EXPLORING CELLS WITH A CENTRIFUGE

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by

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INTRODUCTION

In one of her masterpieces, Nobel Laureate Selma Lagerlöf tells how the little boy Nils Holgersson visited the whole of Sweden, from Skåne to Lappland, on the wings of a friendly white gander.

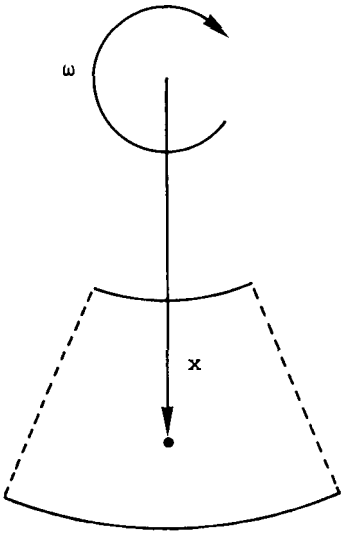
I too have made a wonderful journey, using like Nils Holgersson an unconventional mode of travel. For the last 25 years, I have roamed through living cells, but with the help of a centrifuge rather than of a microscope.

On these trips I was never alone. I want to mention this at the outset, since I owe much to my travelling companions. Some of their names will come up as my tale unfolds; but there are so many of them that I will be quite unable to mention them all. My debt goes also to my early mentors in science: Joseph Bouckaert, Joseph Maisin, Hugo Theorell, Carl and Gerty Cori, Earl Sutherland. Four of them have preceded me on this podium. Three, unfortunately, are not with us any more.

THE DEVELOPMENT OF ANALYTICAL CELL FRACTIONATION

Thirty years ago, much of the living cell still remained virtually unexplored. The reasons for this are simple. Morphological examination was limited downward in the scale of dimensions by the resolving power of the light microscope, whereas chemical analysis stopped upward at the size of the smaller macromolecules. In between, covering almost two orders of magnitude, lay a vast "terra incognita", impenetrable with the means of the day. Invasion of this territory started almost simultaneously on its two frontiers, after electron microscopy became available to morphology and centrifugal fractionation to biochemistry.

When, in 1949, I decided to join the little band of early explorers who had followed Albert Claude in his pioneering expeditions, electron microscopy was still in its infancy. But centrifugal fractionation, the technique I wanted to use, was already well codified. It had been described in detail by Claude himself (1), and had been further refined by Hogeboom, Schneider and Palade (2) and by Schneider (3). According to the scheme developed by these workers, a tissue, generally rat or mouse liver, was first ground with a Potter-Elvehjem homogenizer, in the presence of either 0.88 M (2) or 0.25 M (3) sucrose. The homogenate was then fractionated quantitatively by means of three successive centrifugations and washings, under increasing centrifugal



SEDIMENTATION VELOCITY

$$\frac{dx}{dt} = s.\omega^2.x$$

x = RADIAL DISTANCE (cm)

ω = ANGULAR VELOCITY (rad.sec^{-1})

s = SEDIMENTATION COEFFICIENT OF PARTICLE (sec)

FOR SPHERICAL PARTICLE OF RADIUS r (cm) AND OF DENSITY ρ_p (g.cm^{-3})

IN MEDIUM OF DENSITY ρ_m (g.cm^{-3}) AND OF VISCOSITY η (poises)

$$s = \frac{2\,r^2\,(\rho_p - \rho_m)}{9\,\eta}$$

Fig. 1. The Svedberg equation and its application to a spherical particle.

force x time integrals, to yield "nuclei", "mitochondria", "microsomes" and a final supernatant. The fractions, as well as the original homogenate, could then be analyzed for their chemical composition, enzyme content, and other properties.

All these details were available in the literature, and there seemed little more for us to do than to acquire the necessary equipment and follow instructions carefully, especially since our interest in cell fractionation itself was rather peripheral at that time. All we wanted was to know something about the localization of the enzyme glucose 6-phosphatase, which we thought might provide a possible clue to the mechanism of action, or lack of action, of insulin on the liver cell.

Fortunately, this is not exactly how things happened. Working with me on this project was Jacques Berthet, still a medical student at that time, but with an unusually mature and rigorous mind. He went about the job of setting up the technique in a careful and systematic fashion, paying special attention to all physical parameters. A few practical tips from Claude, who had just returned to Belgium, were also helpful.

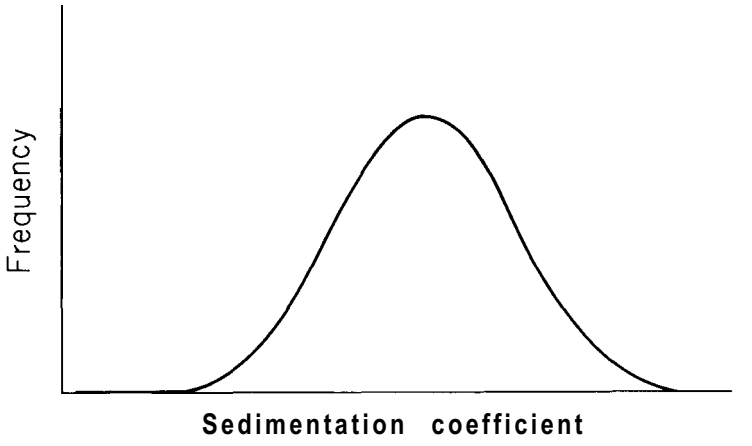


Fig. 2. Image of a polydisperse population of particles. Owing to individual differences in size and/or density, different members of the population do not have the same sedimentation coefficient. The centrifugal properties of the population as a whole are depicted by a frequency distribution curve of sedimentation coefficients. Size and/or density distribution can be similarly represented. Frequency is usually defined as $\frac{dn}{Ndx}$ (or in the case of histograms $\frac{\Delta n}{N\Delta x}$), in which $\frac{dn}{N}$ is the fraction of total particles having an abscissa value comprised between x and x + *dx*. Similar diagrams may be drawn in terms of relative mass, relative enzyme activity, etc ..., instead of relative number.

Particularly important, I now realize in retrospect, was the fact that we took some time to study the theory of centrifugation, as beautifully exposed in the classical book by Svedberg and Pedersen (4).

