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THE STRUCTURE & FUNCTION OF THE MEMBRANES AND SURFACES OF CELLS

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THE STRUCTURE AND FUNCTION OF THE MEMBRANES AND SURFACES OF CELLS

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CONTENTS

INTRODUCTORY REMARKS

By F. G. YOUNG

Department of Biochemistry, University of Cambridge, England

In an oral examination many years ago, more years than I can easily remember or wish to count, I was asked a question that I thought at the time was a little unfair. This question was 'What do you think is the most important discovery that can be made in Biochemistry during the next thirty years or so?' After some thought I offered the suggestion that knowledge of how the metabolism of carbohydrate, fat, and protein was integrated and controlled within cells would to my mind be the most important discovery next to be made. This, however, did not entirely satisfy my questioner, who said that the elucidation of the mechanism of the differential permeability of cell membranes would in his view be the most important advance that could be made during the next generation.

These days I am much more frequently the quizzer than the quizzed, but I have never used this particular inquisition myself. Nevertheless, the memory of this question and of its significance has remained in my mind, and I suppose that the question is the indirect reason why I am in the Chair for the first part of this Symposium on 'The Structure and Function of Membranes and Surfaces of Cells.'

The more immediate reason why I am here today, despite the fact that my own researches have not lain in the field of permeability, is that I suggested to the Committee of the Biochemical Society that the Cambridge meeting of the Society, planned for 12 and 13 July this year, should include a Colloquium on 'The Biochemistry of Cell Membranes'. There is much research going on in Cambridge which is relevant to this subject. I had in mind a Colloquium devoted to comparative aspects of the structures of the membranes rather than to theoretical dissertations on possible mechanisms of permeability. The Committee of the Biochemical Society apparently thought a Colloquium on this or a related subject was so attractive that it should not be held outside the great metropolis itself. Accordingly, about a year ago I was notified that the Committee of the Society had decided that the present Colloquium would be held in London in March 1962, and that we at Cambridge would have to think of another title for the Colloquium on the occasion of the meeting in July. In these circumstances I suppose those responsible for the organization of the present meeting thought that they were bound to give me an opportunity of showing some interest

in it. For my own part, I decided that I ought to take some part in the birth of the infant in whose sireing I had played an unexpected and indeed an involuntary part.

In recent years we have become so accustomed to the separation, by differential centrifugation and other means, of corporate structures that existed originally within the cell, that it is now an accepted and commonplace view that barriers to permeability exist not only at the cell surface but also within the cell itself. Nevertheless, I believe that in a group of biochemists, such as we have here today there are some who do not realize the relationship of permeability barriers to the preparation of black tea, as we habitually drink it in this country. In the preparation of black tea from the green leaf, rolling of the leaf breaks permeability barriers within it so that polyphenol oxidases and other enzymes can then make contact with substrates which they act upon to produce both the dark colour and the aroma and taste which we hope to enjoy this afternoon. This so-called process of fermentation of the tea leaf in the manufacture of black tea is yet another example where intracellular permeability barriers are of significance.

Both cytochemistry and chemotherapy can be said to have benefitted from what might be described as the 'Chinese Box' concept of the cell, whereby a series of membranes may have to be crossed before a substance—a metabolite or a drug—can reach a site of action where it can bring about an action. It is not surprising that in such circumstances no simple and direct relationship is found to exist between the physical and chemical properties of physiologically active substances and their biological actions. Not only is the effectiveness of the molecule when it reaches its point of action of importance, but also the power of penetration of possibly a series of different types of barriers.

When we consider the endoplasmic reticulum and related structure we do not know precisely at what spot we are inside, and at what spot we are outside, the cell. In fact the more we get to know about this question the less capable are we of defining what is intracellular and what is extracellular. This is readily understandable. A cell membrane or any other membrane, must to some extent be an arbitrarily defined volume surrounding the cell or other structure. The limits of this are defined by man rather than by nature. Even though there may be no absolute definition, which is applicable to all biologically significant membranes, I think that we largely agree about what we mean in general terms when we discuss membranes, whether they be around cells or microsomes.

