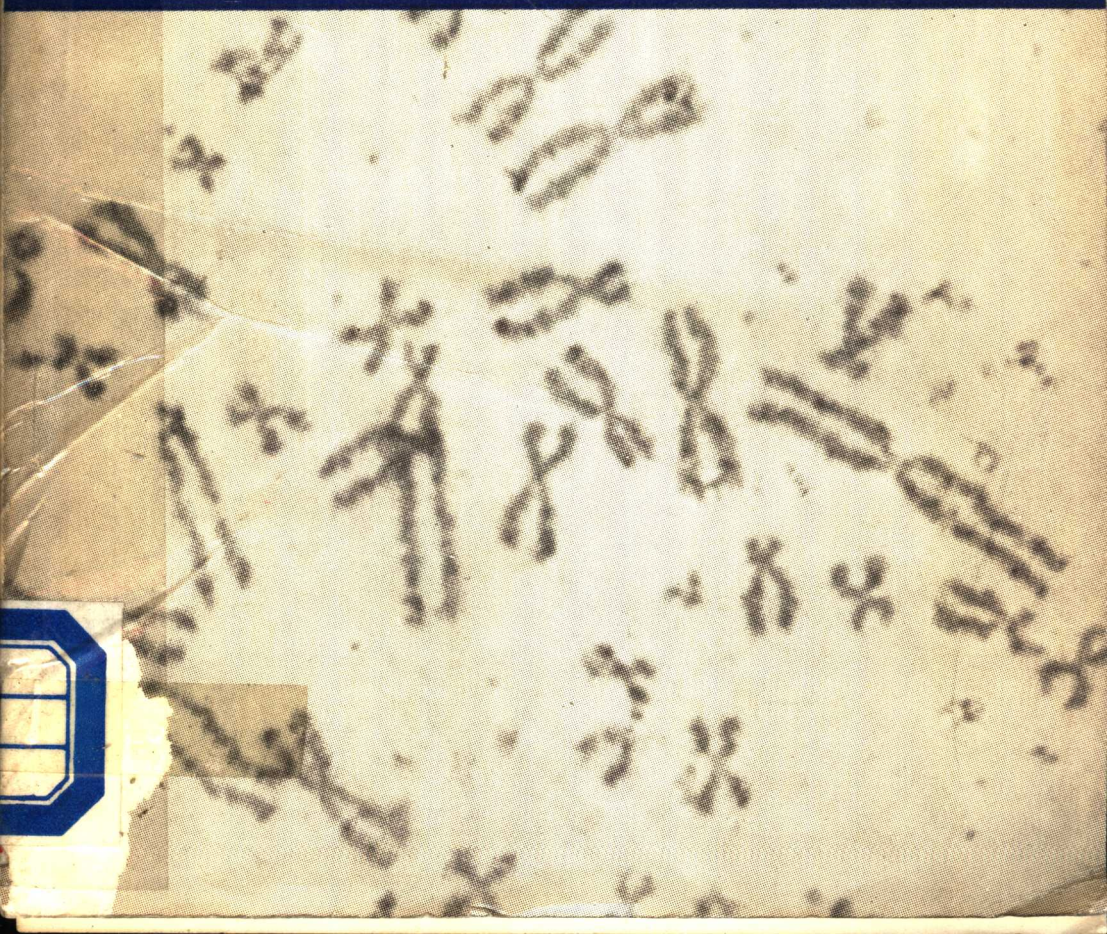
NEW TECHNIQUES IN BIOPHYSICS AND CELL BIOLOGY

Volume 1

Edited by R. H. Pain & B. J. Smith



New Techniques in Biophysics and Cell Biology

Volume 1

Edited by

R. H. Pain

Department of Biochemistry,
University of Newcastle-upon-Tyne

B. J. Smith

Cell Genetics Group,
Imperial Cancer Research Fund Laboratories, London

A Wiley–Interscience Publication

JOHN WILEY & SONS

LONDON · NEW YORK · SYDNEY · TORONTO

Library of Congress catalog card number 72-8611

ISBN 0 471 65757 3

Made and printed in Great Britain by
The Garden City Press Limited
Letchworth, Hertfordshire SG6 1JS

Preface

'Of making many books there is no end'—and the Preacher is undoubtedly preaching today to the converted. It came then, as something of a surprise to us to realize that there exists no collected volume of new techniques particularly suited to the needs of the young researcher entering the field of Molecular and Cell Biology. We were confirmed in this notion by the more than usual willingness of the authors to contribute to filling this gap. Established techniques are well documented. This series, however, is designed to give an account of new techniques which show every promise of being useful tools but most of which have, as yet, to become widely established. Molecular biology moves fast, so the aim has been to reduce publication time to the minimum.

