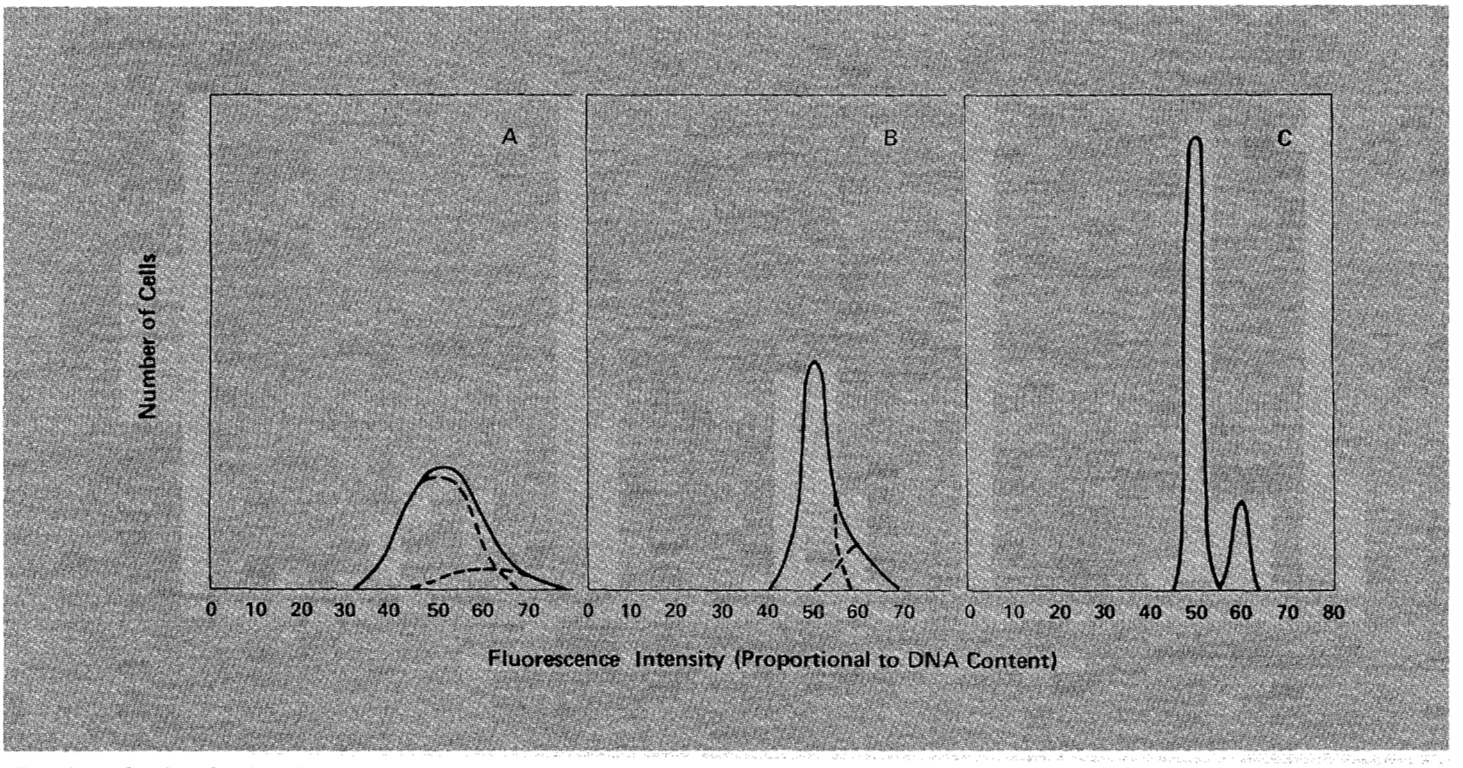

Fig. 9. Pulse-height distributions that might be obtained from two populations of cells stained for DNA content. The two populations are present in a ratio of 5:1 and their pulse-height distributions measured before mixing are shown by dashed and dotted curves. Cells in the less prevalent population have 20% more DNA. The solid lines in A, B, and C represent the pulse-height distribution of the populations mixed together. The distributions in A were measured by a low-resolution flow cytometer (14% cv) and in B by one having twice the resolution (7% cv). The resolution obtained with current instruments, shown in C, was four times the resolution in A. Here the two populations can be resolved completely and measured easily.

had a coefficient of variation (cv) of 14%. A number of possible causes for the poor resolution were examined: nonuniform laser output, low laser beam intensity, low-efficiency photomultipliers, noisy electronics, poor staining procedures, and low-brightness dyes. We concluded that more powerful and stable lasers, brighter dyes, and better staining techniques were needed. With these improvements we routinely obtained a 3-4% cv, and by using two DNA fluorochromes of differing specificity we obtained 1.5-3% cv.

