# QUANTITATIVE APPROACHES TO THE CELL DIVISION PROCESS

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## 1. Introduction

It was only three decades ago that the English scientist Twing expounded some of the basic concepts of computing machines. Today, thanks to a number of outstanding mathematicians, but particularly the late John von Neumann, we have sufficient theoretical knowledge to give instructions for building a machine that could duplicate itself. If one tried to build a self-duplicating machine with our present technical knowledge, this might perhaps be some $10^{15}$ to $10^{18}$ times as large as a typical living cell; yet cells have apparently continuously performed the feat of self-duplication for possibly 500 million years. The actual process of cell division is still almost as obscure to us as it was when Anton van Leeuwenhoek, using his newly invented microscope, recognized a yeast cell as a living entity.

The author cannot make claim to expertness in the field of mathematical statistics. This article is presented merely to expose some of the problems one may encounter when attempting to experiment with unicellular organisms. It will become apparent that not only do we need to refine our quantitative, observational techniques to give us insight into phenomena at the level of single molecules, but also it seems to be of interest further to develop and use mathematical methods for the solution of biophysical problems which can treat many thousands of variables simultaneously.

The problem of cell division and cell proliferation is qualitatively similar for all kinds of cells.

In mitotic division, utilizing nutrient materials and energy supply available in the medium, all essential cell components double themselves and the end result is two more or less identical living cells. Detailed description of the cell proliferation process is available in many books and reviews (see, for example, [1] and [2]). There is, however, enormous variation in the morphological, physiological, and biochemical details of the process. This variation is characteristic not only of each genus of each species, but the same cell can exhibit astonishing

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variation in its "phenotypic" properties when exposed to varying environmental conditions.

The group the author is associated with has for years been interested in one kind of cell, common baker's yeast. There are perhaps 70,000 strains known of this unicellular organism, which can be found practically everywhere on the surface of the earth [3], [4].

The cells of saccaromyces cerevisiae are ellipsoidal in shape. They contain a nucleus, vacuole mitochondria, and cytoplasmic fine structure [5]. They are surrounded by a cell membrane, and a polysaccharide wall. They have mitotic division in a process known as budding.

Genetics tells us that essential properties of the cells are contained in the genes of the cell nucleus. These have enough information to determine essential properties and in the yeast it can be proven by tetrad analysis [6] that the genetic properties replicate very precisely.

Let us now cite a number of more or less quantitative facts about the cells themselves and their proliferation. A haploid yeast cell, in its resting state, is an ellipsoidal object about 5 micron on the larger and 4 micron on the smaller axis. Table I shows that such cells are about 72% water and 28% solid. The

TABLE I

SOME CHARACTERISTICS OF A TYPICAL CELL
HAPLOID SACCAROMYCES CEREVISIAE

Number of copies is listed assuming one double strand of DNA. In polytene nuclei there is assumed to be more than one strand; in higher ploidy cells the number of replicas is proportional to the ploidy.

| | |
|---|---|
| Volume (from [7]) | $46.6 + 5.8 \times 10^{-12}$ cm$^3$ |
| Wet Mass | $49.4 \pm 6.1 \times 10^{-12}$ |
| Dry Mass | $13.8 \pm 8.7 \times 10^{-12}$ g |

| Composition | Amount | Number | Number of Copies | Number of Species |
|---|---|---|---|---|
| DNA (from [8]) | $2.26 \pm 0.23 \times 10^{-14}$ g | $1.6 \times 10^8$ Base pairs | 1 | $1.6 \times 10^8$ |
| RNA (from [8]) | $55.6 \pm 1.6 \times 10^{-14}$ g | $\sim 10^7$ Molecules | 25 – 100 | $1.4 \times 10^6$ |
| Proteins | $845 \times 10^{-14}$ g | $\sim 1.7 \times 10^8$ Molec. | $1.7 \times 10^5$ – $1.7 \times 10^{13}$ | $10^2 - 10^6$ |
| Carbohydrates | $350 \times 10^{-14}$ g | $\sim 10^{10}$ Molecules | $>10^7$ | $<10^3$ |
| Lipids | $67 \times 10^{-14}$ g | $\sim 1.3 \times 10^9$ Molec. | $>1.3 \times 10^6$ | $<10^3$ |
| Water | $3{,}560 \times 10^{-14}$ g | $\sim 1.2 \times 10^{12}$ Molec. | $>1.2 \times 10^{12}$ | 1 |

molecules in the cell have been grouped according to their importance and function. It is generally assumed that the important part of the genetic material of the cell nucleus is DNA (desoxyribose nucleic acid), containing all or most of the inheritable information of the nucleus. It appears likely that in some viruses and unicellular organisms at least part of the essential information is

carried in a single copy, unless nature has insured the cell by endowing it with polytene (multiple stranded) DNA and/or ploidies higher than one, as in most human somatic cells (ploidy 2) or in some plants where ploidy of 16 or 32 is not unusual.