Each technique is described in a way which should enable the research student and research worker to be able to assess its usefulness and applicability and sufficient theoretical and experimental background is provided so that he can quickly master the technique in practice. In addition, it is expected that the advanced undergraduate will find these chapters a useful introduction to the modern tools of the trade and thus broaden his concepts of modern biology as an experimental science.

We wish to thank the authors for their readiness to write and for their punctuality in providing manuscripts.

R. H. PAIN

B. J. SMITH

Contributors to volume 1

J. EDWARDS — *Department of Cell Biology, University of Glasgow, Glasgow, U.K.*

K. W. JONES — *Epigenetics Research Group, University of Edinburgh, U.K.*

N. G. MAROUDAS — *Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, U.K.*

RICHARD G. MILLER — *Department of Medical Biophysics, University of Toronto and The Ontario Cancer Institute, Toronto, Ontario, Canada.*

E. R. MORRIS — *Unilever Research, Colworth/Welwyn Laboratory Colworth House, Sharnbrook, Bedford, England, U.K.*

HOWARD R. MORRIS — *University Chemical Laboratory, Cambridge, U.K.*

P. L. PEARSON — *Department of Human Genetics, University of Leiden, Wassenaarseweg 62, Leiden, The Netherlands.*

G. R. SANDERSON — *Unilever Research, Colworth/Welwyn Laboratory Colworth House, Sharnbrook, Bedford, England, U.K.*

S. P. SPRAGG — *Department of Chemistry, The University of Birmingham, Birmingham B15 2TT, U.K.*

Contents

CHAPTER 1

Intercellular adhesion

John Edwards
Department of Cell Biology,
University of Glasgow,
Glasgow, U.K.

I. INTRODUCTION

A. A New Field for Molecular Biologists

During the development of vertebrate embryos, remarkable movements of cells and groups of cells take place, which require that cells alter their associations, apparently according to a detailed programme. These rearrangements, known as morphogenetic movements, lead to the segregation of the three primary structural layers of cells (the germ layers) and formation of tissues. (For a stimulating introduction to this subject, see Trinkaus, 1969.) Experimental studies, particularly those of Townes and Holtfreter (1955) with amphibian embryos, suggested that an important element in the control

of morphogenetic movements was intercellular adhesion, which was thought to be initially indiscriminate, but during development to acquire tissue specificity. It is now twenty-five years since Paul Weiss (1947) proposed that intercellular adhesion during development is controlled by 'lock and key' interactions between complementary molecules located at cell surfaces. During those years, there has been considerable progress in the identification and characterization of molecular species important in other respects in development (for example, the proteins of microtubules (Tilney, 1968) and microfilaments (Wessels *et al.*, 1970)), implicated in change and maintenance of cell shape, and in cell motility. But although numerous adhesive factors have been reported, and some are being actively investigated, the molecules controlling the adhesive properties of vertebrate cells (which Moscona (1968) has termed cell-ligands) remain almost as obscure as ever. Indeed, one theory of intercellular adhesion, the application to cells of the DLVO theory of stability of lyophobic colloids (Curtis, 1967), dispenses with them entirely, or at least accounts for adhesive phenomena in terms of bulk or averaged properties of cell-surface membranes.

Yet it seems likely that an understanding of intercellular adhesion in molecular terms will illuminate a variety of biological problems beyond that of morphogenetic movements which first gave rise to interest in the subject. For example, there are many cellular interactions which depend, or may depend, on formation of contacts between cells. Several of these, such as contact inhibition of movement (Abercrombie, 1970), 'topoinhibition' (Dulbecco, 1970), transfer of small molecules between cells (Furshpan and Potter, 1968), cell recognition such as precedes myoblast fusion (Okazaki and Holtzer, 1965) and contact effects on expression of differentiated properties of cells (Morris and Moscona, 1970), have been studied particularly with cells cultured outside the animal. It would not be surprising if some of these interactions share molecular apparatus with intercellular adhesion. Or, looked at another way, interactions between macromolecular components of the surfaces of contiguous cells may well result in adhesion of the cells, even if the mechanical linking of cells is only a secondary consequence of their prime function. The specializations observed at intercellular contacts by electron microscopy suggest there is likely to be diversity in adhesive mechanisms. The nature of the molecular architecture which gives rise to the distinctive appearance, for example of the desmosome (Fawcett, 1966), is at present unknown. Moreover, the adhesive specificities long postulated, and now demonstrated experimentally by Roth and Weston (1967), (see Figure 1.7), point to the existence of a largely unexplored molecular specificity of cell surfaces. One reason for active interest in intercellular adhesion is that abnormalities in adhesion or its specificity may contribute to malignancy. Perhaps it is rather optimistic to be addressing attention to problems such as these, when it is doubtful if anyone understands, in terms of the chemistry