The emphasis that Biochemistry has laid on the existence of a common ground plan of metabolic processes throughout the many different types of living cell found in nature has naturally caused those who study

permeability to search for similarities in the permeability barriers which exist in different types of cell. Although similarities undoubtedly exist, chemical differences also are found not only between proteins in the interior, and in the surface of cells, and also between those found in the surfaces of cells from different species. The presence of a high proportion of glutamic acid and alanine in bacterial cell walls in the D- (i.e. less frequently occurring) form of particular interest in this respect. In bacteria, the existence both of cell walls and of protoplast membranes, adds a lively interest to the comparative study of cell surfaces and permeability barriers.

More than twenty years ago Danielli set out in a diagrammatic form a reasonable structure for cell membranes, consisting of orientated lipid between layers of extended protein. Electron microscopy seems to have borne out the existence of this type of structure to a remarkable degree, although as Dr Weiss will later discuss, the limitations of electron microscopy must be kept in mind, including the effects which result from the preparative process and from the bombardment of the preparations by electrons. It seems to me, however, that most of the evidence that has accumulated during the past twenty years supports rather than disproves the courageous interpretation of the physicochemical data which Danielli ventured to illustrate diagrammatrically so long ago. This should be a matter for quiet satisfaction by the Chairman of this afternoon's session.

THE ISOLATION OF MICROSOMAL MEMBRANES

By J. ROTHSCHILD

The Rockefeller Institute, New York, U.S.A., and Physiological Department, Carlsberg Laboratorium, Copenhagen, Denmark

There is a growing appreciation of the importance of membrane structures in the morphology and physiology of the cell. In addition to the nuclear, vacuolar and plasma membranes, other membranes previously overlooked because of the inherent limitations of visible light optics have been revealed by electron microscopy. The organized structure of the mitochondrion, visible in electron micrographs, appears to consist of membranes. In those regions of the cytoplasm which had previously been regarded as occupied by structureless ground substance, an extensive system of membrane-bound cavities has now been recognized in most cells. This complex and varigated family of structures, termed collectively the endoplasmic reticulum (ER)*, comprises a quantity of membranous material often exceeding that previously known to be associated with the cell (see Porter, 1961).

The search for the function of these membrane structures has given rise to a remarkable partnership between morphological and biochemical studies. This combined effort has yielded, in the case of the mitochondrion, an integrated concept of its role in the energy economy of the cell; its essential feature has been the assignment of a related series of biochemical activities to a single clearly identifiable cell structure.

The ER, together with its related microsomal fraction, presents a contrasting picture of complexity. The metabolic activities in which the microsomal fraction has been implicated form no discernible overall pattern. Although a number of the trunk-lines of intermediary metabolism (glycolysis, oxidative phosphorylation, purine and pyrimidine biosyntheses, amino acid metabolism, etc.) have not yet been found to be represented, the list of microsomal enzymes grows longer and more diverse. The small group of esterases which, together with NAD-cytochrome-*c* reductase, had been identified as microsomal ten years ago (Hogeboom & Schneider, 1955), have been joined by a host of

* The abbreviations used in this paper: CDP, cytosine diphosphate; CoA, coenzyme A; ER, endoplasmic reticulum; NAD, nicotinamide adenine dinucleotide; $NADPH_2$, reduced nicotinamide adenine dinucleotide phosphate; RNA, ribose nucleic acid; RNP, ribose nucleoprotein; UDP, uridine diphosphate.