Cell function and cell replication appear to be carried out in some fashion by transferring information from DNA to the molecules which make up the bulk of the cell. Cell interactions occur so fast that kinetically it appears unsound to assume that each of the $5 \times 10^{10}$ amino acid molecules, which are about to be incorporated into the $10^8$ protein molecules, would physically come in contact with each proper part of DNA of each gene. It seems more likely that DNA should have interactions mainly with RNA, which has quite similar structure, or with only some specific protein molecules. As the table indicates, the number of copies of molecular species existing in the cell increases as one moves to less specific forms, as in successive stages of an amplifier.

The structure of DNA has been very extensively studied. Since the analysis of X-ray diffraction patterns by Watson and Crick [9], and very extensive chemical studies, we know that DNA is a double stranded helix, composed of two sets of purine and pyrimidine bases, pentose sugars, and phosphate groups. Specificity is achieved by the order of array of the four bases which compose each of the two strands of DNA. The bases are adenine, thymine, guanine, and cytosine. Efforts are in progress to study the sequence of bases. There seems to be no preference for any particular sequence. However, the positions of purine and pyrimidine molecules on the two parallel strands are related to each other. Adenine is always opposite thymine, whereas guanine is connected to cytosine. Thus, in a single long chain DNA molecule the genetic message appears to be spelled out in duplicate by a four letter alphabet, the letters being the four bases (ad, cy, th, gu). All efforts to determine the exact molecular weight of DNA seem to have failed; it appears to occur in chains of indefinite length. We do know that under certain conditions the nucleus can be fractionated into subnuclear fragments, however. Thus, the DNA is not necessarily continuous; it may occur in various lengths, however, each corresponding to "words" of information content.

It is assumed that the sequence of bases along DNA has the basic message containing instructions for all cell activities including self-duplication. We consider the somewhat narrower problem of the relationship of DNA to information content, as expressed by the sequence of bases in DNA to the information in specific proteins of the cell, as expressed by their amino acid sequence. Assuming that there is a direct translation of one message to another, this code could be deciphered if we knew the exact sequence of nuclear bases in the gene that is responsible for synthesis of a given protein for which the amino acid sequence is known. While amino acid sequences are known in a number of proteins [10] the sequence of bases is not known in DNA and we do not seem to have a suitable method for attacking this problem. Nevertheless, Gamow, Rich, and others (see [11] and [12]) have made intriguing efforts to devise a proper code. As we

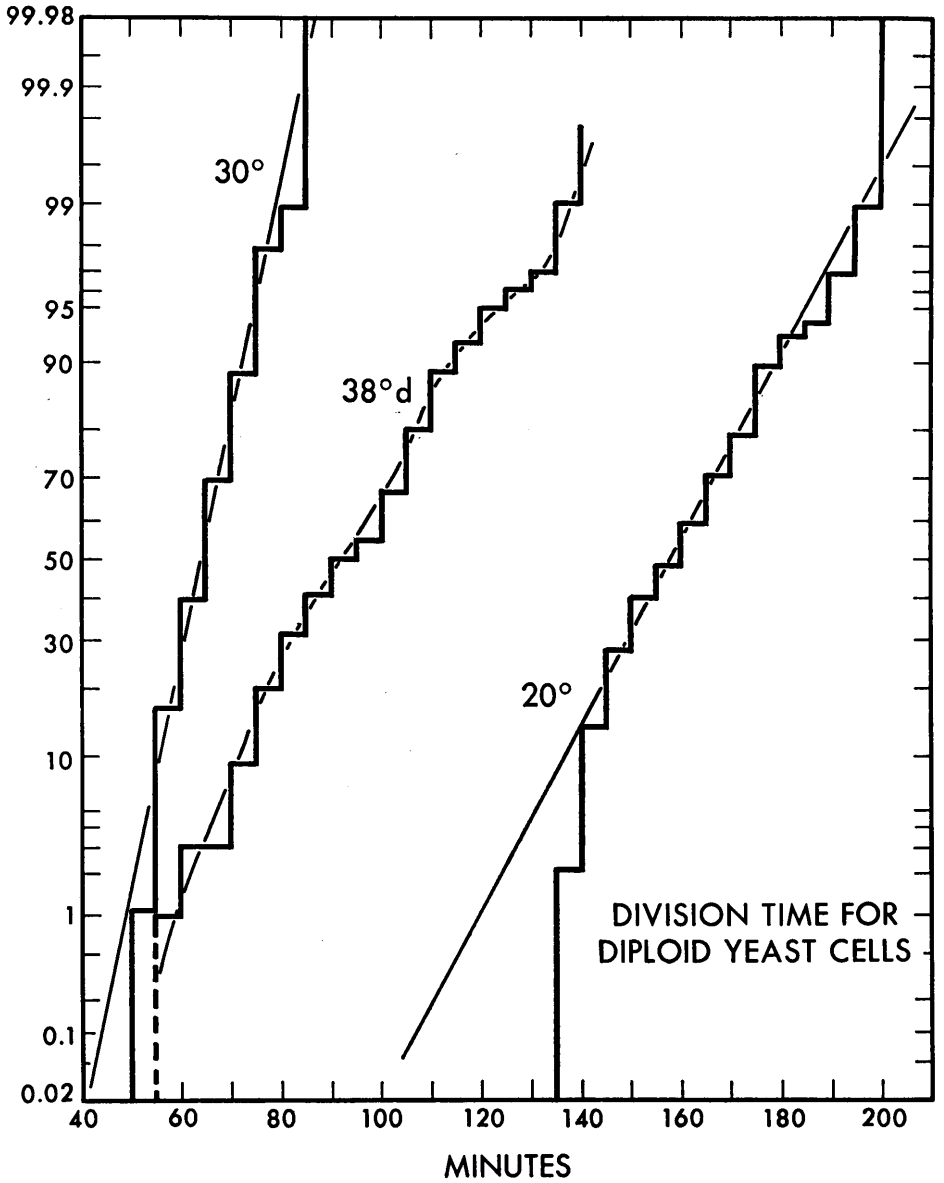


FIGURE 1

Distribution of division times of diploid saccharomyces cerevisiae at three different temperatures on yeast extract medium.