of cell surfaces, why erythrocytes continue their independent existence, and do not change places (for example) with liver parenchymal cells.

However, increasingly detailed information is beginning to become available from biochemical studies, of the kinds of macromolecules present at cell surfaces which could potentially control intercellular adhesion, and confer specificity (of one sort or another) on intercellular contacts. A hypothesis has been proposed (by Roseman, 1970) which differs from most of its predecessors in the greater precision with which it identifies candidates for the adhesive role, and therefore in its vulnerability to experimental test. The fascinating possibility exists that the language into which the developmental programme of adhesive and other interactions between cell surfaces is translated, is the sequence of sugar residues of plasma-membrane glycoproteins and glycolipids. Tests of this and other hypotheses of intercellular adhesion require measurements of cell adhesiveness, which can perhaps also be thought of as assays for a function of surface-located macromolecules.

This article makes no attempt to review the many experimental approaches to this problem (for reviews, see Lilien, 1969; Curtis, 1973). Instead, it commends two advances in technique which have recently proved useful in the field, and which can reasonably be expected to be of value in future developments. These are respectively, the application of electronic counting to the measurement of cell-aggregation kinetics, and the collecting-aggregate method for investigating adhesive specificity. These share a common strategy which attempts to separate adhesion as far as possible from other sorts of cellular activity.

II. THE CELL-ENCOUNTER STRATEGY

A. Separation of Adhesion from Other Cellular Activities

A variety of embryonic tissues, for example from amphibia (Townes and Holtfreter, 1955) and the chick (Moscona and Moscona, 1952) can be dissociated to single viable cells by procedures such as depletion of divalent cations and subjection to proteolytic enzymes. When cultured under appropriate conditions, the cells are able to readhere, and become reorganized within the aggregates. Cells from different species, such as chick and mouse, when aggregated together, form chimerical aggregates (Moscona, 1957) in which they sort out by tissue of origin, but regardless of species. Reaggregation experiments such as these were considered to lend weight to the idea that there may be tissue-specific mechanisms of intercellular adhesion. A curious feature of these experiments is that they seem to ask of the cells a feat of self-recognition which not all are called upon to perform *in vivo*, since embryos are not normally constructed from masses of randomized

cells. Nevertheless, there is evidence that histogenetic reaggregation can reflect cellular activities which are important in development. For example, De Long and Sidman (1970) showed that cells dissociated at the appropriate developmental stage from embryonic brain tissues of the mouse become reorganized in reaggregates. Cells dissociated from brains of mice homozygous for the mutation 'reeler', which affects brain development, show abnormalities of patterning in such reaggregates. It detracts in no way from the importance of such experiments to emphasize that the process of histogenetic reaggregation clearly requires a multiplicity of cellular activities, besides adhesion (particularly resynthesis of surface components altered by the disaggregation procedure, cell motility within the aggregates and changes in cell shape). Indeed it is doubtful if a simple preference by the cells to form like–like adhesions will account for the patterning (as opposed to segregation) of cells in reaggregates (Steinberg, 1970). This complexity opens the door to diverse explanations of the phenomenon, some of which dispense entirely with the notion of tissue-specific adhesion. An example is Steinberg's explanation in terms of quantitative but not qualitative differences in adhesiveness (Steinberg, 1963). For similar reasons, these systems are not very suitable for attempts to identify molecular species responsible for adhesion. It seems inevitable therefore, that attempts be made to separate adhesion experimentally from other cellular activities. Several approaches to this problem share a common strategy: encounters are staged between cells, and the probability that encounters result in adhesion is considered a measure of intercellular adhesiveness (Curtis and Greaves, 1967). It needs to be stressed that it is not immediately relevant whether large or structured aggregates are formed, a point made by Curtis and Greaves when they discussed one form of this strategy, the measurement of kinetics of cell aggregation, which they saw to be probably homologous with flocculation in inanimate colloid systems.

