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Cell Growth and Division

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General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

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Readers' comments will be welcomed by the Education Officer of the Institute.

1982

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Preface

The processes of cell growth and division are of fundamental importance to biology, yet our understanding of them is almost as superficial as it was nearly one hundred years ago when E. B. Wilson first published his magnificent work *The Cell in Development and Heredity* (1896 Macmillan, New York), a book worth consulting today. Many simple questions can be asked – how does a cell 'know' when to divide, and how does it manage to do so into two daughters of the same size? But simple questions do not necessarily have simple answers, as any cell biologist knows.

The intention of this small book is not only to deal with some of the straightforward detail about cell growth and division, but to give some idea as to how some of the problems are being tackled. Intuitively one feels that a subject has been 'grasped' when intelligent questions can be asked about it. This is probably the best attitude to adopt here, as in approaching any scientific problem.

Studies in Biology No. 21 on *Cell Division and Heredity* deals with the segregation of genes to progeny; it did not deal with processes of preparation for division, nor with mitosis. Thus there is no overlap and the two books are entirely complementary.

Aberdeen, 1982

D. N. W.

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1 Introduction

1.1 Unity in diversity

There is an enormous amount of diversity in nature, and much diversity in the cells which make it up. To understand how cells grow and divide, scientists have had to select representatives from many of the major groups of living organisms, some for superficial study, others for in-depth analysis. The latter group includes such celebrated species as the bacterium *Escherichia coli*, the ciliated protozoan known as *Tetrahymena pyriformis*, a division yeast called *Schizosaccharomyces pombe*, *Allium* (onion) meristem cells, a fungus named *Physarum polycephalum*, sea urchin eggs and mouse fibroblasts (e.g. 3T3 cells). In a short book only a glimpse can be given at what these organisms alone have taught us, for each exemplifies some aspect of cell growth and division. We must therefore look for underlying principles. There is no sound theory of cell proliferation, and formulation of one would rely heavily on common features found in comparisons between many different types of cell. But is there any reason to suppose that common principles are at work among diverse organisms?

Broadly speaking, cells have a far greater similarity than diversity when one looks at their organelles or some of their proteins and nucleic acids. It is largely the way these macromolecules are assembled which accounts for the outward diversity of form. At the molecular level, the cytoplasm of onion root cells contains materials which are difficult to distinguish from those found in sea urchin eggs. When life emerged on this planet, it was fashioned by the chemistry of carbon, along with nitrogen, oxygen, hydrogen and a handful of other elements in smaller quantities. Earth scientists recognize four universal attributes of living things: proteins, nucleic acids, cells and membranes. In physiological terms, they recognize three ubiquitous functions: cell growth, cell division and self-replication. However much these arbitrary criteria may be criticised by biologists, it would be difficult to deny that cell growth and division are 'primitive' in nature. The greater the antiquity, the more probable it is that a common mechanism is involved. Evolution has simply produced variations upon this theme.

The process of cell division is intimately concerned with the segregation of chromosomes, the storage modules for the genetic information, which must be passed on from generation to generation. In *Studies in Biology* No 21, Dr Kemp dealt almost exclusively with the importance and strict requirements for the mixing and segregation of genes at division, as seen by the geneticist at meiosis. There is a ring of familiarity about the notion that the cell is the genes' mechanism of perpetuating themselves (DAWKINS, 1978). The reverse would be to argue that the genes are the cell's means of perpetuating itself. Whether we

approach the problem from the viewpoint of the gene or the cell, the process of cell division involves segregating both the cytoplasm and the nuclear contents.

The preparations made by a cell for division should not be underestimated. In general, when cells of a metazoan animal are busy doing one job, e.g. making mucus or sending electrical impulses, they cannot be dividing – this is often called the 'division of labour'. When cells divide, they have to put nearly all their effort and substance into it, leaving little for other specialized functions. In cells which are incessantly dividing, there is virtually no trace of any differentiated function; they have been called undifferentiated or dedifferentiated cells, probably because the process of cell division is so basic that it is rarely thought of as a differentiated state itself.

