Biochimica et Biophysica Acta, 1000 (1989) 317–320
Elsevier

Commentary by

Christian de Duve

International Institute of Cellular and Molecular Pathology, Brussels (Belgium) and The Rockefeller University, New York, NY (U.S.A.)

on 'Intracellular localization of catalase and of some oxidases in rat liver'
by C. de Duve, H. Beaufay, P. Jacques, Y. Rahman-Li, O.Z. Sellinger, R. Wattiaux and S. De Coninck
Biochim. Biophys. Acta 40 (1960) 186–187

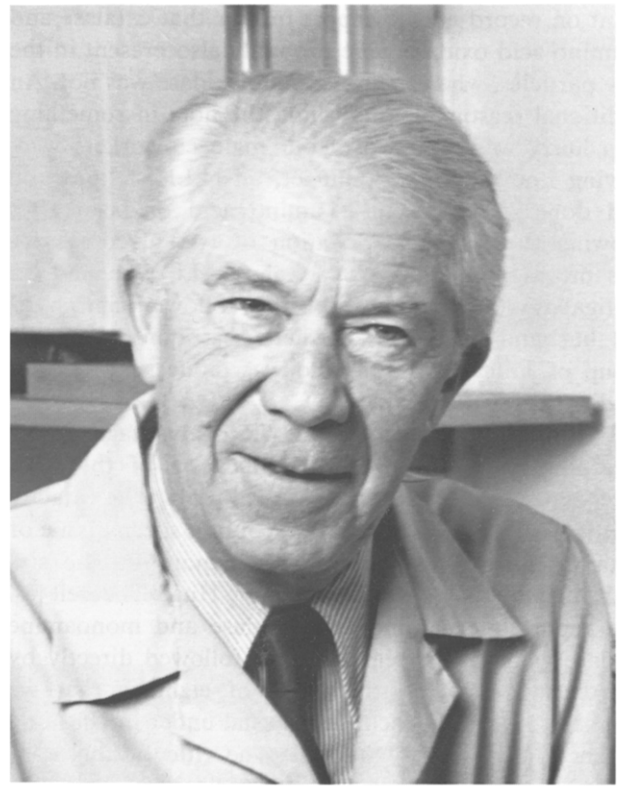
At first sight, this is not a paper to be proud of. It reads like a hodge-podge of recent results, put together mainly for the purpose of getting into print and gaining time for the leisurely writing of a full report. This, I must confess, is very much what it was. We had some excuses, as we were in the midst of a veritable whirlwind of experimentation.

It had all started a few days before Christmas, 1949, when the surprising behaviour of an enzyme that should have been there, but was not, put us on the trail of the lysosome. I have told the story elsewhere [1] and will only recall briefly the two main findings that were made:

(1) Acid phosphatase was largely latent in a rat-liver homogenate prepared by gentle grinding in the presence of 0.25 M sucrose, and in particulate fractions separated from the homogenate by differential centrifugation.

(2) The particle-bound activity was distributed unequally between what were referred to at that time as the mitochondria (about 2/3 of the activity) and the microsomes (about 1/3), which we soon learned to call more cautiously the mitochondrial and microsomal fractions.

In subsequent experiments, the first observation could be explained by the presence of a surrounding membrane that rendered the shrouded enzyme inaccessible to the substrate used for its assay. The second finding was shown to reflect the association of the enzyme with a distinct population of particles having sedimentation properties intermediate between those of mitochondria and those of microsomes. Intrigued by these results, we decided to look for the same unusual features among other enzymes that had been found by others to straddle the mitochondrial and microsomal fractions. We devised a special fractionation procedure for this purpose. In this scheme, a small intermediate fraction particularly rich in acid phosphatase (designated L for light-mitochondrial, now mostly taken to mean lysosomal) was isolated between the main mitochondrial and the microsomal fractions. The enzymes we were looking for were to be likewise concentrated in the L fraction, yielding a characteristic 'submarine' pattern in the kind of histograms that I invented for the representation of tissue-fractionation results. In addition, they were to share, qualitatively and quantitatively, the latency properties of acid phosphatase.