Although separating mitochondria and microsomes might appear worlds apart from the determination of the molecular weight of macromolecules, certain concepts were common to the two operations and could be usefully transposed from the latter to the former.

One was that of *sedimentation coefficient* (Fig. 1), which obviously was applicable to any particle, irrespective of its size.

Another was that of *polydispersity* which, owing to biological variability, was likely to be a property of the populations made up by subcellular organelles. This meant that the centrifugal behavior of such populations could be described only by a frequency distribution curve of sedimentation coefficients (Fig. 2), not by a single s value as for most molecular populations.

A third important point related to the *resolving power* of differential sedimentation, which some elementary calculations revealed to be surprisingly low (Fig. 3).

There was much insistence in those days on the various artifacts that complicate centrifugal fractionation, such as, for instance, breakage or agglutination of particles, adsorption or leakage of soluble constituents. But these were only accidents, no doubt serious, but amenable to experimental correction. The problem, as it appeared to us, was a more fundamental one.

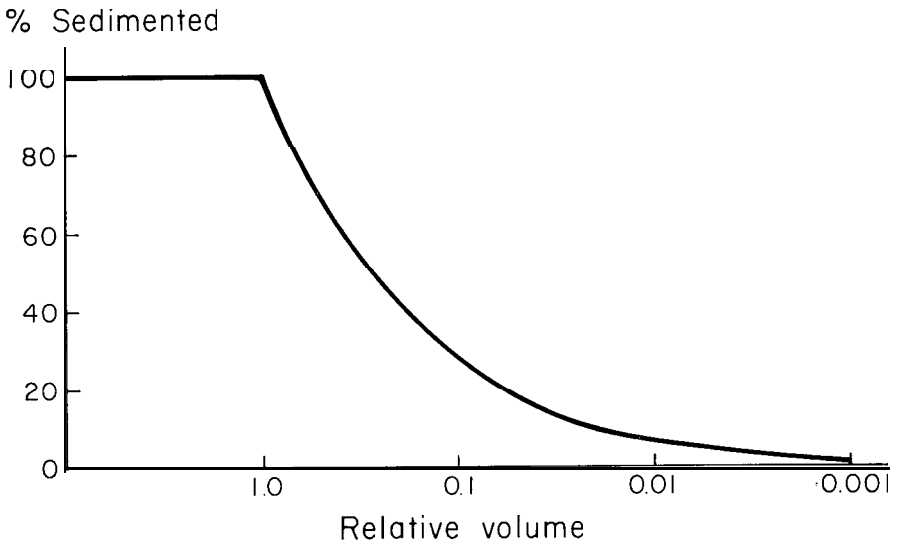


Fig. 3. Graph shows the percentage of particles recovered in a sediment as a function of relative particle volume. Particle density is assumed to be the same for all particles. The meniscus of fluid in the rotating centrifuge is assumed to be half-way between the axis and the bottom of the tube or cell.

What we were doing was trying to separate populations which, owing to overlapping polydispersities, might at best be only partly separable from each other. In addition, we were using a poorly discriminating method for this purpose.

I cannot claim that all this was immediately clear to us. But considerations of this sort undoubtedly colored our approach from the start (5). We fully expected centrifugally isolated fractions to be impure, while suspecting that populations of cell organelles might be difficult, if not impossible, to resolve quantitatively. Conscious also of the severe limitations of light microscopic examination of the fractions, we tried to extend the biochemical interpretation as far as possible. Instead of looking at each fraction separately and focusing on its enzyme content, as was usually done, we looked rather at each individual enzyme and contemplated its distribution between all the fractions.

In order to permit a comprehensive view of enzyme distribution patterns, I introduced a histogram form of representation, illustrated in Fig. 4. In this figure are shown the distribution patterns of three of the first enzymes we studied, on the left as determined by the classical 4-fraction scheme, and on the right as determined by the modified 5-fraction scheme that we worked out in an effort to elucidate the significance of the small difference in distribution observed between acid phosphatase and cytochrome oxidase (6). This difference, as can be seen, is very much magnified by the modification in fractionation scheme.

These histograms turned out to be very revealing, by more or less automatically conveying the notion of polydispersity, illustrated in Fig. 2. In fact,