learn more about cells, it is becoming more and more doubtful whether or not a direct translation of DNA into proteins exists, and if it does whether the code is the same for all organisms, or different for each species.

In our laboratory a number of recent investigations were carried out to study the time relationships of cell division, and here is a brief resume of some of the results.

Given a complete medium, it is of interest to know the statistical distribution of the time required for cell division. It has been known for some time that in various organisms cell division time is closely regulated. Fertilized sea urchin eggs, for example, carry out their division almost synchronously, and biologists and statisticians have been interested in the stochastic processes of cell division for some time. Kelly and Rahn [13] have experimented with normal bacterial populations. Their models prompted Kendall to present a statistical theory of stochastic birth processes [14], [15]. Powell [16] analyzed cell division of six species and pointed out that correlation between generation times of sister cells and other factors must be taken into account. Kubitschek [17] presented additional data on bacteria.

## 2. Statistical distribution of cell division times

The investigation on diploid yeast cells was carried out in our laboratory by Victor Burns [18], who observed single cells under the microscope, waited until they divided, then moved mother and daughter cells apart to fresh places on the nutrient agar medium. The statistical distribution of division times approximates normal distribution and is shown on figure 1. Measurements were made at three different temperatures within the physiological range. Assuming that all division involves completion of $N$ statistically independent events, each taking the same length of time on the average, we can estimate $N$ by assuming that $\sigma$ is the standard error and $\tau$ the mean division time. For 20°C and 30°C the ratio $\sigma/\tau \fallingdotseq N^{-1/2}$ is the same, approximately 0.09, so from this $N \fallingdotseq 123$. All division occurs faster at 30° and this would indicate that if simple mono-molecular reactions were responsible for the events, the activation energy would correspond to about 16 kcal/mole. At 38°C one would expect a mean division time of 29 minutes. Instead the mean division time is considerably delayed and shows much greater statistical variation, as shown in table II and in figure 2. There is

TABLE II

DIVISION OF DIPLOID YEAST CELLS

| Temperature (°C) | $\tau$, Mean Division Time (minutes) | $\sigma$, Standard Deviation (minutes) | $\sigma/\tau$ | Mother and Daughter |
|---|---|---|---|---|
| 20°C | 162 | 15 | 0.092 | synchronous |
| 30°C | 67 | 6 | 0.089 | synchronous |
| 38°C Mother | 73 | 13 | 0.17 | not synchronous |
| Daughter | 96 | 18 | 0.19 | not synchronous |