Table 1. *Some microsomal enzyme activities*

Synthesis of glycerides:	
Triglycerides	Weiss & Kennedy (1956)
Phosphatides, via CDP and UDP intermediates	Kennedy & Weiss (1956); Paulus & Kennedy (1960)
Transmethylation of phosphatides	Gibson, Wilson & Udenfriend (1961); Bremer & Greenberg (1960)
Acylation with acyl-CoA	Kornberg & Pricer (1953); Lands (1960)
Phosphatidic acid metabolism	Hokin & Hokin (1958)
Glycolipids and plasmalogens	Brady, Formica & Koval (1958); Sribney & Kennedy (1958); Burton, Sodd, & Bradey (1958); Kiyasu & Kennedy (1960); Brady & Koval (1958)
Metabolism of plasmalogens:	
Hydrolysis of vinyl ethers	Warner & Lands (1961)
Fatty acid synthesis:	
Reduction of acyl-CoA intermediates	Brady & Koval (1958); Lachance, Popják & Waard (1958)
Steroid biosynthesis:	
Cholesterol biosynthesis	Bucher & McGarrahan (1956); also reviewed by Popják (1958)
Steroid Hydrogenation of unsaturated bonds	Forchielli & Dorfman (1956)
Carbonyl reduction	Hubener, Fukushima & Gallagher (1956); Recknagel (1957)
$NADPH_2 + O_2$-requiring steroid transformations:	
Aromatization	Ryan (1959)
Hydroxylation	Ryan & Engel (1956)
$NADPH_2 + O_2$-requiring drug detoxifications:	
N- and *O*-Dealkylations	La Du, Gaudette, Trousot & Brodie (1955); Axelrod (1956)
Aromatic hydroxylations	Mitoma, Posner, Reits & Udenfriend (1956)
Side-chain oxidation	Gillette (1959)
Deamination	Axelrod (1955)
Thio-ether oxidation	Salzman & Brodie (1956); Gillette & Kamm (1960)
Desulphuration	Davison (1955)
Other drug detoxifications:	
Reduction of aromatic nitro groups	Fouts & Brodie (1957)
Reductive cleavage of azo linkages	Mueller & Miller (1949)
O- and *N*-Glucuronosidation	Dutton (1956); Axelrod, Inscoe & Tomkins (1957)
L-Ascorbic acid synthesis	Hassan & Lehninger (1956); Chatterjee, Chatterjee, Ghosh, Ghosh & Guha (1960)
UDP-uronic acid metabolism	Burton, *et al.* (1958); Pogell & Leloir (1961)
UDP-glucose dephosphorylation	Pogell *et al.* (1961)
Sulphite oxidation	MacLeod, Farkas, Fridovich & Handler (1961)
Aryl- and steroid-sulphatases	Dodgson, Spencer & Thomas (1954); Roy (1957)
Incorporation of iodine into amino acids and protein	Ekholm (1961)

additional enzymic and metabolic activities. In only one case, however, that of the participation of microsomes in protein synthesis, has it been possible to relate biochemical function to cell structure. For the remainder no unifying pattern is evident. At best a few connecting threads may be discerned. Examination of the partial and perhaps unrepresentative list of the more recently reported functions given in *Table 1* reveals the following interconnections:

(1) the CDP- and UDP-nucleotide function in phosphatide bio-

synthesis, hexose metabolism, glucuronide formation and ascorbic acid biosynthesis;

(2) the persistent microsomal intervention in steroid biosynthesis, from the synthesis of mevalonic acid to the final steroid hydroxylations;

(3) the extensive series of reactions linked by a common requirement for $NADPH_2$ and oxygen;

(4) the presence of at least one microsome-mediated step in the synthesis of each glyceride, phospholipid, glycolipid and plasmalogen.

Although their involvement in lipid metabolism may be thought to reflect the high lipid content of the microsomes, a similar basis for microsomal intervention in carbohydrate metabolism, oxidation-reductions and other miscellaneous activities is less evident.

This complex pattern in its outline and its relief probably reflects the historical accident of research as much as the real contours of the natural environment. Sometimes enzymes are reported as 'soluble' (i.e. not sedimentable under the conditions investigated) in fractionations using buffered salt solutions; however, the same enzymes may appear to be 'particulate' in sucrose medium. In many instances, to workers whose object is the isolation and purification of an enzyme, cell structure becomes a nuisance.

This impression of complexity mirrors the findings of the morphologists. In contrast to the membranes of the mitochondrion, those of the endoplasmic reticulum, from which the microsomal fraction is largely derived, show great diversity. Their appearance and disposition, as revealed by electron microscopy of intact cells, varies with the tissue and organism. Even within a single cell it is often possible to distinguish half a dozen local differentiations (reviewed by Porter, 1961; Oberling, 1959; Dalton, 1961). The membranes may be formed into vesicles, tubules or flattened cisternae joined in places to form an interconnecting reticulum. In some regions the outer surface of the tubules is covered with small particles or granules, giving them a rough appearance (Palade, 1958). The granules, which are frequently found lying free in the cytoplasm, have been shown to be rich in RNA; it is these which are the site of microsomal protein synthesis. The smooth-surfaced or agranular ER takes on a variety of forms ranging from lamellar stacking of the cisternae (typical of the Golgi region), through intricate tubules to dispersed vesicles. Not all elements of the ER are found in every kind of cell.