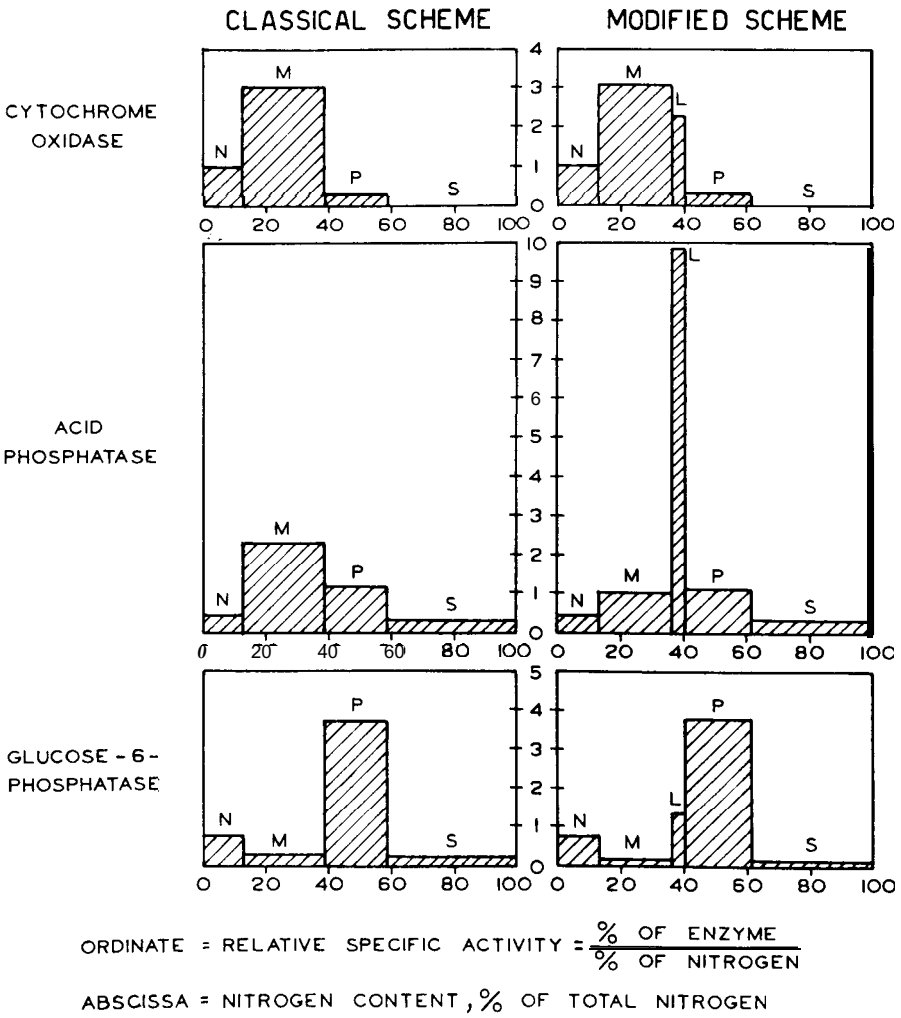


Fig. 4. Enzyme distributions represented in histogram form. The relative specific enzyme content (% activity/% protein) of the fractions is plotted against their relative protein content, inscribed cumulatively from left to right in their order of isolation (decreasing sedimentation coefficient) : nuclear *N*, mitochondrial *M*, microsomal *P*, and supernatant S, in classical 4-fraction scheme; and nuclear *N*, heavy-mitochondrial *M*, light-mitochondrial *L*, microsomal *P* and supernatant *S*, in modified 5-fraction scheme (6). Although very crude, similarity with frequency distribution curves of polydisperse populations can be recognized. Distinction between three populations, now known to consist of mitochondria (cytochrome oxidase), lysosomes (acid phosphatase) and endoplasmic reticulum fragments (glucose 6-phosphatase), is enhanced by use of 5-fraction scheme. From reference 7.

since the fractions are aligned along the abscissa in order of decreasing sedimentation coefficient, one may, in a very crude fashion, look at the abscissa as a deformed scale of sedimentation coefficients, and at the histograms as correspondingly deformed frequency distribution histograms of sedimentation coefficients. The logical next step in this line of reasoning was to assimilate

enzyme distributions to particle distributions, and therefore to interpret, at least tentatively, significant differences in the distribution patterns of two enzymes as reflecting association of the enzymes with distinct particle populations.

Extrapolation from enzymes to particles could, however, not be made without some sort of assumption concerning the relationship between relative enzyme activity, the numerator in the ordinate of Fig. 4, and relative particle number, the numerator in the ordinate of Fig. 2. The simplest, and at the same time most plausible, such assumption was that members of a given particle population have essentially the same biochemical composition, larger particles simply having more of everything than smaller particles. Within the limits of validity of this assumption, which I have called the *postulate of biochemical homogeneity*, the histograms of Fig. 4 could now be likened to distribution diagrams of total particle mass or protein (not of actual particle numbers, it should be noted, although further conversion to numerical distributions can be made with some additional information). We had to assume, of course, that the enzyme distributions were not grossly distorted by translocation artifacts, or to correct for such artifacts as much as possible.

Another postulate we made was that each enzyme is restricted to a single intracellular site. This *postulate of single location* is less essential than that of biochemical homogeneity, since bimodal or multimodal distributions are amenable to the same kind of interpretation. In practice, however, single location made a useful addition to biochemical homogeneity, supporting the use of enzymes as markers of their host-particles.

First used empirically as pure working hypotheses, the above considerations were progressively validated, as more enzymes were studied and a limited number of typical distribution patterns began to emerge. Actually, as shown by the results of Fig. 5, things were not quite as simple, and a number of complications of various sorts tended to blur the picture. But most of these could be dealt with satisfactorily by ancillary experiments (8).

In these studies, a second line of evidence proved very useful, based on *enzyme latency*. Owing to impermeability of particle membranes to one or more of the substrates used in the assay of enzymes, many particle-bound enzymes fail to display activity "in vitro" as long as the membrane surrounding them is intact. Various means, mechanical, physical or chemical, can be used to disrupt the membrane and to release the enzymes, as we first showed for rat-liver acid phosphatase (Fig. 6). If two or more enzymes are present together in the same particles, they will be released together in this kind of experiment; if in different particles, they may come out separately (Fig. 7). In our hands, such studies have been very useful, providing an independent verification of the significance of the similarities and differences revealed by centrifugation experiments.

By 1955, our results were sufficiently advanced to allow us to propose with a certain measure of assurance the existence of a new group of particles with lytic properties, the lysosomes, and to hint at the existence of another group of particles, the future peroxisomes (8). At the same time, we had, from the