The curves in Fig. 9, representing histograms obtained on a pulse-height analyzer, illustrate how instrument resolution influences data quality and the ability to see small differences in the DNA content of mixed cell populations. The histograms show the number of cells vs DNA content per cell.

The motivation for improving instrument resolution came from the desire to monitor the growth of replicating cells and from the need to produce safe human vaccines from cell lines maintained in the laboratory. At the time, it was proposed that only those mammalian cells that faithfully maintain their normal numbers of chromosomes should be used for vaccine production. By assumption, any increase in the number of chromosomes would be reflected by a corresponding increase in DNA content. Consequently, if flow cytometers could measure DNA content more accurately, they could detect abnormal cells with increased DNA content and thus monitor the early stages of chromosome instability. We improved the resolution of our instruments for this purpose and found that a change in the number of chromosomes *does not necessarily* correspond to a change in DNA content. This highly significant result indicates that in some instances a cell can conserve its DNA while repackaging it into a different number of chromosomes.

The need to distinguish cell doublets (two cells stuck together) from a single

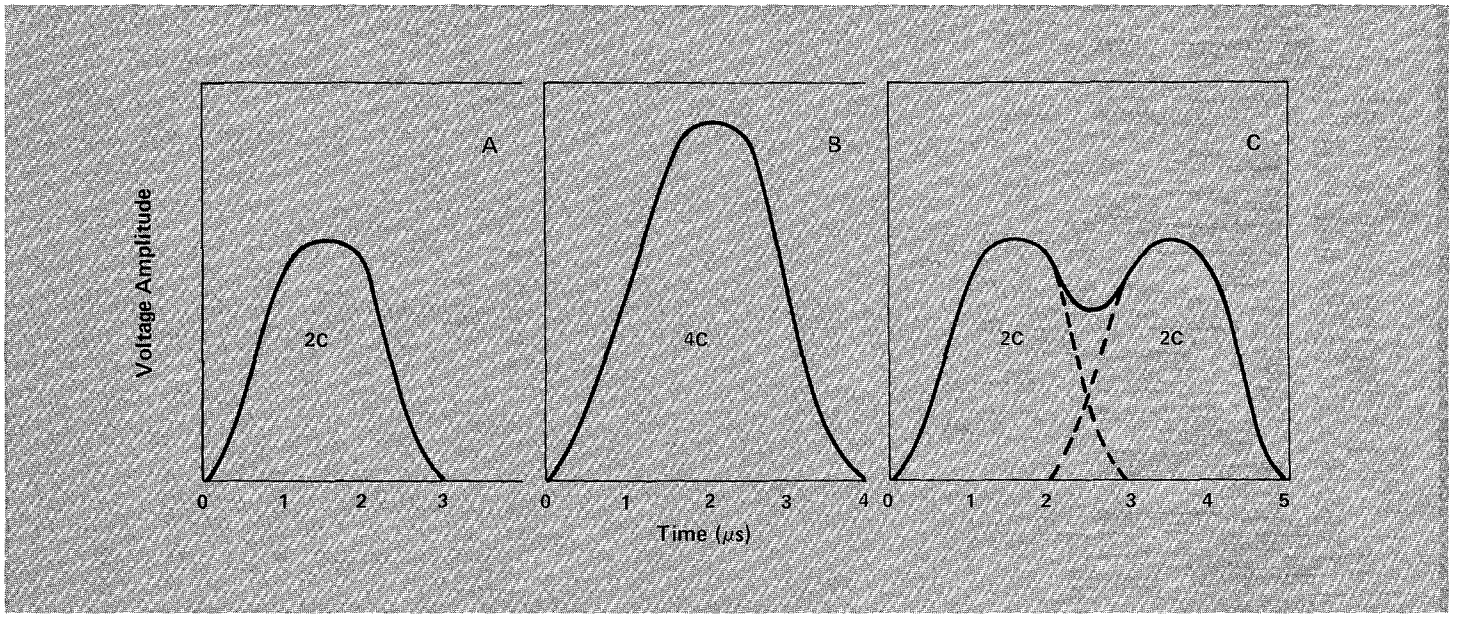

Fig. 10. Pulse shape profiles. A. Pulse from a nucleus of a single cell (2c DNA content) as it passed through a laser beam that is small (7 microns) compared to the diameter of the nucleus. By definition, 1c is the DNA content of a germ cell. B. Pulse from the nucleus of a single cell having twice the amount of DNA (4c). C. Pulse from two cells (each having 2c DNA content) that are stuck together. Since the electronics normally integrates the area of the pulses to get the total amount of fluorescent light, both the one cell in B and the two cells in C would be recorded as a single cell with twice the normal amount of DNA. In reality, the profile in C arises from two cells. The narrow width of the laser beam causes the pulses from the two cells to overlap (dashed part of curve C) to produce a saddle in a single pulse. Electronic circuitry has been designed to detect the saddle between the two peaks so that pulses of this shape can be discriminated from pulses of equal area such as shown in B. Thus, a single cell having twice the amount of DNA can be distinguished from a doublet.