Christian de Duve

The first enzymes to fall into our net were β-glucuronidase and cathepsin D, and, later, acid

0006-3002/89/$03.50

ribonuclease and acid deoxyribonuclease, which, together with acid phosphatase, made up the five acid hydrolases on which the lysosome concept was built [2]. There was an additional enzyme in this early catch, not a hydrolase, but an oxidase: urate oxidase, then known as uricase. It produced a particularly beautiful submarine, which, however, differed somewhat in shape from the distribution patterns of the hydrolases. Furthermore, unlike the acid hydrolases, urate oxidase proved to be insoluble and did not display any latency. These differences led us to envisage right from the start the possibility that urate oxidase may belong to another group of granules, "resembling large microsomes rather than small mitochondria, and differing from lysosomes by a greater uniformity in sedimentation properties" [2].

It took us another 5 years to turn this hypothetical possibility progressively into a near-certainty. Our 1960 Preliminary Note in BBA was written mainly to put this point on record and to report further that catalase and D-amino acid oxidase were probably also present in the new particles, whereas monoamine oxidase was not. An additional reason for publishing the note in something of a hurry was that two of our main co-workers were leaving Louvain. Otto Sellinger, an N.I.H. fellow who had done the work on D-amino acid oxidase (after showing the lysosomal location of two glycosidases), was moving to Rome. Jady Rahman-Li, who had investigated monoamine oxidase, was following her physicist husband to Argonne, where she soon joined the group of John Thomson, who was pioneering density-gradient centrifugation.

I must confess that we took full advantage of the breathing-space afforded by publication of the BBA note, actually stretching it to several years. The catalase results were first published in 1963, in a special issue of Acta Chemica Scandinavica commemorating the sixtieth birthday of my former mentor, Hugo Theorell [3]. The report on D-amino acid oxidase and monoamine oxidase came out a year later [4], followed directly by the closing paper in the series of eighteen that we published in the Biochemical Journal under the heading 'Tissue Fractionation Studies'. The title of this concluding article summarized fifteen years of research: 'Resolution of Mitochondrial Fractions from Rat Liver into Three Distinct Populations of Cytoplasmic Particles by Means of Density Equilibration in Various Gradients.' [5].

This last paper was the culprit responsible for our long publication delay. It contained a comprehensive account of fractionation experiments performed in sucrose gradients, with either H_2O or D_2O as solvent, and in gradients of glycogen made up with sucrose solutions of various concentrations. The results were complex and their presentation proved difficult, especially as we attempted to fit them to theoretical equations that Jacques Berthet, Henri Beaufay and I had worked out a few years before to describe the physical behaviour of osmotically active particles in density gradients [6,7]. It was a laborious exercise, but it proved rewarding in the end, as it allowed us to draw some kind of 'identikit' picture of each group of particles, based exclusively on centrifugal data. Their real pictures were published a year later in a paper in which the identification of lysosomes with pericanalicular dense bodies, that of the particles containing catalase and the two oxidases with microbodies, already suspected before, were established beyond reasonable doubt by electron-microscopic examination of highly purified preparations [8]. A significant factor in this success had been the discovery by Robert Wattiaux that the injection of Triton WR-1339 causes a selective and drastic decrease in the density of the lysosomes (in which the detergent accumulates), and thereby allows peroxisomes to be isolated free of their most bothersome contaminant by density equilibration in a sucrose gradient [9]. I was able to offer a preview of the whole story in a Harvey Lecture held in November 1963 [10]. I have a special affection for this lecture, which is something of a scientific testament, prefiguring the talk that I gave in Stockholm eleven years later under the title 'Exploring Cells with a Centrifuge' [11].

Yet, during all that time, the new particles containing urate oxidase, D-amino acid oxidase and catalase remained unnamed (except for the term 'microbody', which I did not like, as it relayed no information on the function of the particles, nor even on their structure). The H_2O_2 connexion had not escaped us, of course. Already in the 1960 BBA paper it is stated that "the particles concerned could be an important site of H_2O_2 metabolism, since they would contain two oxidases known to form this compound, in association with the most important enzyme destroying it." This consideration had, in fact, been my main motive in choosing to study the distribution of monoamine oxidase. This enzyme showed the right kind of heterogeneous distribution in conventional fractionation experiments, and I firmly expected it to accompany the others. The finding that it did not was one of my greatest scientific disappointments – and lessons – and probably contributed to my hesitation to propose a peroxisome concept. In addition, it seemed to me inappropriate to build a concept on results obtained only on rat liver. I felt that it was necessary first to search for similarly equipped particles in other biological materials.