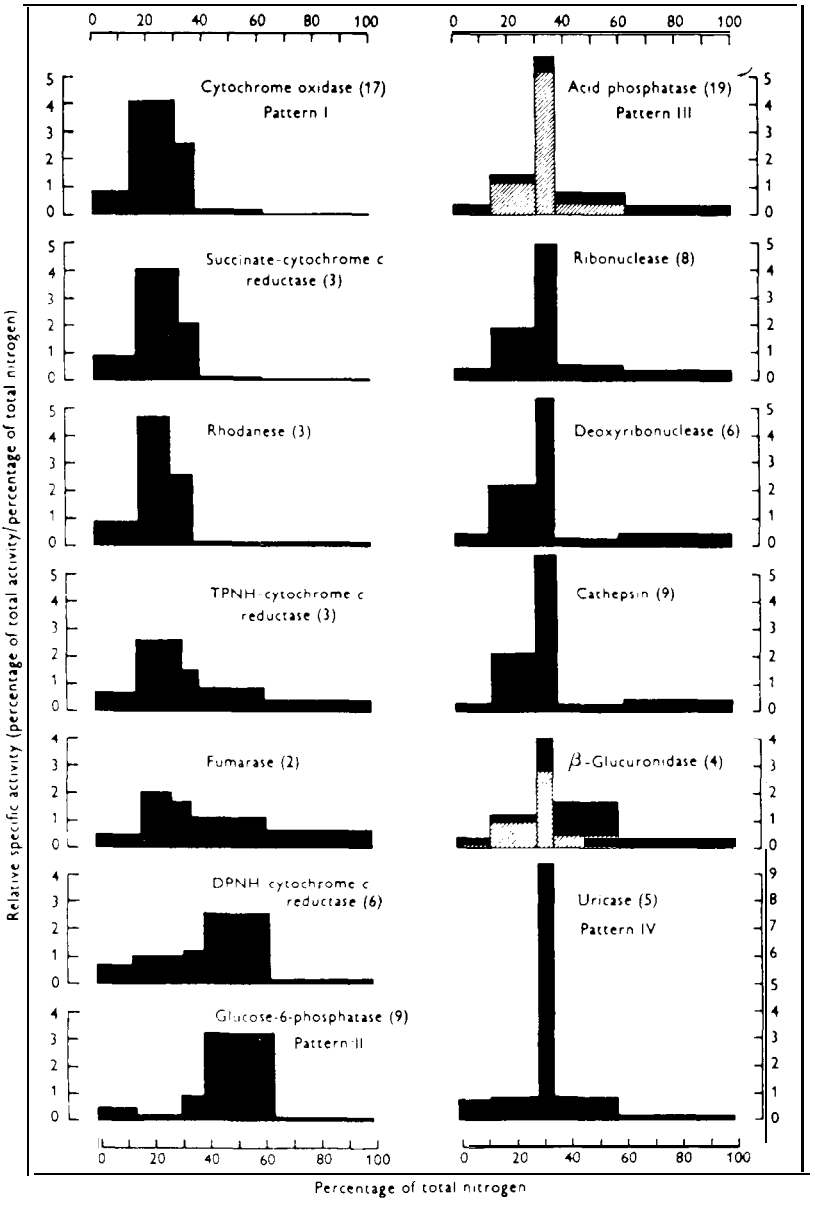


Fig. 5. Distribution patterns of enzymes in rat-liver fractions separated by 5-fraction procedure (see: Fig. 4). Pattern I, shared by 3 enzymes, represents the distribution of mitochondria; pattern II (glucose 6-phosphatase) that of microsomes. In between, in left-hand column, are complex combinations of patterns I and II. Pattern III is shared by 5 lysosomal acid hydrolases, except for β-glucuronidase which has an additional microsomal component. Pattern IV belongs to the peroxisomal urate oxidase. Details are given in original paper (8).

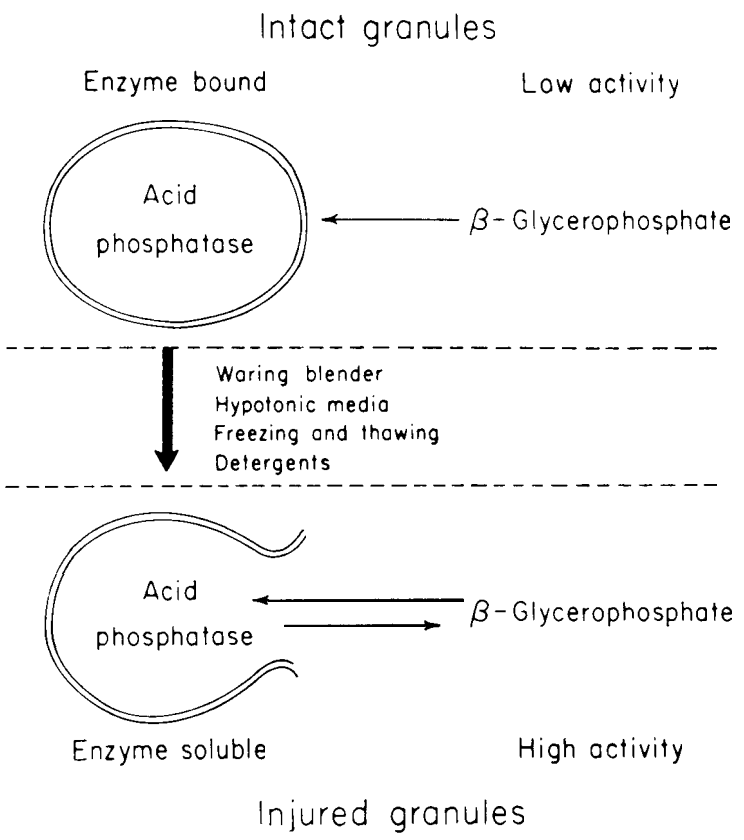


Fig. 6. Model of latency of rat-liver acid phosphatase, as proposed in 1951 (9). From reference 10.

mixture of theoretical considerations and experimental results that I have just briefly recalled, derived a certain "philosophy" of centrifugal fractionation, which I subsequently elaborated in greater detail in several publications *(11)*. The key word here was *"analytical"*. Basically, we felt that our approach was no more than an extension of the classical Svedberg technique from the molecular to the submicroscopic and microscopic level.

A major difficulty at this stage, however, was that available techniques did not measure up to the kind of information we were hoping to extract. The answer to this problem was provided by density gradient centrifugation, which was introduced in the early 1950's. This new technique offered prospects of improved resolution; it allowed the use of density, as well as of sedimentation coefficient, as separation parameter; and, finally, its analytical character was unmistakable (Fig. 8). In fact, as shown as early as 1954 by Hogeboom and Kuff (13), it could even be used successfully for the determination of molecular weights.

Here again, we devoted some time to theoretical studies (12). In this, Berthet and I were joined by another young co-worker, Henri Beaufay, whose skills as a self-taught engineer proved particularly valuable for the design of