Now it is as well to admit at the outset that the aggregation of cells dispersed from tissues is generally very remote from anything that occurs *in vivo*, so there are no *a priori* grounds for supposing it will yield useful information about the mechanism of adhesion and its control *in vivo*. In order that cell encounters shall be independent of cell locomotion, the cells have to be brought into collision by agitating a suspension of them in a fluid medium. The large-scale (though not necessarily molecular) organization of the surfaces of such cells must surely be very different from that of cells in a tissue, or extended on a substrate. For example, as Follett and Goldman (1970) have shown for BHK 21 cells, the excess surface generated by the shape change when the cells are suspended, is accommodated in numerous microvilli. Moreover, since the lamellipodia (Abercrombie, Heaysman and Pegrum, 1970) of fibroblasts and epithelial cells are the front of locomoting cells and cell sheets, they may have special properties with regard to the

initiation of intercellular adhesions. The fate of such specialized surface regions when cells are suspended is obscure.

Another problem is that for experiments to be possible, cells must first be separated from one another, by procedures such as depletion of divalent cations, the use of proteolytic enzymes or some combination of these. Since these procedures are designed to separate cells, their effect (as pointed out by Lilien, 1968) is very likely to alter precisely those components of cell surfaces which it is proposed to investigate. (One system escapes this particular objection. As a result of their natural history, although destined to form a tissue, the cellular slime moulds present themselves to the experimenter as a population of discrete cells (Gerisch, 1968). These organisms, together with the sponges (Humphreys, 1967), have long been considered useful models for the study of intercellular adhesion in higher animals.)

In view of the high degree of artificiality inherent in such experiments, external evidence is needed that the ability of cells to adhere to each other when shaken in suspension genuinely reflects their adhesive properties in some developmentally important context. In other words, that it serves as an assay for surface macromolecules responsible for adhesion *in vivo*. Such evidence seems to exist least equivocally in the studies by Gerisch (1961 and 1968) of adhesion in the cellular slime moulds. These cells acquire the ability to adhere in suspension at the same stage in development at which encounters between them occasioned by their own locomotion on a surface begin to result in formation of stable contacts. A series of mutants exists which are deficient in adhesion. A second important point emerges from this example, namely that it is only under certain restrictive conditions (the presence of EDTA) that the suspension assay is specific for the developmentally relevant adhesive mechanism. Equivalent genetic tools are entirely lacking for studies of adhesion of vertebrate cells, though it is of interest that a lethal mutation in the chick, *talpid*[3], which causes abnormal limb morphogenesis, gives rise to wing-bud mesenchyme cells which aggregate faster than wild-type cells in the first hour from dissociation (Ede and Agerbak, 1968). There are nevertheless some indications that the outcome of encounters between vertebrate cells in suspension can depend on interactions of potential biological interest. By far the best evidence is for the collecting-aggregate technique (see Section IV) where formation of adhesions can be shown to be tissue specific, and sensitive to modification of the cell surface by a specific glycosidase. For experiments which measure only kinetics of aggregation of one cell type, it has been possible to demonstrate some specificity for various factors promoting aggregation (Lilien, 1968; Orr and Roseman, 1969b; Oppenheimer and Humphreys, 1971) and a marked effect of viral transformation of cells (Edwards, Campbell and Williams, 1971).

If we accept that in experiments such as these, measurements of the rate and extent of formation of intercellular adhesions could, at least in principle,

provide an assay system for molecular species responsible for adhesion *in vivo*, then it becomes important to distinguish between assay for the presence of such species, and assay for their synthesis by the suspended cells.