This I was able to do in the new laboratory that I had started at the Rockefeller Institute (now the Rockefeller University), in New York. I had three invaluable co-workers: Pierre Baudhuin, a key participant in much of our work in Belgium; Miklós Müller, a Hungarian investigator who had joined me to look for lysosomes in protozoa and was quite ready to extend his search to other organelles; and Brian Poole, a gifted

graduate student who unfortunately died prematurely in 1981, at the peak of a particularly promising career. We obtained positive results on rat kidney – not too surprising a finding, as microbodies had first been recognized morphologically in kidney – and, much more significantly, on the ciliate, *Tetrahymena pyriformis*. We were also able to add an L-α-hydroxy acid oxidase and, in kidney, an L-amino acid oxidase to the list of enzymes associated with the new particles [12]. These results clinched the matter. I proposed the name 'peroxisome' at the 1965 meeting of the American Society of Cell Biology [13]. One year later, Baudhuin and I published the first review on 'Peroxisomes (Microbodies and Related Particles)' [14]. Finally, thanks to the development of a special zonal rotor by Beaufay [15], we were able to make large-scale preparation of purified peroxisomes and saw their colour for the first time [16]. They were green, the colour of catalase.

On the whole, the peroxisome concept has survived well. It has been strengthened by the identification of other H_2O_2-producing oxidases, including an acyl-CoA oxidase belonging to a β-oxidation system [17], as components of the particles, and it has been found to be applicable to a wide variety of eukaryotes, including animals, plants, fungi, and protozoa. In many instances, additional enzyme systems, involved in, among other important biological processes, transamination, lipid conversion to carbohydrate, cholesterol metabolism, plasmalogen synthesis, photorespiration, and bioluminescence, have been found in peroxisomes, attesting to the multifarious functions that these organelles carry out, in spite of the wasteful nature of their respira-

From top left: Henri Beaufay, Pierre Jacques, Jady Rahman-Li, Otto Sellinger, Robert Wattiaux, Simone De Coninck (Mrs. Wattiaux)

tion. Their properties, functions, genetic regulation, pathological defects, biogenesis and evolutionary history are attracting increasing attention. Space does not permit adequate coverage of these interesting developments. For further information, Refs. 18–22 may be consulted.

Some microbody-like organelles lack the oxidase-catalase combination that is the hallmark of peroxisomes. Among them are the hydrogenosomes of trichomonads [23], the glycosomes of trypanosomatids [24] and, found in filamentous fungi, some peculiar catalase-less microbodies endowed with a β-oxidation system that is not geared to the production of hydrogen peroxide [25]. To what extent these particles are related to authentic peroxisomes is not yet clear. Hydrogenosomes almost certainly are not. In any case, even if the peroxisome concept should turn out not to be universal, it remains the most general and specific common link between the vast majority of microbodies and related particles found in nature. As a biochemical marker, it is superior to β-oxidation, often a property also of mitochondria.

As to the monoamine-oxidase mystery, it was solved after Schnaitman et al. [26] showed that this enzyme is associated with the outer mitochondrial membrane. Its presence in the microsomal fraction could be attributed by Beaufay and co-workers to the contamination of this fraction by outer membrane fragments stripped off from mitochondria in the course of homogenization [27]. Its presence outside the peroxisomes, like that of H_2O_2-generating oxidases in the cytosol, indicates that there must be an extra-peroxisomal H_2O_2 pool detoxified by an enzyme different from catalase, probably glutathione peroxidase [28]. Presumably, peroxisomes can also contribute to this pool when catalase activity is low or absent, thus explaining why genetic deficiencies of catalase are not lethal.

Correspondence: C. de Duve, I.C.P. 75.50, Avenue Hippocrate 75, B-1200 Brussels, Belgium.