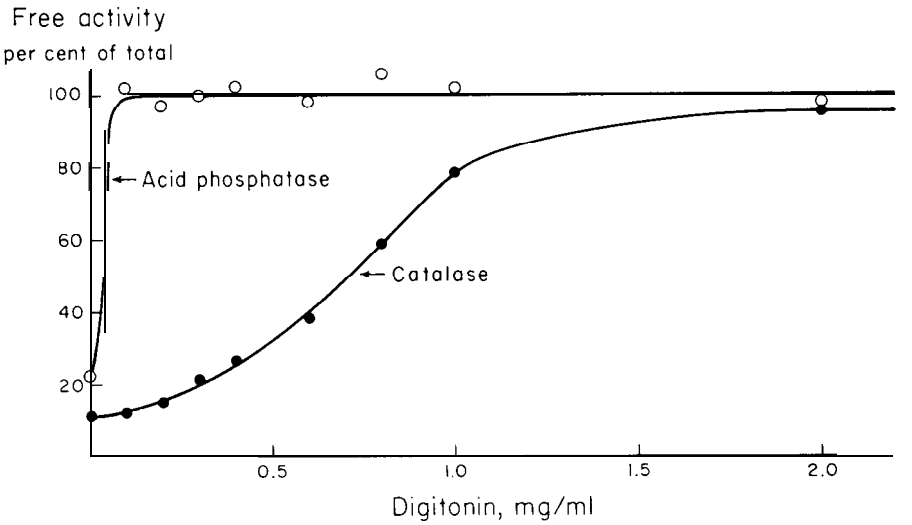


Fig. 7. Differential release of the lysosomal acid phosphatase and of the peroxisomal catalase by increasing concentrations of digitonin. From reference 10.

various accessories, culminating in the construction of a completely automatic rotor (14), different in principle from the zonal rotors built by Norman Anderson (15), and particularly adapted to rapid isopycnic separation at minimum hydrostatic pressure. The importance of the latter advantage has been emphasized by my former collaborator Robert Wattiaux (16).

Particles sedimenting through a density gradient are apt to undergo a progressive increase in density, due to inflow of solute or outflow of water or both, depending on the number and permeability properties of their membranes and on the nature of the solute(s) and solvent used to make the gradient. These factors we tried to incorporate in a theoretical model of particle behavior (12, 17), and at the same time to take into account in the design of our experiments. It appeared from our theoretical considerations that the sucrose concentration of the medium might be a particularly important variable, and that different types of particles might respond differently to changes in sucrose concentration. We therefore subfractionated large granule fractions from rat liver in iso-osmotic glycogen gradients prepared with sucrose solutions of different concentrations as solvent, as well as in sucrose gradients prepared with either H_2O or D_2O (18).

The results of these experiments confirmed and extended our earlier findings, establishing the existence of three distinct groups of enzymes, as defined by their centrifugal behavior. There was little doubt in our minds that these observations reflected the occurrence of three distinct populations of particles in the large granule fraction. By fitting our results to the theoretical equation, we were even able to evaluate a number of physical parameters for each putative particle population, and to construct, from purely biochemical data, a sort of "robot picture" of the particles themselves. This is shown in Table I. Due to heterogeneity within the population, the data given in this table for the

Table I. *typical Physical Properties of Rat-Liver Particles*

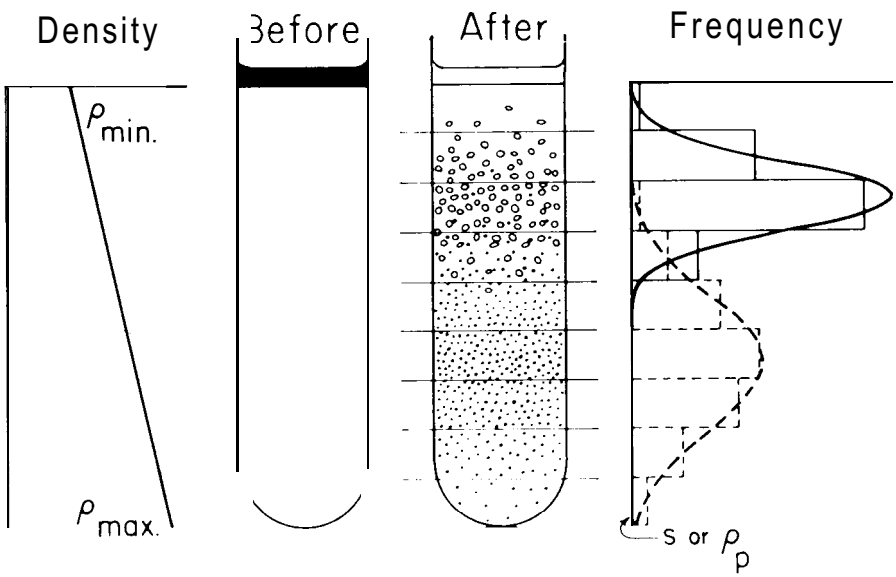
From reference 10

Parameter	Mitochondria	Lysosomes		Peroxisomes		
Reference enzyme:	Cytochrome oxidase	Acid phosphatase	Acid DNase	Urate oxidase	Catalase	D-Amino acid oxidase
Dry weight (μg)	10^{-7}	2.7×10^{-8}	3.6×10^{-8}	2.4×10^{-8}	—	—
Dry density	1.315	1.300	1.331	1.322	1.319	1.315
Osmotically active solutes (milliosmoles/g dry weight)	0.157	0.128	0.334	0	0	0
Water compartments (cm^3/g dry weight)						
Hydration	0.430	0.256	0.212	0.214	0.295	0.296
Sucrose space	0.905	1.075	0.330	2.51	2.68	2.54
Osmotic space in 0.25 M sucrose	0.595	0.485	1.265	0	0	0
Total in 0.25 M sucrose	1.930	1.816	1.807	2.724	2.975	2.836
Sedimentation coefficient in 0.25 M sucrose (Svedberg units)	10^4	4.4×10^3	5×10^3	4.4×10^3	—	
Diameter in 0.25 M sucrose (μm)	0.8	0.51	0.56	0.54	—	—
Density in 0.25 M sucrose	1.099	1.103	1.100	1.095	1.088	1.090

lysosomes are of questionable significance. On the other hand, those listed for mitochondria and peroxisomes agree very well with measurements made by other techniques.

Though analytically satisfactory, the results described so far still fell short from definitive proof, since they had unfortunately confirmed our fear that distinct populations of subcellular particles might prove intrinsically inseparable quantitatively due to overlapping of size and/or density distributions. It was possible to obtain pure samples by cutting off non-overlapping parts of the populations; but this introduced the danger of biased sampling. A means of almost complete separation, although under somewhat artificial conditions, was provided in 1962 by Wattiaux, Wibo and Baudhuin (19), when they discovered that pretreatment of the animals with Triton WR-1339 causes a selective decrease in the density of lysosomes, due to accumulation of the Triton within these particles (Fig. 9). Thanks to this finding and to the Beaufay rotor, large-scale separation of the three populations has now become possible, allowing a variety of biochemical and functional studies that were not feasible before (20).