B. Dependence of Adhesion on Metabolism

If adhesive components are present on the surfaces of freshly dispersed cells (because they survived, at least in part, the dispersal procedure, or because this procedure depended only on immediately reversible effects) then the rate of formation of adhesions should depend on the rate of encounter of the cells and the amount and distribution of the adhesive components only. The situation then should resemble somewhat the assay of an agglutinin, and there is no obvious reason why the rate of formation of adhesions should then depend on biosynthetic processes—it should be insensitive to both specific inhibitors such as cycloheximide, and general inhibitors of ATP generation, such as dinitrophenol and azide. Insensitivity to dinitrophenol was shown by Gerisch for the EDTA-insensitive, stage-specific 'agglomeration' of slime-mould amoebae (referred to in Section II.A). The analogy with agglutination serves to emphasize two aspects. First, that the distribution of adhesive components on cell surfaces may be important in determining whether or not adhesions are formed. This is suggested by the finding by Nicolson (1971) that agglutination of 3T3 cells by the plant lectin concanavalin A probably depends on the grouping on the cell surface, and not simply on the number, of accessible sites for lectin attachment. Secondly, independence of metabolism does not necessarily mean that formation of adhesions will not be inhibited by low temperature, since agglutination of 3T3 cells by concanavalin A is very strongly temperature dependent (Inbar, Ben-Bassatt and Sachs, 1971). One wonders if these aspects may not be related, low temperature inhibiting the grouping of dispersed adhesive components on cell surfaces, by inhibiting their diffusion (Frye and Edidin, 1970). Just as agglutination requires both the exogenous lectin and components of cell surfaces, it seems likely there will be multiple components involved in intercellular adhesion—a 'lock and key' interaction can scarcely depend on less than two. It ceases to be mere semantics whether such components function 'between cells' as seems to be envisaged by Moscona in using the term 'cell-ligands', or whether they are integral parts of cell surfaces. The answer may well prove to be different for different kinds of adhesion. In the latter case, it will presumably be much more difficult to obtain soluble preparations of adhesion-promoting factors. It may be more profitable to look for fragments which act as competitive inhibitors of the formation of adhesions. Jamieson, Urban and Barber (1971) reported that glycopeptides from collagen act in this way on the adhesion of platelets to collagen. Another parallel is the inhibition by glycopeptides of the binding

of various desialylated serum proteins to the surface of liver parenchymal cells (Pricer and Ashwell, 1971).

A different situation is where the rate of formation of adhesions is controlled by the rate of synthesis by the aggregating cells of some surface component. Under these conditions, the rate will be sensitive to both specific and general inhibitors of cellular metabolism. The common finding that aggregation of cells which have been separated by proteolytic enzymes is inhibited by inhibitors of protein synthesis is perhaps not very informative. Of greater interest is the example of the aggregation of trypsinized cells of the mouse teratoma, described by Oppenheimer, Edidin, Orr and Roseman (1969), which requires L-glutamine to act as a source of amino groups of hexosamines (see Section III.C). A distinction, referred to by Lilien (1969), which is difficult to make experimentally, is whether the metabolic requirement in systems such as this, is purely to make good the inactivation or removal of surface components by the dispersal procedure, or whether it results from their metabolic turnover by the cells. In either case, it needs to be stressed that if the formation of adhesions is dependent on cellular metabolism in a particular experimental system, then this represents an incomplete separation between different cellular activities. Several difficulties of interpretation arise as a result. It cannot be ruled out that there is regulation of the synthesis of cell-surface components, such that restriction in the supply of one may switch off synthesis of the others. For example, in the glutamine-dependent aggregation of teratoma cells referred to above, it is possible to argue that although hexosamines are indeed required for cell-surface renewal, they do not themselves form part of the actual adhesive species. Further, the requirements for synthesis of the components can obscure the requirements for their function. For example, metabolic-dependent aggregation of BHK 21 cells (Vicker, Campbell and Edwards, 1973) requires divalent cations. This is not surprising, since they are presumably required for a variety of intracellular reactions supporting the biosynthesis of components of cell surfaces, and probably also to maintain impermeability of surface membranes. The question remains whether the functioning of adhesive components on the external surface (once synthesized) requires divalent cations. The fact that metabolism-independent aggregation is insensitive to EDTA suggests it probably does not.

It seems likely that the aggregation of many types of cells shortly after dissociation, will, in common with BHK cells, show a mixture of both kinds of rate determination. At early times, adhesion depends on surface components which have survived the dispersal procedure. Later, continued aggregation depends on synthesis of more of the same (or conceivably different) species and therefore becomes increasingly sensitive to metabolic inhibitors, as preexisting adhesive components are sequestered in cell contacts.