When the microsomal fraction isolated from liver is examined with the electron microscope three main components can be recognized: (1) small electron-dense particles about 15 mμ in diameter which are apparently identical with the ones seen in tissue sections; (2) smooth-surfaced membrane-bound vesicles ranging in size from 50 to 300 mμ;

and (3) rough-surfaced vesicles in a somewhat more restricted range of size (Palade & Siekevitz, 1956*a*; Palade, 1958). While the rough-surfaced vesicles have been identified, from their characteristic appearance, as being derived from the rough-surfaced ER, it is more difficult to trace the derivation of the smooth-surfaced vesicles. The gross configuration of the smooth ER, by which some of its regions can be distinguished, seems to be lost during the isolation process, the tubules, flattened lamella and cisternæ being converted into a collection of more-or-less identical appearing vesicles (Palade & Siekevitz, 1956*a*). It seems likely, however, that this seeming uniformity conceals a diversity of origins (e.g. Golgi, other regional differentiations of the ER, and plasma membrane), which may be reflected in the previously noted biochemical complexity. A number of other components may be found depending on the tissue and its physiological state. The most characteristic of these in liver microsomes are glycogen, lipid droplets and ferritin (Kuff & Dalton, 1957).

The microsomal fraction is clearly a mixture of structural elements of differing morphological character, derivation and physiological function. It is, moreover, a variable mixture. Whereas the procedures for the preparation of the nuclear and mitochondrial fractions have been evolved in an effort to isolate certain identifiable structures, those commonly used for the microsomes (Hogeboom, 1955; Schneider & Hogeboom, 1950; Keller & Zamecnik, 1956) were established at a time when a clear description of the microsomal components was not available. Hence the definition of the microsomal fraction is an operational one (Palade, 1958); the fraction is a more or less arbitrary sampling of the particulate matter remaining in suspension after the mitochondria have been removed. Its composition reflects the peculiarities of the tissue sample, the choice of suspending medium (a selection usually made with the mitochondrial rather than the microsomal fraction in mind), and the particular centrifugation procedure. The last is usually determined by the availability of equipment and by patience.

A meaningful interpretation of experiments performed with isolated cellular components requires a clear identification of the cellular origin of the isolated materials and their separation into homogeneous fractions. It is evident that the microsomal mixture as at present prepared does not meet these criteria; a new series of cell fractions are required. Since the disposition of the ER, the major source of the microsomes, shows such variation with tissue and organism, it follows that the new procedures will have to be modified accordingly. Indeed the choice of tissue may be an important element in the solution of a difficult fractionation problem (see Kuff & Dalton, 1959; Palade & Siekevitz, 1956*b*). Our earliest information concerning the composition of the

plasma membrane was derived from studies on the red blood cell (Ponder, 1948; Parpart & Ballantine 1952) and on bacterial protoplasts (Gilby, Few & McQuillen, 1958; Weibull & Bergström, 1958), studies which were dependent upon the absence of an internal membrane system in these cells. With more complex cells the need for careful fractionation, and its difficulty, increases. It should be noted that the discussion which follows applies mainly to mammalian liver and specifically to that of the rat.

PREVIOUS EFFORTS TO SUBFRACTIONATE THE MICROSOMES

The early opinions or suspicions that the microsomal fraction was a mixture of particles of differing physical dimensions, chemical composition, and presumably, biological function (Chantrenne, 1947; Barnum & Huseby, 1948; Hogeboom, Schneider & Palade, 1948), tended to be obscured in the rush to exploit its biochemical potentialities. But in the early 1950's, with interest growing in microsomal RNA, Petermann and her co-workers undertook to separate the RNA-rich portion of the microsomes, and to characterize it by ultracentrifugal analysis (Petermann & Hamilton, 1952; Petermann, Mizen & Hamilton, 1956). Similarly, the growing catalogue of microsomal enzymes impelled Novikoff, Podber, Ryan & Noe (1953) to investigate their distribution within the fraction. A systematic fractionation of the microsomal material, however, required a clearer conception of its nature and physical properties. Since separation of a mixture of particles by centrifugal techniques utilizes differences in size, density and shape, a description of the microsomal materials in these terms was necessary.