While the biochemical approach I have outlined was being developed in our laboratory, electron microscopy was making great strides of its own, soon becoming available for the examination of subcellular fractions. For obvious



1. Differential sedimentation

Gradient: *Shallow stabilizing,* $\rho_{max.} < \rho_{p\ min.}$

Centrifugation: ⟶ *Incomplete sedimentation*

Abscissa of frequency distribution: *Sedimentation coefficient*

2. Density equilibration

Gradient: *Steep,* $\rho_{max} > \rho_{p\ max.}$

Centrifugation: *Prolonged, high speed*

Abscissa of frequency distribution: *Equilibrium density*

Fig. 8. Schematic representation of density gradient centrifugation, with initial top-layering of the sample. Two forms, based on differences in sedimentation coefficient and density respectively, are shown. Diagram at the right pictures frequency distribution of particles or markers as a function of tube height. Conversion to frequency distributions of sedimentation coefficients or densities generally requires readjustment of ordinate and abscissa values, leaving surface area of each block (% content in fraction) unchanged. For details of calculations, see reference 12. From reference 10.

reasons we were very anxious to take a look at our purest fractions, in order to test our conclusions and eventually identify our hypothetical particles. Already in 1955, thanks to the expert collaboration of Alex Novikoff and to the facilities of Albert Claude in Brussels and of Wilhelm Bernhardt in Paris, we were able to do this for lysosome-rich fractions, which were found to contain dense bodies, surrounded by a membrane and of about the size predicted for lysosomes (21). Later, we were able to acquire an instrument of our own, and Henri Beaufay taught himself another skill, which he later perfected under

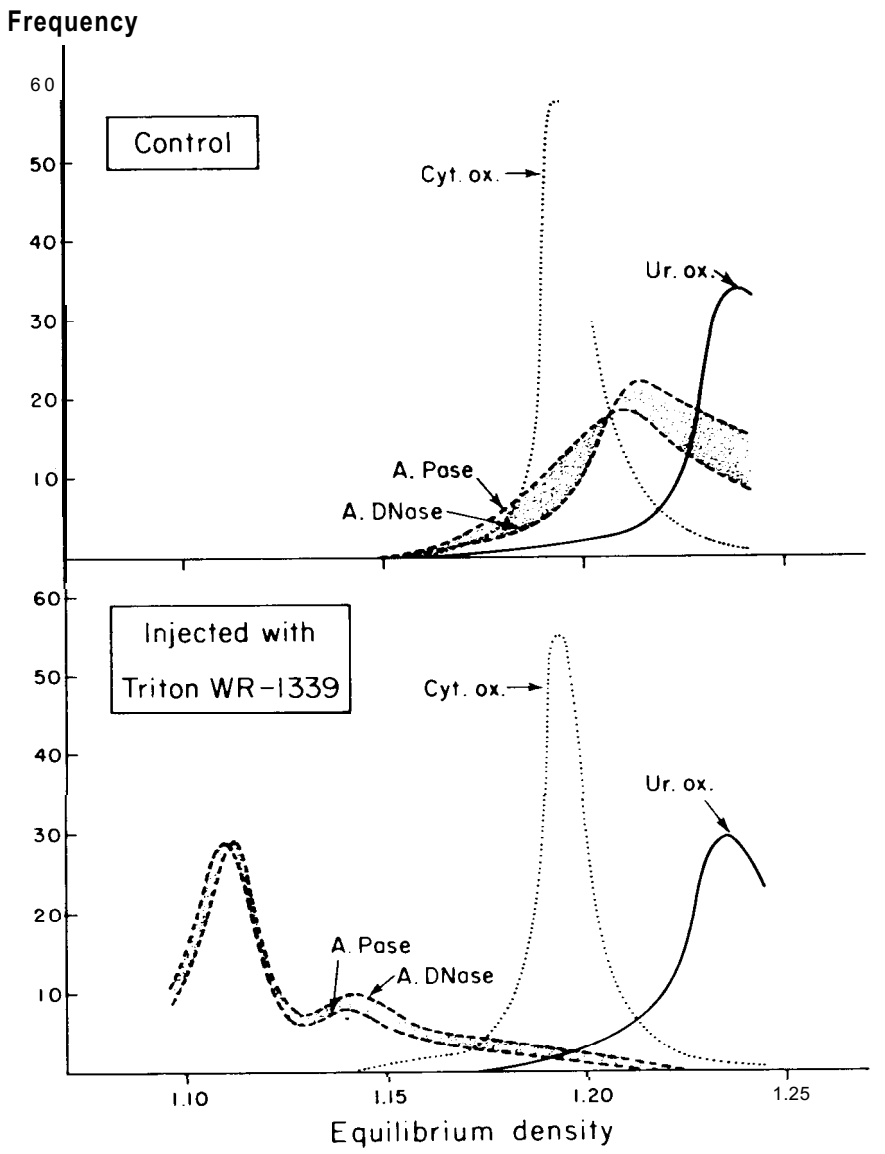


Fig. 9. Influence of a previous injection of Triton WR-1339 on the equilibrium density of rat-liver particles equilibrated in an aqueous sucrose gradient. Upper graph shows overlapping of lysosomes. (A.Pase = acid phosphatase; A.DNase = acid deoxyribonuclease) with mitochondria (Cyt.ox. = cytochrome oxidase) and peroxisomes (Ur.ox. = urate oxidase). Four days after intravenous injection of 170 mg of Triton WR-1339 to the animals, the density of the lysosomes has decreased drastically, that of mitochondria and of peroxisomes remains unchanged. Graph constructed from results of Wattiaux *et al.* (19), reproduced from reference 10.

the guidance of George Palade. With Pierre Baudhuin, he confirmed the identification of lysosomes as "pericanalicular dense bodies" and showed that the peroxisomes correspond to the particles known as "microbodies" (22).

Thus, the gap between biochemistry and morphology was finally bridged, after some 15 years of research.