In cells which are continuously dividing, growth occurs between successive mitoses in a period called interphase. It represents a period almost entirely devoted to active preparation for the next division. An incorrect impression about proliferating cells is that preparations for division occur in the last few minutes before an impending division. The well-prepared cell goes into division in a co-ordinated manner and each of the offspring to emerge should have roughly (but not necessarily exactly) the same amount of inheritance and an equal chance of survival. If the preparations for division are disturbed, all kinds of aberrations may occur at division leading to grossly abnormal or non-viable offspring.

Daniel Mazia, one of the foremost exponents on cell division, considers the problem of ensuring the accurate division of the cell as one of the most fundamental of biological problems (DIRKSEN *et al.*, 1978). The discovery of how a cell divides remains an enormous challenge to the scientist. And scientist is used here intentionally rather than biologist because those investigating the problem today include biophysicists, crystallographers, geneticists, organic chemists, molecular biologists and many others. It is a truly interdisciplinary area of research, and its discoveries have impact on all biological and medical sciences.

1.2 Growth and entropy

Some questions about cells have no easy answer, for example, why do cells grow? In speculating about the features of the earliest living organisms in evolution and knowing how 'primitive' organisms behave today, it seems clear that they must not only abide by the same laws of thermodynamics as everything else but that they have been limited by carbon (organic) chemistry. In a system in which polymerization is a major feature, molecular randomness is lost, i.e. entropy decreases as more orderly structures evolve. Under these conditions, rapid development and evolution must have continued because the opposite would otherwise have occurred and any developing system would therefore have reverted to complete chaos (maximum entropy). There is no option for a system with decreasing entropy but to evolve. With the passage of time, those systems which were best suited to continuing to reduce the entropy persisted while other systems (species) disappeared. The adaptability of a protein-based life form

combined with mutable nucleic acids saw the evolution of many regulatory controls which have allowed cells to grow and divide, and to colonise many extreme and bizarre niches of this planet.

1.3 Self-assembly

One important concept which needs to be fully appreciated is that cells grow by a self-assembly process. Unlike a sweater or a house, the cell has first to obtain its raw material (nutrients), synthesize its own building units (macromolecules), and then construct itself from the inside out without any reference to some pattern or external plan. There is no schedule at hand to consult as to when events should occur in relation to one another, and there is no timekeeper or quantity surveyor to give the go ahead for division. The cell does not have a balance with which to ensure that its daughters will be the same size at division. Too often we take all these processes for granted because of our familiarity with growth and division, but it is important to contemplate on how they all take place. It has often been presumed that the cell is programmed, i.e. acts in a deterministic manner at the molecular level to make the right things happen within the cell. But there is nothing magical about the behaviour of the molecules within a cell. Growth is achieved through a remarkable process of self-assembly steps within an otherwise random system. This may be difficult to understand but a simple example will serve to show how assembly and regulation may be achieved in the growth of a cell without external control. Imagine that a cell grows cytoplasmic processes just 6 μm in length. What regulates this growth so that it stops at 6 μm? In Fig. 1–1 a possible mechanism is given in molecular terms based on a very simple principle, in which the subunits have only one way of linking together and need no external regulation to ensure accurate length. This is, of course, hypothetical but similar processes will be met in Chapter 4.

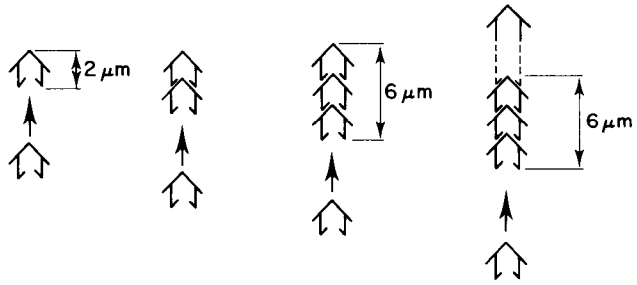

Fig. 1–1 Growth to a constant length of 6 microns. As a fourth subunit joins the chain, the bonds of the first are weakened and the subunit is lost.

1.4 The idealized cell

There are two main classes of organism occurring in nature, *prokaryotes* and *eukaryotes*. Prokaryotic cells do not have their chromosomal material enclosed within a membrane-bound nucleus whereas eukaryotic cells do. Not a great deal of difference is found in their growth processes but division is different, with most eukaryotic cells losing their nuclear membranes for a short period during chromosome segregation. Fig. 1–2 shows a cell of each type in an idealized simplified diagrammatic form. Both have cell coats, cell membranes, cytoplasm containing ribosomes and many other structures.