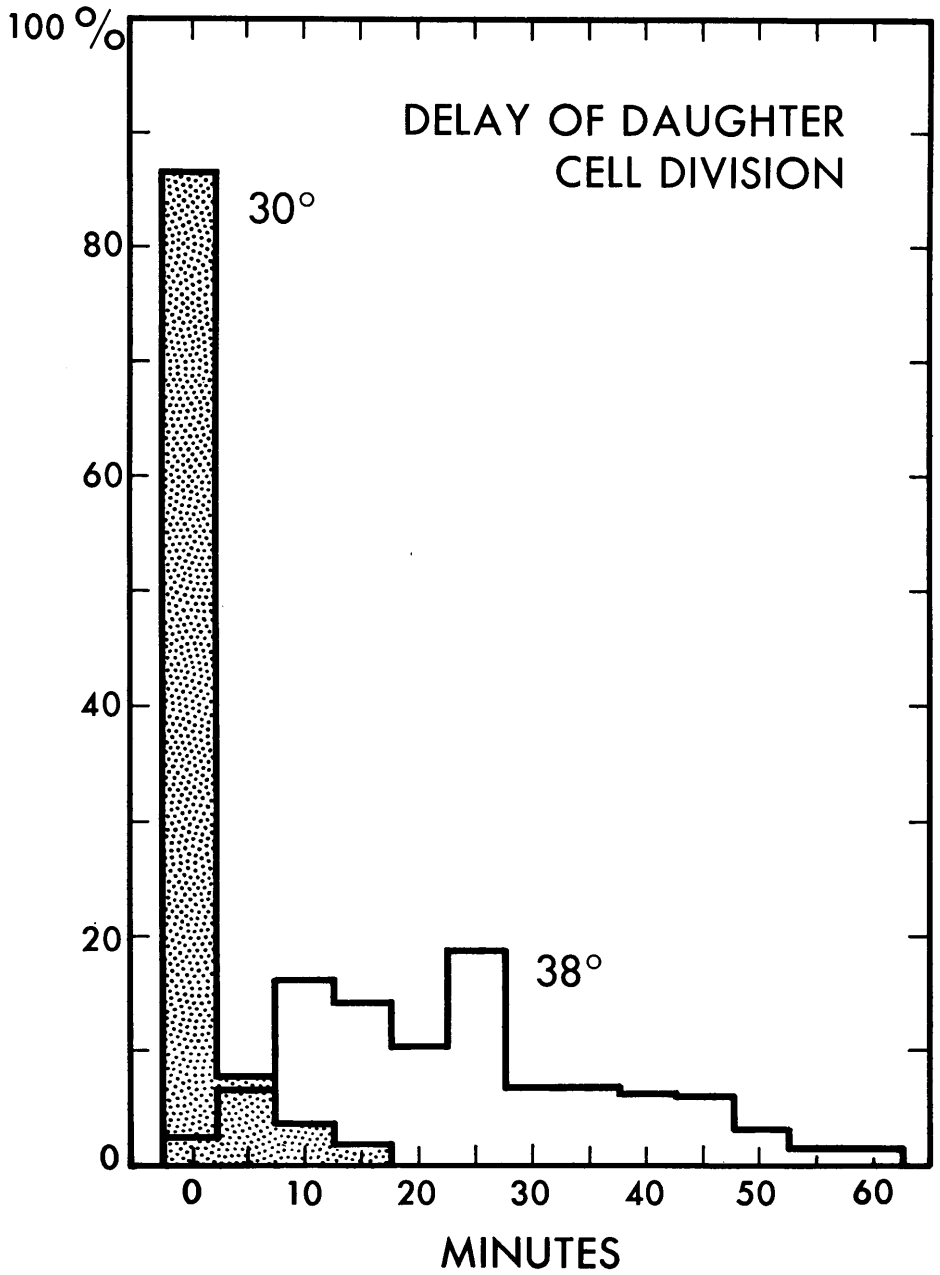


FIGURE 2

Delay of daughter cell division compared to division of mother cell at 38°C.

reason to believe that at 38° the biochemical reactions participating in cell division have gotten out of phase (see table II).

Since new cells in yeast come about by bud formation it is possible to determine which is the "mother" and which is the "daughter" cell. It is true that we can tell this with certainty only of the cell wall; the molecules of the mother and daughter nucleus might be undistinguishable. The question arises whether a cell which divided faster than others will retain this faster rate in consecutive divisions. One may observe the same cell in consecutive divisions exhibit similar fluctuation in cell division time as a group of cells, except for the "aging" phenomenon, which causes cells to die after a large number of divisions [19]. One will then wonder if statistical fluctuations exist between mother and daughter cells. At 30°, the optimum temperature for yeast cells, the time for mother and daughter cell division is almost exactly identical. This gave rise to speculation that some critical events essential to the cell division process are determined not in the cell's own lifetime but during the previous cell generation.

### 3. Delay in cell division time

The nearly synchronous cell division described is exhibited only when genetically competent cells are grown on full nutrient media for several generations. Many agents can be applied which will alter the mean division time and the statistical variation in the process [2]. These are of interest not only because they alter the dynamics of the process but also because the coupling between nucleus and cytoplasm, responsible for harmonious development of cells, appears to be modified. Cell division can be blocked or delayed by many toxic agents which may attack one of the many cell division stages. Colchicine, for example, acts in metaphase by breaking down the mitotic figures. Narcotic agents not only depress respiration but can inhibit the cells' energy reservoir mechanisms. Radiomimetic drugs apparently act directly or indirectly on synthesis of new DNA. Best known among these are nitrogen mustard and triethylene melamin. We shall only cite the case of $X$ radiation, for which all division delay has been quantitatively studied in yeast cells [18], [20]. A given dose of radiation can inhibit cell division and thus lead to lethal effects; it will also delay cell division time from which recovery is possible. The major delay in all division appears not in the generation where the cell is irradiated, but in the ones following; another proof that for cell division rate processes the cell's memory can carry from one division to the next. As shown by combined efforts of various investigators (see [21], [22]), the nuclear synthesis is temporarily inhibited, but cytoplasmic growth and protein synthesis goes on leading to delayed cell division and formation of giant cells [22]. Most striking about the cell division delay thus produced is that the mean delay can be expressed for the cells under discussion as a "saturation" curve as function of dose. The maximum mean delay obtainable is 350 minutes at 30°C or about five normal division periods. As longer and longer delays are produced, more and more of the cells are perma-

nently inhibited from division. The mean delay $d$ is a function of dose $D$; according to Burns [18],

$$d = 350(1 - e^{-1.5 \times 10^{-4}D}), \tag{1}$$

where $D$ is in roentgens. The size of the cells continues to grow until their mean volume is three times normal. Protein is also synthesized in this period. Some of the giant cells thus produced are limited in size by their polysaccharide walls which have limited elasticity. It was observed in our laboratory by Anger, Zirkle, and myself, in time lapse motion pictures, that some of these cells die by exploding. Apparently the synthesis and enrichment of substances continues in the cells until the internal osmotic pressure overcomes the limiting strength of the wall.