More recently, Baudhuin has adapted quantitative morphometric methods to the examination of subcellular fractions, making it possible to compare measurements derived from biochemical data with those obtained by direct mensuration (23). In several instances, excellent agreement has been found between the two sets of data (20, 23, 24, 25).

Applications to Biology

I have chosen to dwell at some length on our theoretical and technical studies, because they were, I believe, the key to whatever achievements were made by our group. I know that others have accomplished important advances by the alternative process of first purifying a subcellular component and then analyzing it. For example, nuclei, secretion granules, plasma membranes and Golgi elements have been largely characterized in this fashion. But purification is generally a laborious procedure, it is difficult to control, and it is rarely quantitative. The advantage of the analytical approach is that it is widely applicable, and it can provide a considerable amount of quantitative information even with a relatively poor resolving power. The important point is that with this kind of methodology, we derive the information, not from the properties of specific fractions believed to approximate a given intracellular component, but from the manner in which properties are distributed over a large number of fractions, which together represent the whole tissue.

In our laboratories, this general approach has been applied to a variety of biological materials and for the study of many different problems. In continuation of the work on liver, already described, it has supported a number of studies concerned with the functions of lysosomes, including those of Robert Wattiaux on intralysosomal storage (26), of Pierre Jacques on pinocytosis (27), of Russell Deter on autophagy (28), of Jack Coffey, Nick Aronson and Stanley Fowler on lysosomal digestion (29), and of Andre Trouet and Paul Tulkens on the effects of anti-lysosome antibodies (30). It has also allowed Brian Poole, Federico Leighton, Tokuhiko Higashi and Paul Lazarow to make a searching analysis of the biogenesis and turnover of peroxisomes (24, 31). In recent years, a large team grouped around Henri Beaufay and Jacques Berthet, and including Alain Amar-Costesec, Ernest Feytmans, Mariette Robbi, Denise Thinès-Sempoux and Maurice Wibo, has launched a major attack on microsomal and other membrane fractions, with the aim of characterizing physically, chemically, enzymically and immunologically the various types of cytomembranes occurring in these fractions (32).

In its applications to other mammalian tissues and cell types, analytical cell fractionation has allowed Pierre Baudhuin and Brian Poole to recognize peroxisomes in kidney (33); Gilbert Vaes to carry out a thorough study of bone lysosomes, leading to very revealing observations on the role of these particles in bone resorption (34); Bill Bowers to make a comprehensive biochemical dissection of lymphoid tissues and lymphocytes, as a preliminary to

an analysis of cell-mediated immune cytotoxicity (35); Marco Baggiolini to characterize the two types of granules present in neutrophil polymorphonuclear leucocytes (36); Richard Schultz and Pierre Jacques to unravel some of the complexities of placental tissue (37); Tim Peters to fractionate aortic smooth muscle cells (38) and enterocytes (39); and Paul Tulkens to do the same for cultured fibroblasts (40), a system also used by Brian Poole and Maurice Wibo for investigations of protein turnover (41).

Under the leadership of Miklós Müller, a series of fascinating studies have been performed in New York on a number of different protozoa. In *Tetrahymena pyriformis*, Müller was able to identify two types of lysosomes, which discharge their enzymes, one in phagocytic vacuoles and the other in the outside medium (42). In collaboration with Pierre Baudhuin and later with Jim Hogg, he has shown the existence in the same organism of peroxisomes which, like plant glyoxysomes, contain enzymes of the glyoxylate cycle (33, 43). More recently, with Don Lindmark, he has characterized in *Trichomonads* a completely new type of cytoplasmic particle, with the capacity of converting pyruvate to acetate, CO_2 and molecular hydrogen, the hydrogenosome (44).

Other studies have dealt with the role of lysosomes in tissue regression, notably those of Denise Scheib-Pfleger and Robert Wattiaux on Müllerian ducts in chick embryos (45), and those of Yves Eeckhout on the tail of metamorphosing tadpoles (46).

It has been my good fortune to participate in most of these investigations, sometimes actively and sometimes simply in an advisory capacity, and to watch at the same time the growing interest of other laboratories in similar problems. After trying, with increasing difficulty, to review the field of lysosomes at regular intervals (7, 47), I welcomed with some relief the appearance in 1969, under the editorship of Professor John Dingle and Dame Honor Fell, of the multi-author treatise "Lysosomes in Biology and Pathology", of which volume 4 is now in press (48). The literature on peroxisomes and related particles has grown more slowly, but has now also reached an appreciable size (49).

It must be pointed out that many of these advances have been made by means of morphological rather than by biochemical methods, or by a combination of both. In this respect, the development of cytochemical staining reactions for enzymes previously identified biochemically as specific particle markers has been an invaluable aid, thanks to the pioneering work of Alex Novikoff, Stanley Holt, Werner Straus, Fritz Miller, Sidney Goldfischer, Marilyn Farquhar and many others.

Applications to Pathology and Therapeutics

In recent years, we have become increasingly concerned with the possible medical applications of our findings. The possibility that lysosomes might accidentally become ruptured under certain conditions, and kill or injure their host-cells as a result, was considered right after we got our first clues to the existence of these particles. We even made a number of attempts to test this

hypothesis in ischemic tissue and in the livers of animals subjected to hepatotoxic treatments or to carcinogenic diets (50). But we became discouraged by problems of interpretation (47). Even today, clear-cut demonstration of the so-called "suicide bag" hypothesis remains very difficult, although there seem to be at least a few authenticated cases involving this mechanism of cell death. Much more clearly documented is the mechanism of tissue injury through extracellular release of lysosomal enzymes, a field which has been pioneered by Honor Fell and her co-workers.

The two mechanisms mentioned above rely on the very plausible instance of lysosomal enzymes exerting their lytic effect at abnormal sites. What we did not suspect in the beginning was that the failure of lysosomal enzymes to act at their normal site could also cause serious diseases. This fact was brought home to us in a rather surprising fashion through the work of my colleague Géry Hers, who in 1962 diagnosed glycogen storage disease type II as being due to a severe deficiency of a lysosomal enzyme (51). This finding initiated a series of fruitful investigations on other storage diseases, in which Francois Van Hoof played a major part (52). It also provided useful guidelines to the chemists and pathologists who, in various parts of the world, were trying to unravel the pathogeny of hereditary lipidoses and mucopolysaccharidoses. Today, with more than twenty distinct congenital lysosomal enzyme deficiencies identified, this mysterious chapter of pathology has been largely elucidated (53).