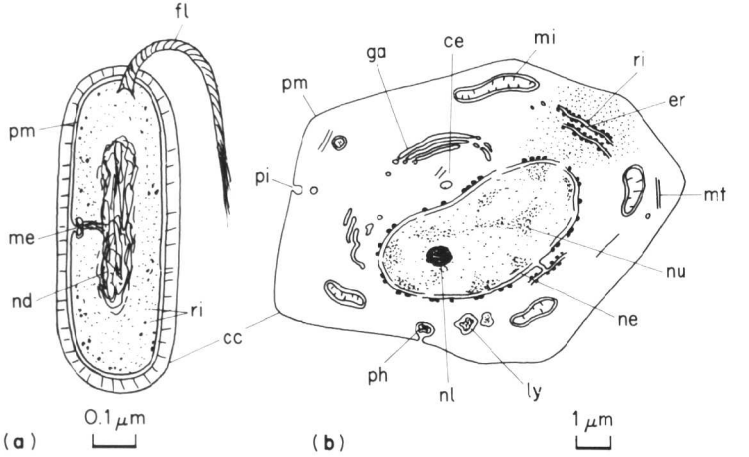

Fig. 1–2 Diagram of a rod-shaped, prokaryotic bacterium (**a**) and an eukaryotic animal cell (**b**). cc, cell coat; ce, centrioles in cell centre; er, endoplasmic reticulum; fl, flagellum; ga, Golgi apparatus; ly, lysosomes; me, mesosome; mi, mitochondrion; mt, microtubule; nd, nucleoid; nl, nucleolus; nm, nuclear membrane; nu, nucleus; ph, phagocytic vacuole; pi, pinocytotic vacuole; pm, plasma membrane; ri, plasma membrane.

Most prokaryotes are *autotrophs*, organisms which can synthesize their food from simple carbon and nitrogen sources. Plant cells (eukaryotes) are also autotrophs whereas animal cells (eukaryotes) are not. The latter require more complex precursors or nutrients and are known as *heterotrophs*, being ultimately dependent upon autotrophs for organic precursors. All organisms are also dependent upon energy to assimilate materials into their biomass and this comes directly or indirectly from the sun. The efficient conversion of solar energy into chemical energy has allowed the successful colonization and adaptation of green plants throughout this planet.

1.5 The control of growth

There are three aspects to be looked at in the control of growth. The first concerns the intrinsic factors which influence the rate at which biomass increases according to the availability of a supply of food or raw material and also to the environmental conditions (Chapters 2 and 3). The second is the mechanism which initiates and co-ordinates division (Chapters 4 and 6). And the third deals with the control of cell numbers in the populations of cells which arise from division (Chapters 2 and 7). All three aspects must be carefully co-ordinated but the mechanisms by which this is achieved remain relatively obscure.

1.6 Degeneration and death

Although growth is concerned with the increase of units of biomass, it is not without its important counterpart, cell death. If a bacterium such as *E. coli* is dropped into a nutrient broth, it will multiply rapidly until it has exhausted all assimilable substances. Since life requires that a continuous supply of substrates for energy generation be available, the effect of uncontrolled proliferation in the organisms would be the cutting off of their own life-line. To avoid this consequence, cells usually slow down their growth when conditions become overcrowded and start to deteriorate. Bacteria often sporulate. This reduces to a

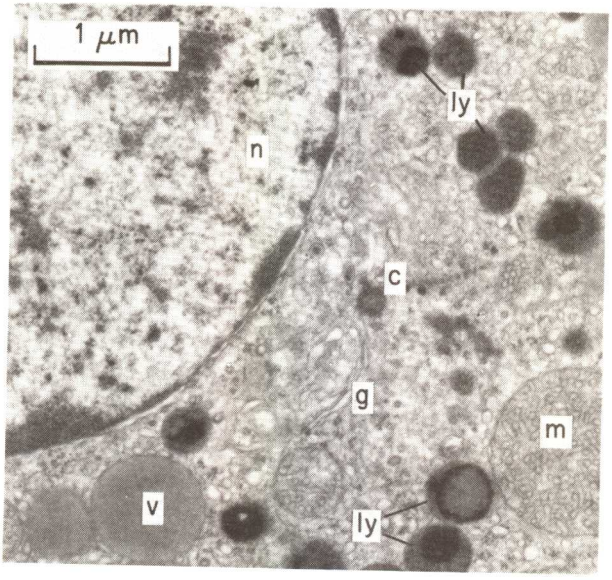

Fig. 1–3 Electron micrograph of an adrenal cell with many lysosomes (ly). Also seen are the nucleus (n), mitochondrion (m), lipid vacuole (v), Golgi apparatus (g) and a centriole (c).