References

1 De Duve, C. (1969) in Lysosomes in Biology and Pathology (Dingle, J.T. and Fell, H.B., eds.), Vol. 1, 3, North-Holland, Amsterdam.
2 De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J., 60, 604.
3 De Duve, C., Beaufay, H. and Baudhuin, P. (1963) Acta Chem. Scand. 17, 210.
4 Baudhuin, P., Beaufay, H., Rahman-Li, Y., Sellinger, O.Z., Wattiaux, R., Jacques, P. and De Duve, C. (1964) Biochem. J. 92, 179.
5 Beaufay, H., Jacques, P., Baudhuin, P., Sellinger, O.Z., Berthet, J. and De Duve, C. (1964) Biochem. J. 92, 184.
6 De Duve, C., Berthet, J. and Beaufay, H. (1959) Progr. Biophys. Biophys. Chem. 9, 325.
7 Beaufay, H. and Berthet, J. (1963) Biochem. Soc. Symp. 23, 66.
8 Baudhuin, P., Beaufay, H. and De Duve, C. (1965) J. Cell Biol. 26, 219.
9 Wattiaux, R., Wibo, M. and Baudhuin, P. (1963) in Ciba Foundation Symposium on Lysosomes (De Reuck, A.V.S. and Cameron, M.P., eds.), p. 176, J.&A. Churchill, London.
10 De Duve, C. (1965) The Harvey Lectures 59, 49.
11 De Duve, C. (1975) Science 189, 186.
12 Baudhuin, P., Müller, M., Poole, B. and De Duve, C. (1965) Biochem. Biophys. Res. Commun. 20, 53.
13 De Duve, C. (1965) J. Cell Biol., 27, 25A.
14 De Duve, C. and Baudhuin, P. (1966) Physiol. Rev. 46, 323.
15 Beaufay, H (1966) La Centrifugation en Gradient de Densité, Ceuterick, Louvain.
16 Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, S. and De Duve, C. (1968) J. Cell Biol. 37, 482.
17 Lazarow, P.B. and De Duve, C. (1976) Proc. Natl. Acad. Sci. USA 73, 2043.
18 Hogg, J.F. (ed.) (1969) Ann. N.Y. Acad. Sci. 168, 209.
19 Tolbert, N.E. (1981) Annu. Rev. Biochem. 50, 133.
20 Kindl, H. and Lazarow, P.B. (eds.) (1982) Ann. N.Y. Acad. Sci. Vol. 386.
21 Lazarow, P.B. and Fujiki, Y. (1985) Annu. Rev. Cell Biol. 1, 489.
22 Fahimi, H.D. and Siest, H. (eds.) (1987) Peroxisomes in Biology and Medicine, Springer, Berlin.
23 Müller, M. (1980) in The Eukaryotic Microbial Cell (Gooday, G.W., Lloyd, D. and Trinci, A.P.J., eds.), p. 127, Cambridge University Press, Cambridge.
24 Opperdoes, F.R. (1987) Annu. Rev. Microbiol. 41, 127.
25 Kunau, W.-H., Kionka, C., Ledebur, A., Mateblowski, M., Moreno de la Garza, M., Schultz-Borchard, U., Thieringer, R. and Veenhuis, M., in Ref. 22, p. 128.
26 Schnaitman, C., Erwin, V.G. and Greenawalt, J.W. (1967) J. Cell Biol. 32, 719.
27 Beaufay, H., Amar-Costesec, A., Thines-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) J. Cell Biol. 61, 213.
28 Mills, G.C. (1960) Arch. Biochem. Biophys. 86, 1.

Intracellular localization of catalase and of some oxidases in rat liver

In rat liver, the intracellular distribution of uricase has been found by several workers to resemble that of acid phosphatase, without, however, being entirely identical with it. The significance to be attached to these findings has been diversely appreciated. While HOGEBOOM *et al.*[1] have favoured the view that the two enzymes belong to the same particles, other authors like THOMSON AND MOSS[2] have preferred to leave the matter open. In this laboratory, acid phosphatase has been found to be associated, together with a number of other acid hydrolases, with a special group of cytoplasmic particles which have been called *lysosomes* (for a review see ref. 3). The general parallelism between uricase and the lysosomal enzymes has been confirmed, but attention has been drawn to the greater homogeneity and higher D_2O-binding capacity of the uricase-containing particles, as possible indications that they may be distinct from the lysosomes[3–5].