The rate of intracellular processes depends a good deal on the external chemical environment as well. When the cell is placed from one medium to another of different composition it will have to readjust its enzyme systems to utilize the second medium optimally. Usually there is a delay, labeled "phenotypic lag," in the first and second cell division, but if the medium is sufficient at all then the cells will soon divide again at the optimum rate for the particular temperature. It is through experiments of this sort that we learned that the genetic apparatus of yeast cells is capable of many more functions that it is called to do in a given environment. Below is a partial list of accomplishments for a typical cell:

(1) Vegetative proliferation: proliferation of the cell by mitotic division.

(2) Mating: zygote formation with a cell of opposite mating type and, in saccharomyces cerevisiae vegetative colony, formation of diploid cells (if the mating types are haploid).

(3) Reductive cell division or spore formation: derivation of haploid spores from diploid cells, in response to somewhat specific biochemical challenge.

(4) Synthesis of an elaborate set of enzymes in response to the chemical nature of medium: yeast cells can synthesize all important enzymes and vitamins except biotin from simple carbon sources (sugar), nitrogen source (ammonia). If the chemical nature of the environment changes, the cell may adapt to the new environment by induction of growth of a set of new enzymes.

(5) Fermentation of certain sugars to alcohol, or in presence of oxygen to acid.

(6) Production of some antibiotics.

(7) Induction of antibody production.

The above functions have been selected for yeast cells only, yet they represent many of the functions cells are capable of, in a general sense. Omitted is photosynthesis, a property of plants, and differentiation, usually found in multicellular organisms. Yet a colony of yeast cells, when grown on agar gel, exhibits certain primitive features of differentiation, has characteristic shape, and its cells are in different states of proliferation depending on the cell's position in the colony.

Of the above-listed variety of specific properties, relatively few have been studied sufficiently to have definite assignments on the genetic linkage map.

Nearly 200 specific loci have been mapped. Usually there are several loci for a given metabolic requirement. In order to synthesize adenine, for example, seven different genetic loci are known; each locus in the sense of interpretation by Beadle and Tatum is supposed to be associated with production of one of the enzymes which participates in biosynthesis of adenine. When the yeast cells are exposed to a new milieu, for example, a different sugar is placed in the medium as a source of carbon, the cells have to adapt themselves by manufacturing enzymes for metabolism of the particular product. This phenomenon is called enzyme induction and it has been shown by Novick [23] and others that provided the requisite genes are present, the induction results in exponential rise of the induced enzyme until it is present in sufficient concentration to supply activity in step with other biochemical processes in the cells. Induction seems to require presence of the enzyme initially in small amounts and it seems logical to postulate that such an inducible enzyme serves as partial template for itself. A similar induction process has recently been proposed [24], [25], for the induction of antibodies in cells challenged by antigens. Once induced, the enzymes or antibodies proliferate and maintain steps with the cell division process, so when challenged again several generations later, the proper enzyme or antibody is available promptly. Conversely, a cell adapted to a certain environment and having been filled by certain enzyme systems will require considerable time to readapt by modifying its own enzyme systems.

Dynamic interactions of this type show up the role of the genes not merely as possessing the code, but also having control over the intensity and rapidity of synthetic action on the part of the cytoplasm. The latter in turn acquires greater importance, since it appears to be able to act as template for new cytoplasmic products.

The kinetics of interaction of cells with the external medium are of interest, particularly since these differ from ordinary chemical kinetics. In laboratory chemistry the rate of interaction of two molecules $A$ and $B$ usually depends on their respective concentrations (or activities) $C_A$ and $C_B$; the initial rate is often proportional to $C_AC_B$.

For yeast cells one may get an interesting measure of the rate of utilization of substrates by measuring the initial rate of cell division when starving cells are placed on complete or incomplete media. Under these conditions cell division is in partial synchrony, as shown in figure 3. Experiments of this type were carried out in our laboratory in buffered yeast extract media, varying the concentration of the yeast extract medium over a 1000 fold range, so that they would support $10^8$, $10^7$, or $10^6$ cells per $cm^3$. When the starting concentration of cells was low, for example $2 \times 10^5$ cells/$cm^3$, then the same first division time was obtained for each concentration of medium of $120 \pm 10$ minutes. It was also possible to obtain an index of the overall uptake of untraviolet absorbing amino acids by cells when they are starved, by ultraviolet microspectroscopy. This work, performed in collaboration with $B$. Thorell [26] showed equal rate of uptake, independent of the concentration of external medium. These experiments pointed