minimum the amount of energy required to support life, at least for some of the cells which can survive until favourable conditions return. Many of the cells, however, will die off during the process. In all cultures this process of drop-out can be occurring at a much less significant level. If growth is slower, and drop-out is occurring, there will be a point at which the two processes balance – a 'steady state'. While our primary concern is with cell growth, much has to be learned about degradation, degeneration, atrophy and death in biological systems. These have been much less popular areas of research and our knowledge is only fragmentary. Sometimes, cell death can be seen as an active process, usually involving organelles called *lysosomes* (Figs. 1–2 & 1–3), which behave like suicide capsules in the cytoplasm. On other occasions, cells undergo a protracted involution by a process called apoptosis, which describes their being shed like falling leaves. The relationship of cell death to the regulation of cell populations will be discussed in section 7.7.

2 Cell Growth and Multiplication

2.1 Growth and cell size

2.1.1 General features

Cell size can increase in several ways, the most usual being by the synthesis and accumulation of new macromolecules and their attendant substances so that the consistency and specific gravity remain constant. Although this seems self-evident, increase can also occur by the accumulation of water. Absorption of water can be a regulated process up to a certain level but if it goes too far, the cell membrane becomes extended and blebbing occurs. Finally the cell bursts and its contents come spewing out (lysis). Oedematous change, as transient wateriness is called, is reversible in tissues which have been injured. Although cells in this condition obviously get larger, this can scarcely be called cell growth. For similar reasons, we might also consider that adipocytes (fat cells), which store large quantities of lipid in intracellular vacuoles, or plant cells, in which a large central vacuole filled with watery sap produces marked elongation, 'grow' in the broadest sense of the word. Clearly 'growth' requires qualification in different circumstances, and when used by biologists without further qualification it generally implies a *co-ordinated accumulation of cellular components with time*. It also implies a simultaneous and equivalent increase in functional capacity.

With the cell as the basic unit of growth, an increase in size usually leads to division and an increase in cell number; this relationship is one of the central issues in biology. Cells of a particular type have characteristic features by which they are recognized, one of which is size. This alone helps us distinguish one species of bacillus from another, or platelets from erythrocytes in the blood.

2.1.2 Cell weight and composition

Weight (mass) is an obvious means of measuring growth. Tissues or organisms weighed in their natural state are measured in grammes as a *wet weight*, often expressed in milligrammes (mg; 10^{-3}g), microgrammes (μg; 10^{-6}g), nanogrammes (ng; 10^{-9}g) or even picogrammes (pg; 10^{-12}g) because cells are very small. Evaporating off water gives the *dry weight*, which is usually expressed as an absolute weight relative to a known wet weight from which it was derived. Sometimes the dry weights of similar organs may be closer than their wet weights, which could simply reflect hydration differences. An example of this is the endometrium or lining of the uterus, which shows considerable changes in the degree of hydration of the cells during the menstrual cycle.

Dry weight can be further analysed by combustion or ashing of the material.

This drives off many of the more volatile products of elements such as carbon, hydrogen, oxygen and nitrogen of the macromolecules and leaves salts and oxides of other elements, e.g. K, Na, Ca, Zn, Cu, Mg, of which the rarer ones are called *trace elements* (e.g. Zn, Co) because of their exceptionally low levels in living materials. Nevertheless, they are vital to metabolism, and growth cannot occur in their absence. Ashing concentrates these substances, making assays more accurate.

Although it is easy to weigh a growing baby or plant, it is not so easy to weigh a cell; and it is also of dubious practical value. But the cell has been weighed by applying the Cartesian diver principle. The late Professor Zeuthen loaded individual amoebae on finely-drawn capillary tubes, giving them buoyancy in a column of water by the inclusion of a small bubble of air. By applying hydrostatic pressure, the diver could be kept at a definite mark. The difference in pressure required to keep the unloaded capillary at the calibration mark could be expressed as a weight difference down to 10 pg.