C. Arrangements for Bringing About Collisions

Early experiments with reaggregating cells, such as those of Wilson (1907) with sponges, required active cellular motility to bring about encounters between cells. In order to avoid this (and incidentally, also the complication of chemotaxis) Gerisch (1961) introduced the use of roller cultures for bringing about the agglomeration of slime-mould amoebae. A variety of other methods of generating velocity gradients, and hence collisions in cell suspensions has since been adopted. Moscona (1961) introduced the use of shakers which moved the culture flasks in a circular path. This gyratory action has been much the most widely used. Where light scattering is used to follow the progress of aggregation (see Section III.A), the need to avoid a moving culture vessel favours the use of a magnetic stirrer within the cell suspension. In our own work with BHK 21 cells (Edwards and Campbell, 1971) we have used a linear shaking action (reciprocating or oscillatory shaking) in the hope of avoiding some of the complications introduced by the centrifugal segregation by size of cells and clusters which occurs in gyrated flasks. Roth and Weston (1967) compared the effectiveness of gyratory and oscillating actions for the collection of cells by preformed aggregates (see Section III.D and Figure 1.7). With gyratory action, rather surprisingly, the rate of collection of cells by aggregates was inversely related to aggregate size, whereas in oscillated flasks, the more easily understood relation held, i.e. larger aggregates collected cells faster. This difference the authors attributed to zoning of cells and clusters by size in the gyrated flasks, with single cells concentrated at the centre and aggregates orbiting farther out, the larger their size. Another possible difference between the two modes of shaking is that there could be rather high transient velocity gradients in reciprocally shaken flasks, resulting from the sharp changes of velocity at each reversal of direction. Curtis (1969) has pointed out that in all these systems, rates of formation of adhesions can be affected by circumstances which have no bearing on cell adhesiveness. This is because collision rates (and indeed, adhesive probabilities) depend on the distribution of velocity gradients in the agitated fluid. This in its turn will depend not only on the rate and pattern of movement of the culture flask, but also on the viscosity of the medium. Thus quite low concentrations of substances with high intrinsic viscosities, such as hyaluronic acid, DNA or methyl cellulose may be expected to slow aggregation considerably, yet at longer times lead to the stabilization of larger aggregates. To avoid these problems, Curtis (1969) introduced the use of the Couette viscometer, which can generate known, uniform, velocity gradients, independently of the viscosity of the medium. This technique is not without its own difficulties, however. The nature of the equipment limits the number of suspensions which can be worked with at one time, and for some cell types, problems of settling occur.

III. AGGREGATION KINETICS AS A MEASURE OF ADHESION

A. Assessment of Aggregation

If collisions between cells result in adhesions, cells in a shaken suspension aggregate. The progress curve of aggregation provides an operational definition of adhesiveness (Curtis and Greaves, 1967), though it has to be borne in mind that extraneous factors, such as change in size of cells, or viscosity of media can also affect the time course. During aggregation, the number of single cells declines while that of the largest aggregates increases, but there are intermediate sizes of which the numbers rise at first and then fall. A complete description of this process would require knowledge of the progress curves for all sizes of aggregates, and in principle, this could be obtained. In practice, however, it is a good deal simpler to choose some single index of the shifting size distribution, the choice being partly dictated by the available methods of measurement. Now the ideal method of measuring aggregation would be continuous. (The advantages of continuous assays over those requiring periodic sampling will be familiar to anyone who has measured enzymic reactions.) The measurement of light scattering by aggregating suspensions provides such a continuous method, which works well for platelets (Born, 1962) and has been applied to slime-mould amoebae (Born and Garrod, 1968) and to embryonic chick cells (Jones and Morrison, 1969). In all these instances, changes in light scattering have been detected as changes in light transmission by the cell suspensions. In the last example, some disadvantages are apparent—first, that the changes in transmission due to aggregation are rather small; second, and more serious, the sense of the response appears to be discontinuous. The initial formation of small clusters results in a decrease in transmission but further progress in aggregation causes an increase.

The alternative is to resort to sampling the suspension and counting selected aspects of the distribution of cells among aggregates of various sizes. This can be done by placing the sample in a calibrated blood-counting chamber, observing the cells and aggregates by phase microscopy, and counting them one by one. Such counting methods are not entirely free of subjectivity, although this can be eliminated by coding samples. The main disadvantage is sheer laboriousness, since the precision of a count is limited by the number of cells or aggregates counted, the statistics of the situation resembling those of a measurement of radioactivity. Since the problem is one of counting large numbers, automation in the form of an electronic sensing-zone counter, such as the Coulter counter, provides the obvious solution.