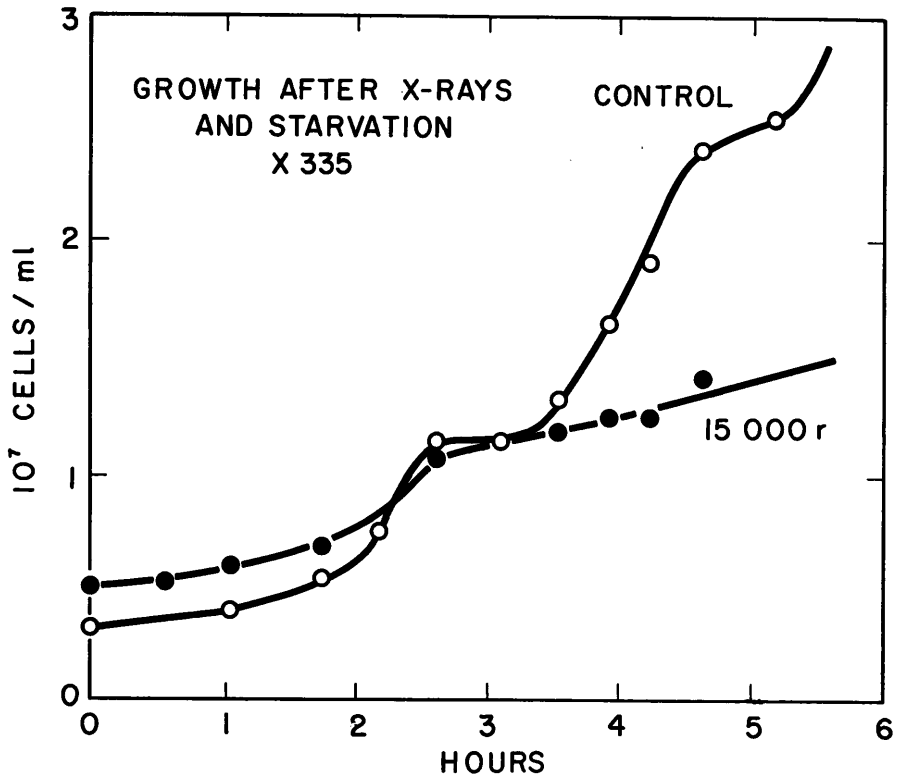


FIGURE 3

Number of diploid cells on complete medium
following a period of nitrogen starvation.
A normal culture and an irradiated culture are given.

to the already known somewhat trivial fact that cell division time, at least in the so called "logarithmic" phase of growth, is independent of the concentration of substrates in the medium.

Another simple experiment may be performed in a similar fashion by placing the cell in synthetic nutrient medium, but omitting one of the nutrients essential to cell division. If the cell is able to synthesize this nutrient it will do so, but the adaptation process takes time and "lag" occurs. Nevertheless the rate of uptake of other substrates present, as indicated by ultraviolet microspectroscopy, is the same as on the complete medium at the same temperature. Exceptions to this rule are known, however. When two substrates are present which feed into the same metabolic cycle, the presence of one may inhibit utilization of the other, as shown by inhibition of yeast cell growth with canavanine in the nutrient medium and by reversal of the inhibition by arginine [27].

Nevertheless, we can make three generalizations which may help in mathematical models of cellular processes: (a) There is a range of nutrients which enter

the starved yeast cells, essentially independently of each other unless they resemble each other chemically and are "analogs." (b) The rate of uptake of a number of substrates by starved yeast cells is independent of concentration of substrates in the medium over a wide range of physiological concentrations. The rate determining factors are within the cells and are either determined by "active sites" on the cell surface or by some other intracellular factors. It is interesting to note that in the chemostat of Novick and Szilard [28], the growth rate is limited by decreasing the concentration of a single nutrient until it governs the rate of the entire division process. (c) Cell division time is independent of the concentration of nutrients in the medium over a large range of concentrations.

By observing the submicroscopic structure of the cell interior, one obtains definite ideas of the possible ways intracellular enzyme-catalyzed reactions occur. The enzymes presumably are located in the lamellar structures of mitochondria, or along the membranes of the endoplasmic reticulum condensed in particles known as "microsomes" [29].

We suspect, and in cases of mitochondria and of chloroplasts we know, that localization of electronic excitation can very rapidly move to other molecules along the membranes of subcellular structures. We also know that the mitochondria contain energy-rich structures, for example, ATP adenosine triphosphate, ready to drive reactions catalyzed by enzymes.

It became obvious in the course of studies on cell division that with respect to the cell's interactions with external media, there are two somewhat distinct phases. The first phase is uptake and storage of nutrients and energy-rich molecules. In this phase interaction with external medium is essential. The second phase is synthesis and organizing of the structures directly leading to cell division. No exchange with external nutrients need be involved, except in some instances of oxygen, which may govern the rate of the division process.

Figure 4 illustrates the occurrence of the "first" cell division from single yeast cells, "starved" on nitrogenless medium for six hours. The cells are exposed to yeast extract medium for varying times (plotted on abscissa), then are placed in buffer, which alone is not capable of cell division. Yet, if the cells were exposed for at least 2/3 cell division time to full medium, they will go ahead and divide; if a cell has been exposed for two cell division times, it may be able to divide two more times from materials stored in the cytoplasm. When the medium is incomplete, so new enzymes are to be synthesized, then the kinetics are more complex, since the first phase of the interaction is interfered with.