An alternative to the slow, technically-demanding diver method is to use *interference microscopy.*. This requires a special microscope designed to send one cone of light from the condenser through the specimen, where the macromolecules in cells refract the light to different degrees. The waves coming to the eye-piece are retarded to a greater or lesser extent compared with those of an outer cone of light not passing through the specimen. Interference occurs when the two beams of white light are re-converged and a distinctive coloration similar to the effect of petrol spread on water is produced. Using monochromatic light, it is possible to measure this interference since it is proportional to both the thickness of the object refracting the light and the density of proteins and other macromolecules which are causing this refraction within the specimen. The technique is very sensitive and can be applied at the subcellular level to measure organelles.

But without these specialized techniques, the easiest way of deriving an approximate cell weight is simply by weighing a large, known number of cells, and calculating an average value per cell.

Cells consist of about 70–75% water and 16–20% protein, with the remaining 7–10% including DNA, RNA, lipid, sugars, polysaccharides, vitamins, ions, trace elements, etc. The predominance of protein makes it a useful guide to overall biomass and is often used to monitor the growth characteristics of living organisms.

2.1.3 Analysis of macromolecular increase during growth

Chemical and biochemical assays Straightforward chemical and biochemical procedures can be used to study macromolecular changes during cell growth. The limitation here is sensitivity because many of the more traditional procedures are too insensitive for assays on small numbers of cells. In general a plentiful supply of material is required, and results are usually expressed as average amounts per unit weight or number of cells. There is complete loss of data at the subcellular level unless fractionation of cellular components is

carried out by *differential centrifugation*. But organelles in different fractions often acquire or lose materials during preparation and therefore estimates are not entirely reliable by this technique.

Standard procedures for protein include the celebrated Lowry test for protein which gives a blue coloration when first Cu^{2+} and then a molybdotungstate complex are reacted with aromatic residues in protein. Other similar tests such as the orcinol or diphenylamine reactions allow one to assay RNA and DNA respectively.

But much more sophisticated analytical procedures are now in use following extraction of the specific type of macromolecule of interest, e.g. lipid or polysaccharide. Using, for example, gas chromatography, ion exchange resins, automatic amino acid analysers, protein sequencers, and immunoassay procedures, characteristic groups or regions within major macromolecules can be clearly identified and usually quantified.

Enzymes are macromolecules which readily lend themselves to assay because they react with specific substrates. By formation of an identifiable product from a known concentration of substrate, reaction rates can be estimated. The rate of reaction under standard incubation conditions is, within limits, proportional to the amount of enzyme present. In fact, the estimate is only a rough guide to the number of enzyme molecules in an active state at the time of incubation. Unfortunately, biological material is usually homogenized before assay which means that a true value corresponding to the native enzymes in the original cells is unlikely to be obtained because many previously active molecules are denatured and inactivated. This can be avoided to a certain extent by carrying out the reaction *in situ* at the cellular level, as described below.

Single cell analysis This relies on extremely sensitive techniques for detecting small amounts of macromolecules, by exploiting some characteristic grouping with a unique absorption spectrum, by attaching a fluorescent substance ('dye') to them, or by some similar procedure. Coupled with a scanner, the *fluorescence microscope* and the *integrating microdensitometer* can, for example, measure emitted light of different wavelengths from fluorescent labels which tag on to certain molecules, thereby quantifying the amount per cell. Obviously the best results are obtained when the tag titrates specifically with a known macromolecular species. Use of fluorescent tagged antibodies now makes *immunoassay techniques* potentially capable of measuring with a high degree of accuracy many of the cellular proteins that occur at extremely low concentrations within the cell. Progress in electronics has also led to automation of fluorescent-binding assays. For example, it is possible to measure the amount of DNA in a living cell by exposing it to a substance such as mithramycin which titrates specifically with DNA. It then passes through a beam of UV light and the bound mithramycin fluoresces, the emitted light being picked up by a photosensitive cell and the signal amplified. The process allows a whole population of cells to be analysed in a very short space of time, using an apparatus called a *flow cytofluorimeter* (see also Chapter 3).

