvograms,' and in our own as 'submarines.' Altogether 13 distinct enzyme distribution patterns obtained with a new fractionation scheme were presented in this manner, together with a number of additional kinetic and latency results ....

"In a classical fractionation experiment, the tissue, usually liver, was homogenized, and fractionated into 'nuclei,' 'mitochondria,' microsomes,' and 'supernatant ' The fractions were analyzed and the results reported as reflecting the properties of the corresponding cell components, with some distortion allowed for experimental artifacts. This manner of interpreting the results had two major weaknesses in my opinion: 1) it assumed that the fractions were pure, which they could not be with the crude separation procedures used, and obviously were not; 2) it assumed that the cytoplasm contained only two kinds of particular components, which was certainly not proven and even appeared unlikely I therefore decided to use other assumptions, equally unproven, but at least more plausible, and, what was more important, capable of being experimentally disproved (or falsified, as Karl Popper would put it). These assumptions were that specific enzymic species have single intracellular locations and that granules of a given class are enzymically homogeneous Within the limits of validity of these assumptions, which I later called the postulates of single location and of biochemical homogeneity, the enzymes themselves could be used as 'markers'–we said reference-for their host-particles The 'submarines' provided a convenient immediate global view of the manner in which each enzyme, and therefore its host-particle, was distributed between the fractions (distribution pattern).

"Here, I believe, was the truly innovative aspect of this paper In practice, of course, it led to the identification of lysosomes as a new group of cytoplasmic particles, and already hinted at the existence of yet another group of particles, now known as peroxisomes. I might add that the word 'lysosome' appears in print for the first time in this paper. This presumably explains why the paper has enjoyed such a high citation frequency."