cell that has doubled its DNA content before cell division or contains double the normal amount of DNA led to another important improvement in instrument resolution. The original spherical optics that produced an illumination area much larger than a single cell were changed to optics that shaped the laser beam into an elliptical slit (as described above) much smaller than the cell nucleus. Figure 10 illustrates the difference in pulse shape obtained using a 7-micron laser beam for a single cell with double the DNA content of a nonproliferating cell and two nonproliferating cells stuck together. Electronics were incorporated to discriminate between the two pulse shapes.

The ability of flow cytometers to measure DNA content with high statistical accuracy has been crucial for many biological studies, in particular, those related to the nature of cancer and its diagnosis and treatment.

Nature of Cancer

Cancer is a general term used to describe what is probably a number of diseases. The common manifestation is growth of the cancer cell beyond the condition of simple repair and replacement to a condition that leads to interference with normal biological processes and eventual death. It appears certain that several steps are required for a cell to become malignant. Some of the steps involve growth characteristics, and others involve immunological alterations. Although most normal cells in a mature organism are not growing (that is, dividing to produce new cells) there is some continuing amount of cell proliferation. Therefore, finding a very slight increase in cell proliferation in inappropriate cells (for early cancer detection) is a very difficult problem. However, prevalent opinion holds that there must be at least one, and perhaps more, unique and measureable properties of cells that are specific in cancer. A great deal of effort has been made to find such properties.

Because tumors can arise from a single cancer cell, all of the information that defines a particular cancer probably is contained within a single cell. This is one rationale for performing cellular

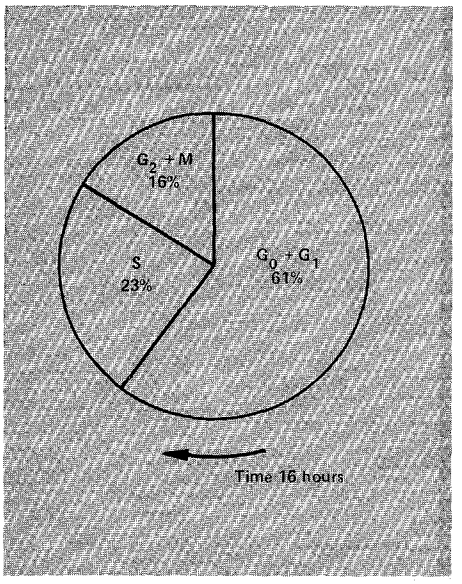

Fig. 11. A conceptual diagram of how Chinese hamster ovary cells (CHO), in exponential growth, are distributed throughout their life cycle.

research to find an unequivocal marker for a cancer cell. A suitable marker could be any of the following: the presence of specific antibodies or enzymes, an unknown chemical substance, a change in electrical properties, a change in the DNA, a loss of chromosome stability, or some presently unimagined feature. Experiments with DNA have high priority because the central cancer problem is thought to be one of altered gene expressions.