This became possible as methods for preparing biological material for electron microscopy became available. First Slautterback (1953) and Bernard, Gautier & Rouiller (1954), using spread and shadowed films, and then Palade & Siekevitz (1956*a*) and Novikoff (1957) working with thin sections cut from fixed centrifuged pellets, confirmed the heterogeneous nature of the microsomal material. Palade & Siekevitz (1956*a*), using a highly refined technique of preparation of their specimens, provided the description of the three microsomal components given above. This has afforded both the physical information necessary for the design of centrifugal methods of separation and the indispensable means for examination of the resulting fractions for cross-contamination.

The impact on cytochemical technique was immediate. Within a few years microsomal fractionations were reported using differential centrifugation (Palade & Siekevitz, 1956*a*; Kuff & Dalton, 1957; Kuff & Ziegel, 1960; Moulé, Rouiller & Chauveau 1960), gradient differential

centrifugation (Thomson & Mikuta, 1954, Kuff, Hogeboom & Dalton, 1956), and isopycnic centrifugation in various kinds of density gradient (Schneider, Dalton, Kuff & Felix, 1953; Kuff & Dalton, 1959; Hanzon & Toschi, 1960). Of these techniques, differential centrifugation is the simplest to perform, and the only one for which the angle rotor is well suited. It is the method generally used for the gross fractionation of the cell into the nuclear, mitochondrial and microsomal fractions. It has been used for those preparations which have yielded the bulk of our present biochemical knowledge about the microsomal fraction. Hence it is not surprising that the early efforts to subfractionate microsomes should have taken differential centrifugation as their starting-point. This method has, however, been subjected to considerable criticism.

(*a*) *Differential centrifugation*

Differential centrifugation relies upon differences between the sedimentation rates of the different particles. Although this rate is influenced by a number of particle characteristics, the most important are size and density. The morphological evidence provided by electron microscopy, together with some more recently acquired data on vesicle densities, and the sedimentation analysis of liver RNP particles (Petermann, Hamilton, Balis, Samarth & Pecora, 1958) makes possible some prediction of the behaviour of liver microsomal particles during differential centrifugation. Information regarding the RNP particles or ribosomes is the most complete. These particles, derived from microorganisms, higher plants and various mammalian tissues, exhibit rather similar sedimentation properties (see Roberts, 1958). Analysis of patterns obtained with the ultracentrifuge yields a sedimentation coefficient of about 80 $s_{20,w}$. This agrees well with the 15 mμ dimension found by electron microscopy, and corresponds to a density in the vicinity of 1·6 g/ml. It has been noted, however, that the 80 *s* particle appears to dissociate reversibly to give smaller components. These smaller fragments sediment more slowly and the resulting contamination may escape detection in electron micrographs.

Electron micrographs of the remaining components, the smooth and rough-surfaced vesicles, indicate considerable variation in size, shape and relative abundance depending upon the source. Those isolated from rat liver are polydisperse in size, with the ranges for the two categories overlapping. The rough vesicles vary from about 50 mμ to about 100 mμ, with a few reaching 200 mμ. The smooth vesicles range from about 50 mμ to about 300 mμ with some smaller granules of indeterminant character. (In some cases these latter profiles may represent sections cut tangentially to larger vesicles.)

Until recently, little was known about the vesicle densities, although it could be assumed that they were substantially less dense than the 80 *s* particles. From this it could also be assumed that the presence of the latter would augment the density of the rough vesicles to which they were attached. Work done over the last few years in Palade's laboratory at The Rockefeller Institute has demonstrated that the smooth vesicles from rat liver are polydisperse in density as well as size. They appear to fall into well-defined density groups, ranging from less than 1·06 to more than 1·18. It should be noted that these densities are for vesicles which have equilibrated with sucrose solutions of the same density. Nothing is known concerning the densities of these vesicles when suspended in sucrose at other concentrations, or in other media. Calculations made by applying these densities either to sedimentation behaviour in less concentrated sucrose or to flotation in more concentrated sucrose are therefore subject to corresponding uncertainties. The rough vesicles all appear to have densities exceeding 1·20.