Spectrometric methods are still of great use in studying enzymes at the cellular

level. Provided a specific enzyme action can be carried out yielding either a coloured product or a product which can be identified in some other way, then a method is available for quantifying the amount of enzyme per cell. *Cytochemistry* demonstrates an important point. Two cells lying side by side in the same tissue may look alike but one cell can frequently show intense activity for an enzyme whereas its neighbour shows none. It can also give intracellular location with considerable accuracy in many cases, e.g. acid phosphatase in the Golgi apparatus and lysosomes. Thus the spatial distribution of enzymes within tissues can be resolved whereas biochemical techniques provide no such information.

Radioactive precursor substances are particularly useful at the single cell level when they become incorporated into macromolecules being synthesized. Treated cells are washed, fixed and overlaid with a photographic film or emulsion, which visualizes the sites that have taken up radioactivity (see Fig. 2–1). *Autoradiography*, as this process is called, and cytochemistry have both been applied at the electron microscope level to give high resolution to the localization of substances.

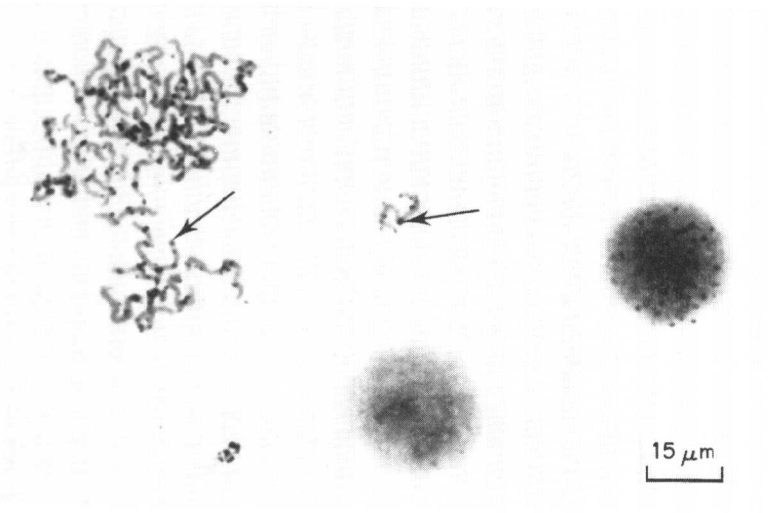

Fig. 2–1 Autoradiograph of 3 nuclei from human lymphocytes stimulated with the lecten, phytohaemagglutinin. One labelled nucleus on right; below, unlabelled nucleus. The third is in mitosis, with grains on individual chromosomes (arrows). By courtesy of Professor J. Swanson Beck.

2.1.4 Comparative aspects of macromolecular synthesis

The accretion of new macromolecules in growing cells is a highly organized affair, not just an indiscriminate accumulation of more molecules. Obviously it would be preferable to gauge increase by an internal yardstick in addition to having it recorded on a per cell basis. The DNA content of a cell is often used in

this capacity because its amount per cell remains more or less constant. Thus the alteration of, for example, sphingomyelin, mRNA or arginine kinase in growing cells is often expressed as an amount per unit of DNA and therefore allows each to be independently related to one another.

The fact that DNA content per cell is constant, and each cell type possesses a characteristic amount, suggests that it determines exactly how big a cell will grow. But DNA content is not that invariable. Apart from the content doubling during each cell cycle (Chapter 3), maturation and differentiation of cells from a growing stock sometimes leads to various types of abortive cell division. For example, DNA may double in content and the chromosomes can become segregated within the cell, but the rest of division fails, leaving the cell with twice its normal complement. The usual *complement* of DNA is 2C, but before division this increases to 4C and the two daughters therefore return to the 2C level. The 'divided' cell which has undergone the poorly understood process of *endomitosis* remains at 4C amount of DNA. The process can be repeated several times giving cells of 8, 16, 32 and higher complement numbers or 'ploidy'. *Polyploidization* is particularly frequent in such places as liver cells, many cultivated plants and large protozoa, but it is a widespread phenomenon and occurs to some degree in most eukaryotic cells. Ploidy refers to the number of chromosome sets possessed by cells; haploid is a half-set (n) equivalent to C DNA, diploid is 2n (equivalent to 2C), tetraploid 4n (equivalent to 4C). You may wonder why two expressions have come into use. Ploidy is a biological expression relating to numbers of sets of chromosomes (n) whereas complement (C) refers to a biochemical measure of DNA content. While they generally agree, it is possible for these values to be disparate.