Based on experiments of similar character, one may arrive at some very crude kinetic models of cell division of yeast cells in complete media and optimum temperatures; in figure 5 we are giving one such model. Cell division is characterized as consisting of a "storage and exchange" phase, signifying reversible exchange with external medium and enrichment of essential precursors within the cell. In the second phase the actual cell division process takes place. This part of the process, in the absence of certain fatty acids, is greatly accelerated by the presence of oxygen and retarded by certain inhibitors, for example

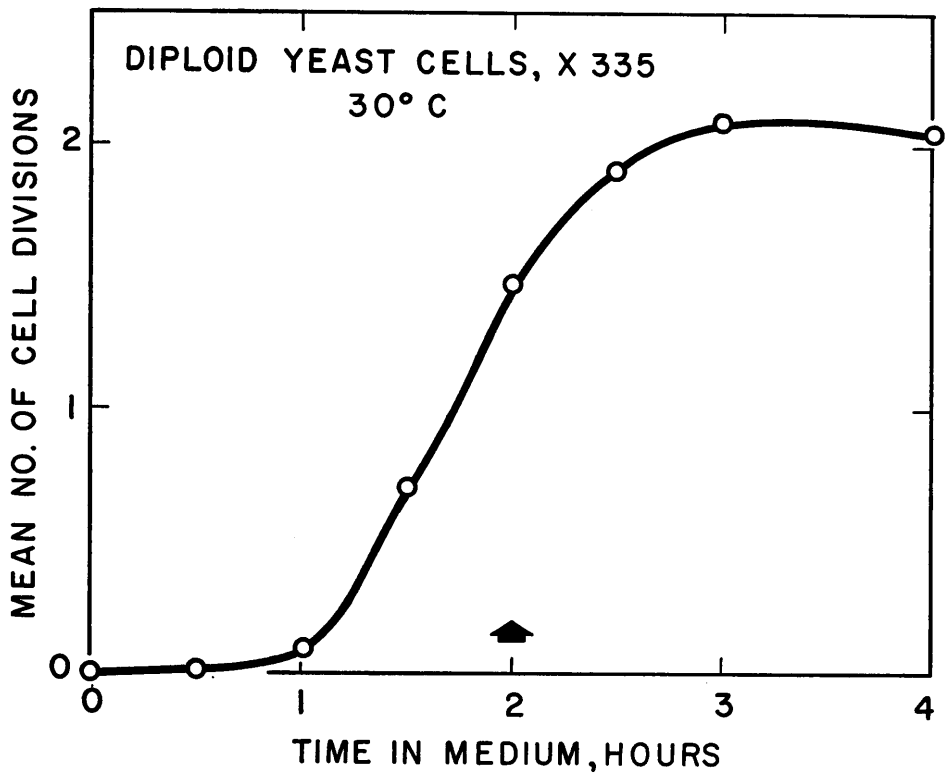


FIGURE 4

Number of cell divisions following a period of time on complete nutrient medium. When the period of nutrition is over, the cells are placed again on nitrogenous medium. New buds appearing thereafter are scored as "divisions."

colchicine and kinetin [30], [31]. It does not appear experimentally "reversible"; however, when too drastic chemical interference takes place both mother and daughter cell may die, or if there has been a deficiency in the nutrients furnished the daughter cell alone may find itself without nucleus, with the mother cell still capable of dividing again.

A similar but more detailed model was proposed by Scherbaum [32].

There must exist a fairly definite relationship between nuclear division and proliferation of cytoplasmic structures. Since the nucleus appears to be controlling the entire process of cell division, we suspect that the controlling influence has some elements of feedback. In fact it has been observed, working with bacteria and with yeast cells, that the rate of accumulation of precursors of certain substances essential for cell division was modified by the concentration of the substance within the cell itself [33].

Close interrelation of cytoplasmic and nuclear division is indicative of strong

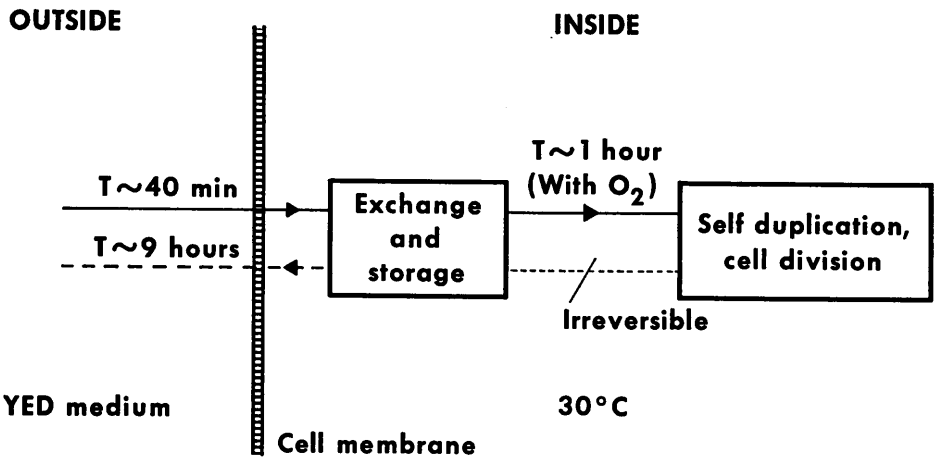


FIGURE 5

Model for exchange of nutrients and for cell division in yeast.

coupling between these two processes; on the other hand deleterious influences could decrease the strong coupling. Sherman [34] has observed that the great statistical variation of cell division times at high temperatures, referred to earlier in this paper (figure 2), is a direct consequence of the fact that cells proliferating at high temperature can lose an important cytoplasmic particle, carrying part of the cytochrome system. The reason such loss occurs in the course of cell division is that the cytoplasmic particle which, at normal temperatures, can easily keep step in its proliferation with that of other parts of the cell, cannot keep in step with cell division at high temperatures. The result is cytochromeless "petite" cells which proliferate less rapidly and with greater time variation than normal cells [35].