The Cell Cycle

To define an abnormal or cancerous condition, we must understand the range of normal conditions. Therefore it is not truly meaningful to separate cancer research from basic research directed to the understanding and quantification of normal biological processes. Undoubtedly a detailed understanding of cellular growth throughout the life cycle of a cell holds the key to an understanding of where the process goes astray and produces uncontrolled tumor growth. One gross change that can be monitored during the life cycle is DNA content. Each phase of a cell's life cycle (Fig. 11) is characterized by the amount of DNA present in the cell nucleus. DNA is measured in units of c, the number of picograms of DNA in the sperm or egg cell of a particular species. All other cells in that species contain double this amount, or 2c. In Figs. 11 and 12, G_0 represents the stage of a mature cell that is not multiplying (for example, a lymphocyte). If the cell receives a signal to replicate (the presence of a foreign material might initiate such a signal), it goes into stage G_1, in which several biochemical activities including the production of protein in the cytoplasm take place. Upon completion of these activities, the cell goes into stage S, in which the DNA replicates itself and the amount of DNA increases from 2c to 4c. Stage S is followed by G_2, a resting stage, and then by M, the mitosis stage, in which the DNA condenses into chromosomes and the cell divides.

Figure 12 shows the distribution of DNA content for a population of normal multiplying cells measured with a flow cytometer. The large peak, containing most of the cells at 2c DNA content, corresponds to stage G_1. The smaller peak around 4c corresponds to cells in stages G_2 and M. The region between the peaks contains the cells in stage S, undergoing DNA synthesis. In the normal life cycle, cells remain between 2c and 4c. However, when things go astray as in malignant growth, abnormal amounts of DNA are sometimes observed. As an organism ages or is subjected to certain chemicals or ultraviolet light and other environmental stresses, both the amount of DNA and the way it is packaged into individual chromosomes may change.

The measurement of DNA distributions therefore is extremely useful for tracking cells through the life cycle and for assaying the effects on the cell cycle of radiation, environmental conditions, and chemical drugs. The technique also

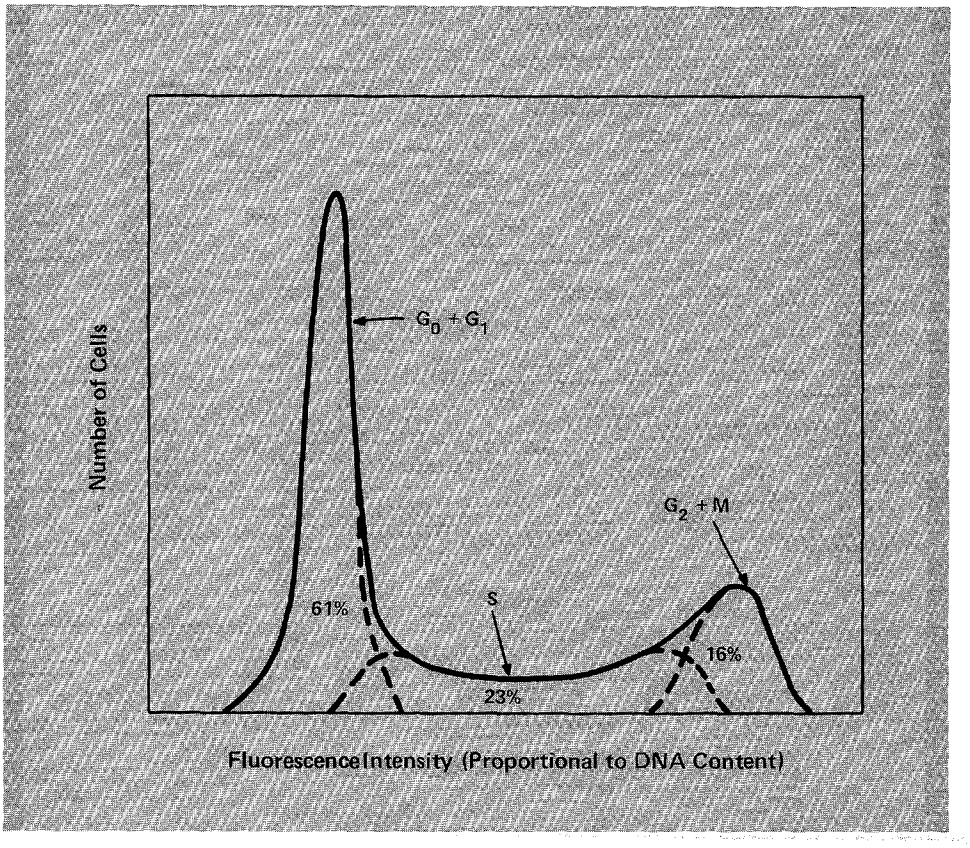

Fig. 12. The pulse-height distribution from exponential growing cells showing how the integral curve can be unfolded into its component parts to determine the percentage of cells in $G_0 + G_1$, S, and $G_2 + M$.