At first sight this picture appears to support the frequently expressed concepts of 'heavy' and 'light', or 'large' and 'small' microsomes, which have guided numerous efforts to fractionate microsomes by differential centrifugation. Indeed there can be little doubt that the first materials to sediment will normally be enriched with rough vesicles while later fractions will contain more smooth vesicles and free particles. If, however, the sedimentation coefficient, S, for the smallest rough vesicles (assume 50 mμ diameter and particle densities, dp = 1·22 and 1·27), for the smooth vesicles (assume 50 and 200 mμ, dp = 1·18 and 1·06), and for the 80 *s* RNP particles are calculated from the usual equation (1), the difficulties become evident

$$S = \frac{2r^2(dp - dm)}{9\eta}, \qquad (1)$$

where S = sedimentation coefficient in seconds; r = particle radius in centimetres; dp = density of the particle in g./ml.; dm = density of the medium in g./ml.; η = viscosity of the medium in poises.

In 0·25 M-sucrose the 200 mμ smooth vesicles of both densities will sediment faster than the rough vesicles, while sedimentation rates of the 50 mμ smooth vesicles overlap that of the 80 *s* RNP particle. In 0·88 M-sucrose the rates for the higher density smooth vesicles overlap those of the rough vesicles. In this medium the lower density smooth vesicles float. Over a range of size and density combinations the sedimentation rate of the smooth vesicles will be identical to that of the 80 *s* particle.

This predicted behaviour corresponds with the actual composition of sediments obtained in 0·88 M-sucrose (Palade & Siekevitz, 1956*a*).

The pellet obtained after 60 min. centrifugation at 105000g exhibits a preponderance of rough vesicles contaminated with smooth vesicles and free RNP particles. When the supernatant is centrifuged again for 180 min. at 105000g the second sediment contains smooth vesicles and free RNP particles with a scattering of rough vesicles. Upon examination of the 180 min. supernatant, both smooth vesicles and RNP particles are found and it is concluded that 'nothing was gained towards separation of small particles by using conventional centrifugation techniques.'

No comparable study has been made of microsomal fractions isolated in the more commonly used 0·25 M-sucrose medium. But Kuff & Ziegel (1960) conclude from analysis of ultracentrifuge patterns found with microsomal material from Novikoff hepatoma extracts, that both membranous material and free particles are present in material sedimented from 0·25 M-sucrose after 17 min. at a calculated centrifugal force ($g_{av.}$) of 105000. While the absence of some rotor dimensions in the report by Moulé *et al.* (1960) makes reconstruction of their experiments difficult, their observations appear to be in accord with these findings.

It can be concluded that differential centrifugation in the suspending media commonly used will separate neither the smooth from the rough vesicles, nor the membranous materials from the free RNP particles. This conclusion is based on quite different considerations from the observation of de Duve & Berthet (1954) that a complete separation of materials with different sedimentation rates can not be achieved in a single differential sedimentation since materials originating at the bottom of the centrifuge tube travel a shorter path. Unlike that noted by de Duve & Berthet, this limitation, due to the overlapping of sedimentation rates, cannot be circumvented by repeated resuspension and centrifugation, nor by sedimentation from a thin layer of suspension at the top of the tube (see Thomson & Mikuta, 1954).

(*b*) *Density gradient centrifugation*

The overlapping of sedimentation rates shown by the two vesicle populations reflects their extreme size range. It is only with respect to their density that the smooth and rough vesicles can be completely differentiated. Hence attention has been directed to equilibrium centrifugation in density gradients as a means of separating particle populations according to their density alone (Brakke, 1951; Holter, 1952). The use of aqueous density gradients in cell fractionation has not, however, been restricted to equilibrium centrifugation. Stabilizing gradients have been used as adjuncts to differential centrifugation (Anderson, 1955, 1956), either with the particle mixture suspended in

the gradient at the start of centrifugation (Kuff *et al.* 1956), or placed at one end of the gradient, usually the top (Thomson & Mikuta, 1954). Although an improved separation may result from the use of the gradient in these cases, it is a separation based on sedimentation rate rather than on density. Even where an equilibrium separation is attempted, failure to reach equilibrium will leave some or all of the migrating particulates in positions determined by their sedimentation rates. Since achievement of equilibrium may be difficult to prove, actual cases of the separation of microsomal constituents according to their density alone may be rare.