Conclusive evidence supporting the latter view has now been obtained by submitting mitochondrial fractions from rat liver to density equilibration in a gradient of glycogen, extending linearly from 0–0.23 g/ml in 0.5 *M* aq. sucrose. In this system, the uricase particles accumulate in the layers immediately below the sedimentation boundary of the glycogen and are almost completely separated from the lysosomes, which are concentrated at the bottom of the tube. The bulk of the mitochondria occupy an intermediate position. Like the lysosomes, the uricase-containing particles are associated with a very small proportion of the total cell nitrogen.

Two other enzymes, catalase and D-amino acid oxidase, have been found to share the main properties of uricase. As already indicated by the investigations of THOMSON AND KLIPFEL[6] on catalase and of PAIGEN[7] on D-amino acid oxidase, they tend to concentrate in the light mitochondrial fraction in fractionations performed according to DE DUVE *et al.*[4]. In addition, they show a higher equilibrium density than the mitochondria and even than the lysosomes in a sucrose–D_2O gradient prepared according to BEAUFAY *et al.*[5], and a lower one in the glycogen–0.5 *M* sucrose gradient described above. Their density–distribution curves do not, however, coincide perfectly with that of uricase in the same systems and it must therefore be concluded that the three enzymes either belong to separate particles showing very similar properties or that they are associated together, but in varying proportion, with a single group of particles. In the latter event, the particles concerned could be an important site of H_2O_2 metabolism, since they would contain two oxidases known to form this compound, in association with the most important enzyme destroying it. Another implication of these findings is that the protection of cellular constituents against H_2O_2, formed, for instance, under the influence of ionizing radiations, may be restricted to very small areas of the liver cell.

In contrast with the oxidases mentioned above, monoamine oxidase shows a true bimodal distribution. The mitochondrial component, which accounts for about 70 % of the total activity, accompanies cytochrome oxidase in several types of gradients and must be considered truly mitochondrial. The microsomal component, on the other hand, can be largely separated from the bulk of the microsomal protein, phospholipid and RNA, as well as from several other enzymes present in this fraction (glucose-6-phosphatase, esterase, DPNH-cytochrome *c* reductase, β-glucuronidase,

Abbreviations: DPNH, reduced diphosphopyridine nucleotide; RNA, ribonucleic acid.

fumarase) by density equilibration in an aqueous sucrose gradient. In this system, monoamine oxidase shows a distinctly lower equilibrium density than the other components. Fumarase, on the other hand, shows an exceptionally high equilibrium density, which depends on the presence of intact RNA.

Department of Physiological Chemistry, University of Louvain, Louvain (Belgium)

C. DE DUVE
H. BEAUFAY*
P. JACQUES**
Y. RAHMAN-LI***
O. Z. SELLINGER§
R. WATTIAUX
S. DE CONINCK

1 G. H. HOGEBOOM, E. L. KUFF AND W. C. SCHNEIDER, *Intern. Rev. Cytol.*, 6 (1957) 425.
2 J. F. THOMSON AND E. M. MOSS, *Arch. Biochem. Biophys.*, 61 (1956) 456.
3 C. DE DUVE, in T. HAYASHI, *Subcellular Particles*, Ronald Press, New York, 1959, p. 128.
4 C. DE DUVE, B. C. PRESSMAN, R. GIANETTO, R. WATTIAUX AND F. APPELMANS, *Biochem. J.*, 60 (1955) 604.
5 H. BEAUFAY, D. S. BENDALL, P. BAUDHUIN, R. WATTIAUX AND C. DE DUVE, *Biochem. J.*, 73 (1959) 628.
6 J. F. THOMSON AND F. J. KLIPFEL, *Arch. Biochem. Biophys.*, 70 (1957) 224.
7 K. PAIGEN, *J. Biol. Chem.*, 206 (1954) 945.

Received March 10th, 1960

* Chargé de Recherches du F.N.R.S.
** Aspirant du F.N.R.S.
*** Present address: Argonne National Laboratory, Medical and Biological Division, Lemont, Ill. (U.S.A.).
§ Post-doctoral fellow of the U.S. National Institutes of Health. Present address: Instituto superiore di Sanita, Rome (Italy).