Rise in ploidy class is accompanied by an increase in cell mass because cytoplasmic division fails to follow the doubling of all the essential components. Clearly a hexadecaploid (16C) cell is much larger than a diploid (2C) cell, but not necessarily 8 times bigger. The size of the cell could be dictated by the number of chromosomes, since everything is ultimately dependent upon the genes they carry. This idea is by no means new; Hertwig in 1908 had already commented that the size of a cell seemed to be closely related to the size of its nucleus, and after seventy years, we seem little further advanced in understanding the basis of this relationship. Indeed the relationships between (a) nuclear and cell size, and (b) cell size and the initiation of division, present two of the most basic and challenging questions in biology today.

2.2 Cell number

2.2.1 Cell counting and sizing

Since increase in cell size and number occurs in growing populations, accurate methods of measuring these parameters are essential. The laborious method of counting cells in a haemocytometer (see Fig. 2–2) has been largely superseded by electronic methods. Nevertheless, the haemocytometer is inexpensive, allows the investigator to see the cells being counted, requires a very small sample and allows the cells to be recovered for further studies if desired. Differential counts

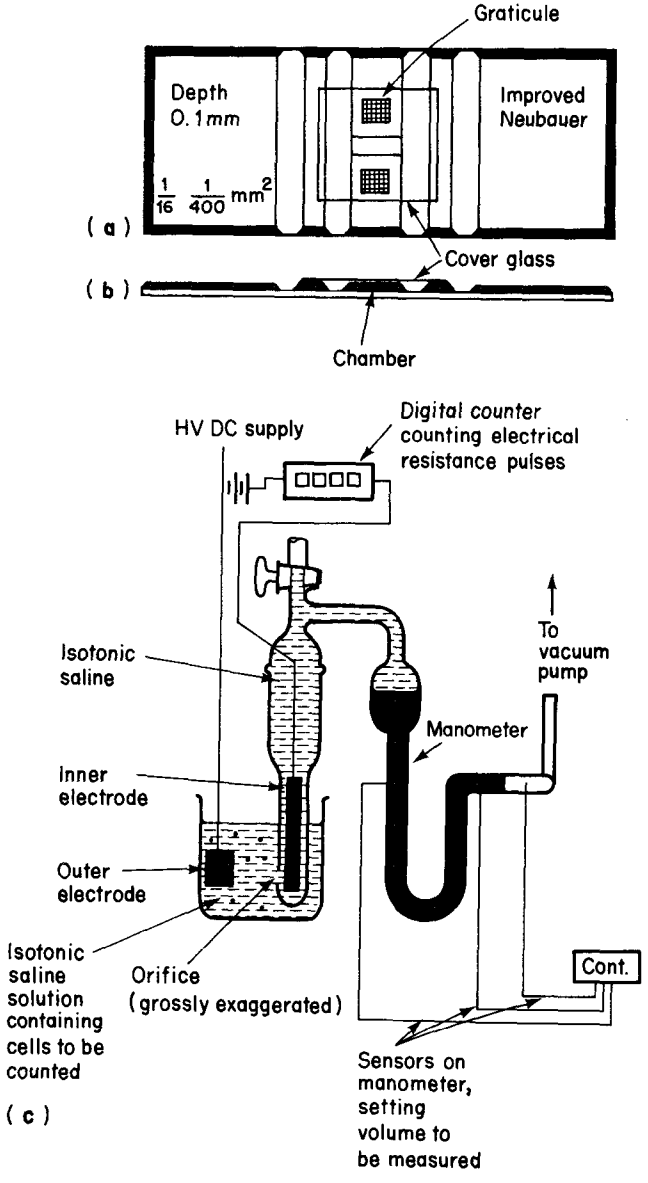

Fig. 2–2 (a) is a plan view, and (b) an elevation of a Neubauer haemocytometer. (c) is a diagrammatic representation of the essential working parts of a Coulter counter. The mercury in the manometer is drawn back over a set of electrical contacts, starting and stopping the counting process over a 0.5 or 0.1 ml volume, the cell suspension being drawn through the orifice by this action.