According to some results obtained over the last few years by Tim Peters, Miklós Müller, Tatsuya Takano, Bill Black and Helen Shio, with the collaboration of Marilyn Farquhar, lipid accumulation in arterial cells during the development of atherosclerosis could well be due to a mechanism similar to that involved in congenital lipidoses. At least in cholesterol-fed rabbits, there is strong evidence, both biochemical and morphological, that the lysosomes of the aortic smooth muscle cells are the main site of intracellular cholesterol ester accumulation, and there are indications that a relative deficiency of the lysosomal cholesteryl esterase may be responsible for this phenomenon (38, 54). Fig. 10 shows some of the biochemical evidence: after cholesterol feeding, lysosomes become considerably less dense due to lipid accumulation. This figure also illustrates the sensitivity of our present techniques. These fractionations were performed on a total of about 1 mg of cell protein. Similar experiments can be, and have been successfully, carried out on a needle biopsy.

Other interesting applications of the lysosome concept are in pharmacology and therapeutics. In line with the "suicide bag" hypothesis, early investigations in this area focused on "labilizers" and "stabilizers" of the lysosomal membrane (55). One outcome of this work has been the suggestion that certain anti-inflammatory agents, such as cortisone and hydrocortisone, might owe at least part of their pharmacological properties to their effect on the lysosomal membrane.

More recently, we have extended our interest to the various substances that are taken up selectively into lysosomes and owe some of their main pharma-

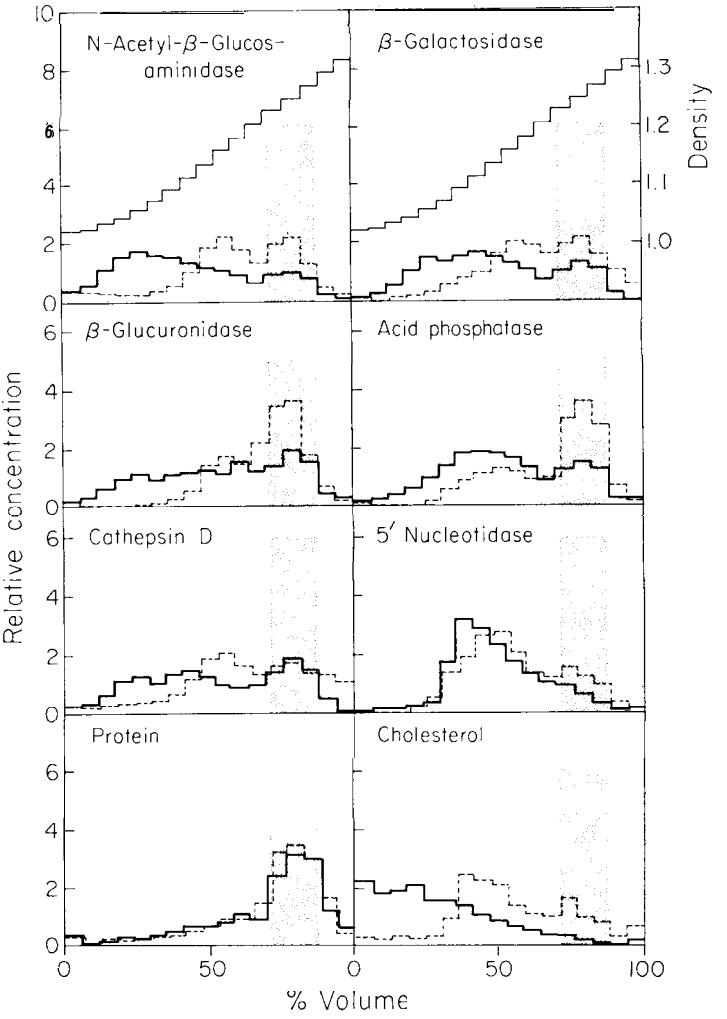


Fig. 10. Influence of cholesterol feeding on density of aortic smooth muscle cel l lysosomes. Graphs show distribution patterns of enzymes after density equilibration (see: Fig. 8) in sucrose density gradient depicted by "staircase" on top. Starting material was a postnuclear supernatant of rabbit aortic cells brought to a density of 1.26 and layered initially at outer edge of gradient (dotted area). Broken lines give distributions in normal preparations, solid lines in preparation from a rabbit showing grade IV atheroma as a result of cholesterol feeding. Note extensive shift to the left of 5 acid hydrolases, indicating lowered density of lysosomes due to lipid accumulation. Distribution of protein, 5′-nucleotidase (plasma membranes) and mitochondrial cytochrome oxidase (not shown) was unchanged. From Peters and de Duve (54).

cological properties to this phenomenon. These "lysosomotropic" agents are surprisingly numerous, including such variegated compounds as neutral red, chloroquine, streptomycin, dextran, polyvinylpyrrolidone, Triton WR-1339 and trypan blue (56). Particularly interesting is the use of certain lysosomotropic agents as carriers for drugs. In Louvain, Andre Trouet has applied this principle to leukemia and cancer chemotherapy, by using DNA as carrier

for the drugs daunorubicin and adriamycin. Experimentally, these DNA complexes proved less toxic and more effective on L1210 leukemia than the free drugs (57). Clinical trials under way over the last two years in several hospitals have given very encouraging results (58).

Conclusion

In the conclusion of his Nobel lecture delivered in 1955, Hugo Theorell asks the question: "What is the final goal of enzyme research?"

"The first stage", he answers, "is to investigate the entire steric constitution of all enzymes . ."

"In the second stage," he continues, "it is a matter of deciding how the enzymes are arranged in the cell-structures. This implies, as a matter of fact, the filling of the yawning gulf between biochemistry and morphology".

The gulf still yawns today. But it is a particular pleasure for me to be able to tell my old friend Theo that it yawns a little less. In our efforts to narrow it, my co-workers and I have been privileged to contemplate many marvelous aspects of the structural and functional organization of living cells. In addition, we have the deep satisfaction of seeing that our findings do not simply enrich knowledge, but may also help to conquer disease.

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