B. Application of Electronic Counting to Measurement of Cell Aggregation

Electronic particle counters have been used extensively for a number of years in investigations of the state of dispersal of liquid droplets and solid particles. Early applications to cell aggregation were to follow the immune agglutination of platelets (Halloran, Harrington, Minnich and Arimura, 1961) but the technique seems not to have been used in the field of cell adhesion until Ball (1966) reported its use to measure the aggregation of embryonic chick cells.

The principle on which the Coulter counter works has been amply described elsewhere (Mattern, Brackett and Olson, 1957). Briefly, particles to be counted, suspended in a suitable electrolyte, are drawn by a pressure difference through an aperture. Passage of a single particle through a zone which includes the aperture itself and a small space on either side (the sensing zone) increases the electrical resistance between electrodes placed on either side of the hole. This gives rise to a voltage pulse (proportional to the volume of the particle) which is amplified and counted if it is within preselected size limits. The applicability of this principle to measurements of cell aggregation depends on the response of the instrument to cell clusters or aggregates of various sizes. The possibilities can be illustrated by considering the response to a cell pair. Ideally, when two cells which have formed an adhesion during aggregation pass through the aperture, the instrument should register a single pulse, of twice the size of that from a single cell. If, on the other hand, the pair are separated on their way through the sensing zone, two unit pulses could result, and the technique would then be useless. Another possibility, which would still detect the adhesion, is that the second cell, though separated, could pass through within a dead time created by the passage of the first, leading to a single unit pulse. Occurrences such as this would lead to an apparent reduction in total volume of cells present as aggregation proceeds. It has been found quite generally that a reduction in count occurs when cells of various kinds aggregate (whether the count is of pulses corresponding in size to single cells, or is of cells and clusters of all sizes). This shows that the technique does indeed register aggregation. In the case of chick embryonic neural retina cells aggregated at low temperature in the presence of a factor isolated from horse serum, Orr and Roseman (1969b) were able to show that the measured volume of cells present remained constant, so that counts lost from the size class of single cells were adequately accounted for by increased counts at larger sizes. This still does not prove that there is no degradation of the spectrum of aggregate sizes as a consequence of shearing in the instrument. In our own work with BHK 21 cells (Edwards and Campbell, 1971), we were able to establish that the decrease in total number of pulses when cells aggregate corresponds to the reduction in number of particles (i.e. single cells and clusters of various sizes, each

counted as one) found by microscopic counting (Figure 1.1). This shows that most clusters give rise to single pulses, but not whether these pulses are of the full expected height. Moreover it does not show that some of the larger clusters do not give rise to multiple pulses, since the count is dominated by the more numerous smaller ones. We investigated this further by fractionating aggregated suspensions on Ficoll density gradients, to obtain fractions enriched in large clusters. This showed clearly that as the sizes of clusters

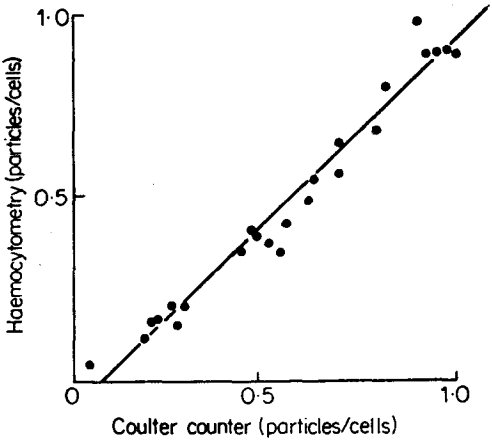


Figure 1.1. Response of the Coulter counter to aggregated suspensions. The extent of aggregation of a series of cell suspensions was determined both by haemocytometry and electronic counting. The more highly aggregated suspensions (7 points at low values) were obtained by aggregating suspension-grown cells in growth medium; the remainder were suspensions of trypsinized cells aggregated in Hanks solution. The units are the number of particles (cells plus aggregates) divided by the total number of cells. [Reproduced, by permission of the copyright holders, from Edwards and Campbell, *J. Cell. Sci.*, **8**, 53–71 (1971)]

of these cells increased, so did the heights of pulses obtained from them with the Coulter counter. Since each fraction still contained a distribution of cluster sizes, we could not show simply that the pulse heights were proportional to the number of cells per cluster. However, the pulse heights bisecting the spectrum of each fraction did increase roughly in proportion with the mean number of cells per cluster determined by microscopic counting