There are, however, a number of special cases where density separations may be achieved without attaining equilibrium conditions. One of these occurs where two density populations are to be separated in a two-layer discontinuous gradient whose discontinuity lies intermediate between their specific gravities. If the mixture is suspended in the upper layer, and the density of the lower layer is made great enough to float the lighter population, complete sedimentation of the heavier is not necessary, provided the sedimentation boundary of the heavier species clears the interface sufficiently to permit sampling. Petermann (1954) has applied this method to the separation of the dense RNP particles from the vesicular elements of the microsomal fraction. A microsomal suspension in 0·88 M-sucrose was layered over 2·05 M-sucrose and centrifuged for 20 hr. at 142000 g. Since a complete account of this interesting experiment does not seem to have been published, evaluation of its success is difficult. Where gradients of any sort are used it is particularly important that a precise description of the centrifugal conditions should be reported. The growing practice of reporting merely a calculated centrifugal force ($g_{av.}$), and the time of centrifugation is quite inadequate. Assuming that the reported $g_{av.}$ is correct (this is usually calculated for the mid-point in the tube, regardless of the actual volume of suspension and resulting position of the miniscus), it is often difficult or impossible to relate it to the forces operative in the critical parts of the gradient. Where the tube is not completely filled, the manufacturers' statement of $g_{av.}$ and minimum centrifugal radius ($R_{min.}$) does not apply, and considerable adjustment of centrifugation times may be necessary, even where no gradient is used. It would seem better to adopt some variant of de Duve & Berthet's (1954) proposal that the actual $R_{max.}$ and $R_{min.}$ be given together with an appropriate statement of centrifugal field and time. When $R_{max.}$ and $R_{min.}$ are available, the simple statement of angular velocity (usually given in r.p.m.) will adequately define the field occurring when constant speed has been attained. The time from beginning of acceleration to beginning of deceleration should suffice where the time of acceleration approximates

that of deceleration, and both together are a small fraction of the total. Where a standard rotor is used, and no confusion is possible concerning the measurements of the rotor and tube, specification of rotor and a volumetric description of the homogeneous suspension or gradient system may suffice. (The usual Spinco rotors meet this requirement, while the International rotors do not, since a large variety of inserts and tubes have been used.) The statement of conditions of centrifugation in terms of relative centrifugal forces (g or $g_{av.}$) or of an integrated term for centrifugal field and time (g-min.) may be useful in discussion of results, but does not provide adequate information when used in description of experiments. Further, it might be noted that the viscosities of concentrated sucrose solutions exhibit large temperature coefficients in the vicinity of 0°. Sedimentation rates may vary by as much as 5% for a difference in temperature of 1°. Hence an exact statement of rotor temperature before and after centrifugation may also be appropriate.

When the procedure of Petermann (1954) was repeated by Littlefield, Keller, Gross & Zamecnik (1955), the 2 M-sucrose layer was found to contain over 50% of the microsomal RNA. The RNA accounted, however, for only 20% of the material in the layer, a rather low value for a preparation containing only RNP particles. A possible explanation is suggested by the short time (90 min.) of centrifugation when only about 50% of the RNP particles could be expected to traverse the rather viscous 0·88 M-sucrose and enter the lower layer. The remaining RNA found in the layer could well have been provided by rough membranes, since the incomplete sedimentation of the RNP particles would necessitate sampling the lower layer very close to the interface. If the animals were not fasted before removal of the livers, the RNP particles may have been further contaminated with glycogen (see Luck, 1961).

Kuff & Ziegel (1960), on the other hand, using 20 hr. of centrifugation, found about 75% of the microsomal RNA in the 2·0 M-sucrose layer. While Novikoff hepatoma was chosen on account of its high ratio of free RNP particles to rough endoplasmic reticulum, as judged from electron micrographs, the higher yield of RNA may also reflect the longer times of centrifugation. It is interesting that these authors report, on the basis of electron microscopy, the presence of very few membranous elements in the 2·0 M-sucrose layer. This seems to suggest that rough vesicles of density greater than 1·27 are not abundant in the hepatoma extracts, since an 80 s RNP particle will sediment little faster than a 100 mμ vesicle with density of 1·28 in this medium.

By deliberately selecting short times of centrifugation, gradients of this kind have been applied to an interesting separation of liver