The existence of viruses may signify the possibility of loss of nuclear control of proliferation of some otherwise normal cell constituent. That such a relationship exists is clearly indicated by induction of phage particles by ultraviolet light, as in the work of Luria and his associates [36]. In this case a cellular particle, the "prophage," apparently exists normally and proliferates with the host cells, without hurting the host cells. A very small disturbance to the cell may destroy the coupling and the phage particles then start uncontrolled proliferation, thereby killing the host cells.

## 4. Radiobiology of yeast cells

Modification of the cell division process by penetrating radiation is particularly relevant because these radiations act at the level of the nucleus and the cell division process shows the most sensitive variation of all cellular systems, following radiation. The work with yeast cells has been reviewed in detail [33].

so we shall refer to it here only very briefly. Table I indicates that in some cells (haploid) the genetic information of DNA may be present in a single copy only. When by chance event an essential part of the chromosomes is destroyed, cell division may be inhibited. This can occur by absorption of a single photon by an appropriate molecule or by interaction with a single ionizing particle, thus giving proof that there is no redundancy of information, at least in some parts of the chromosomes. The probability of survival $p$ is found from the number of lethal interactions of an assumed $n$ equally sensitive, essential parts of the chromosomes. When the dose is $D$ then the mean number of lethal interactions for each of the $n$ sites is $\alpha$, with $\alpha$ a constant. Using the Poisson distribution, we obtain for the probability $p$ that none of the $n$ sites are injured, $\exp(-\alpha D)$. It has been shown that for cells with $m$ sets of chromosomes (ploidy $m = 2, 3, 4, 5, 6$) the probability of survival $P_m$ can be expressed adequately by

$$P_m = [1 - (-e^{-\alpha D})^m]^{n-\beta m D}, \tag{2}$$

when we assume identical constants $\alpha$ and $n$ for haploid or ploidy $m$. The first part of the expression for $P_m$ accounts for the so-called "recessive lethal effect"; this effect causes inhibition of cell division only if all $m$ replicas of a given chromosome site are inactivated independently. The second part of the expression, characterized by a constant $\beta$, accounts for dominant lethal effect: any one of the $m$ replicas of the $n$ sites may be injured in such a way as to cause death of the cell. Actually the dominant part probably expresses chromosome breaks and abnormal rejoinings which prevent normal cell division from occurring. The above model, though reasonably well checked for "resting cells," must be modified when radiation is carried out during various phases of the cell division process, since radiosensitivity varies during the cell division process.

## 5. Discussion

5.1. I attempted to select a few types of measurement on a single-cell species as an illustration of the type of quantitative data obtainable by direct experiment. There are, of course, very many other facts known including many biochemical observations on specific enzyme systems, and probably there are still more details not as yet known to us. We do not have any satisfactory mathematical model for cell replication; however, certain general observations may be made with respect to some of the properties of the type models one needs, and it is possible that these may have intrinsic mathematical interest. The model should be simple at first, but potentially capable of expansion.

5.2. *Characterization of the genetic apparatus.* The genes appear to contain a message specifying structure of all specific molecules within the cell, and morphological and dynamic properties of the cell, as briefly described in this paper.

The genetic message ultimately may be characterized by the DNA code, perhaps in terms of the four nucleotides, making up DNA. Radiation experimentation, however, points to larger subunits, perhaps parts of chromosomes;

as we begin to understand from Kendall's and Powell's analyses of bacterial data and from the work with yeast cells, perhaps there are 20–200 subunits essential to cell division.

When a clone of cells is present, we must represent the genetic apparatus, together with its viable variations: that is, the viable mutants present.

Thus, description of the genetic apparatus can be done by description of the sequence of its subunits and the normal variations of these.

5.3. *Self duplication.* One generally assumes that the genetic apparatus duplicates itself exactly during all reproduction. In fact frequently it may fail in the attempt of self duplication (lethals), particularly following deleterious environmental influence (heat, poison, radiation). Thus, mathematical description of the genetic apparatus should include the probabilities for nonviable mutations.

5.4. Cytoplasmic constituents, for example enzymes, also must be characterized mathematically in terms of their specific structures. Since the genetic apparatus appears to determine specificity, a "dictionary" must exist translating the DNA code to protein code and to morphological codes. The transformation from DNA codes to protein codes should include the possibilities of redundance in the codes and also the possibility that some codes may not be completely determinative.

5.5. In the course of self duplication coupling and feedback must exist between genetic apparatus and nongenetic parts. Phenotypic variation of measurable quantities in the cell is an indication of the looseness of the coupling; synchronous behavior of many cells points to tight coupling. The models should allow for abnormal coupling, for example, lag of cytoplasmic components as described in this paper for the cytochrome system; in the case of lysogenecity the previously existing coupling is destroyed and the phage can multiply unchecked.

5.6. Ultimately the laws of physics must enter into the mathematical description. Gene reproduction will perhaps be described as a process of negative entropy production at the expense of a larger positive entropy change. The rate of enzyme catalyzed reactions will be considered in the framework of the lamellar, membranous structure of cell organelles.

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