Is there an infant mortality in bacteria?

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Abstract
This manuscript proposes a significant step in our long-run investigation of infant mortality across species. Since 2016 (Berrut et al. 2016) a succession of studies (Bois et al. 2019) have traced infant mortality from organisms of high complexity (e.g. mammals) down to unicellular organisms. Infant mortality may be considered as a filtering process through which organisms with potentially lethal congenital defects are eliminated. Such defects may have many causes but here we focus particularly on mishaps resulting from non-optimal conditions in the production of proteins, enzymes and other crucial macromolecules.

The statistical signature of infant mortality consists in a falling age-specific death rate.

The question we address here is whether infant mortality episodes take place in bacteria in the minutes preceding or following cell division. It will be shown that while experiments carried out in the 20th century tried but failed to detect such an effect (mostly because of limited sample size), more recent observations provided consistent evidence of a sizeable mortality, with a rate of the order of 0.7 per 1,000 per hour, in the exponential growth phase of E. coli. A further crucial test will be to measure the age-specific, post-division death rate. An experiment is outlined for that purpose. It is based on the selection of stained cells through flow cytometry and the derivation of their ages at death from their sizes.

If an infant mortality effect can be identified in E. coli it can be conjectured that a similar effect also exists in other unicellular organisms, both prokaryote and eukaryote.

Key-words: Bacteria, division, death, flow cytometry, congenital anomalies

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The juxtaposition in the title of the expressions “infant mortality” along with “bacteria” may at first appear puzzling. However, recall that (i) “infant mortality” is a notion also used in reliability science and (ii) that the question raised in the title has already been investigated (in these very terms) 89 years ago (Kelly et al. 1932). Yet, it is only in the past two decades that some preliminary answers have slowly started to emerge (see below). The specific definition of infant mortality to which we refer is a phase following birth during which the age-specific mortality is a decreasing function of age.

1 Introduction

Within the architectural master plan provided by the genome, the information stored on each gene is to serve as a blueprint for the cell to use when building a specific protein. However, during implementation of this process there can be many pitfalls.

Consider for instance the parallel case of the construction of a building based on an architectural blueprint. In hot weather concrete may dry too fast, thus leading to walls with impaired mechanical characteristics. Freezing weather may also cause problems. In other words, at each step prevailing conditions may not be optimum or, even worse, may fall outside permissible bounds, thus leading to structural flaws. Similarly, inappropriate temperature or pH conditions may lead to incorrect protein production: too little, too much, not at the right location or not an appropriate 3-d molecular structure, any of these defects may possibly lead to life-threatening abnormalities at cellular level. While we are unable to follow such processes at the level of biochemical reactions, visible defects at phenotype level will tell us that “something” went wrong.

1.1 What kind of death?

For lack of a better word, in earlier studies such events were referred to as “production incidents” or “manufacturing mishaps” (Bois et al. 2020). In such incidents the blueprint is correct but not well implemented.

Cells and bacteria may experience several kinds of death. Our focus is on the deaths resulting from manufacturing mishaps. This leaves aside several others forms of death, e.g. deaths due to genetic mutations, senescence deaths due to wear out and tear in growth-arrested bacteria (Yang et al. 2019), death due to apoptosis, deaths due to harmful exogenous factors.

Our target could be described by saying that the deaths we are interested in occur in the screening process of new individuals and are deaths for which no other identifiable cause can be found.

While most of the paper will focus on how to count such deaths, we should begin by briefly explain some of their basic features (additional explanations can be found in Bois et al. 2020).

1.2 Motivation

The first question which comes to mind concerns the motivation for defining this new category of defects? A very direct answer can be found in Fontaine et al. (2008, p.2) where it is expressed as follows.

“Even in the absence of identifiable exogenous stress, there remains a measurable, basal death frequency in [exponentially] growing E. coli populations”.

In this respect see Kibota et al. (1996). The question of the respective weights of genetic versus non-genetic factors was discussed more broadly, based on observations of real twins, in the Appendix of a paper by Bois et al. (2020)
Our first purpose is to account for such unexplained deaths.

1.3 Parallels with reliability science

It is a fairly natural conjecture to assume that the rules developed in reliability science, process management and industrial engineering also apply here. It would be difficult to illustrate this assertion through examples of biochemical reactions at work in the replication process. A typical schematic representation of those complex processes is shown in Fig. 2a from which it is clear that such mechanisms are obviously too complicated to allow a clear insight.

Nevertheless a feeling of what is at stake may be seen by considering the incubation of an egg. This process seems much simpler than the replication of bacteria and yet when one looks closely one realizes that there are many requirements. The creation of new structures relies on a precise spatio-temporal organization. Self-assembly processes must take place at multiple length and time scales.

- Because an egg shell is a technical object its manufacturing process allows a fairly clear insight. Obviously, if too thin or too soft the shell will break during incubation; on the contrary, if too hard, the chick will be unable to break it. In fact the egg’s characteristics must be consistent with the strength of the chick. How this interconnection is implemented, we largely ignore.

- As an example of spatio-temporal organization, it can be mentioned that the amount of yolk must be correlated with the timing of the chick’s development. Too little yolk means that the chick will starve or will not have enough energy to break the shell.

Although to some readers this case may seem simplistic, it has the great advantage that at each step one knows exactly what are the optimal conditions. Moreover, one knows also what will be the effect of non-optimal parameters. Needless to say, for biochemical replication processes we ignore both the optimal conditions and the consequences of non-optimal parameters.

Neither industrial production lines nor broody hens can rely on chance. From basic ingredients to the final product, controls must be set at each step. Similarly, during the brooding process temperature and humidity must remain within narrow bounds, something that can be achieved only through appropriate controls. However, the controls should not be too numerous or take too much time for otherwise the end product will not be ready in time, e.g. the yolk and albumin will be exhausted before the growth process is completed.

1.4 Rules of manufacturing volatility

Will the introduction of the notion of manufacturing mishaps give us a better understanding and even allow us to offer predictions? We believe so and the following points give some hints in this respect.

- Randomness versus deterministic rules In the occurrence of manufacturing mishaps there is both a random and a deterministic part. The random part reflects the fact, already mentioned, that usually the optimal production parameters are not known. The assumption of randomness (or, if one prefers, stochasticity that is randomness in time-dependent processes) emphasizes that, except possibly in special cases, it would be a hopeless task to question the exact origin of abnormalities.

The deterministic part results from the general rules of production management already mentioned and of which illustrations are given below.

- Why high volatility is to be expected in long pathways. The reproduction of a cell comprises many separate tasks, each of which is a sequential process involving a succession of steps. At this point a remark is in order. There is a common analogy in which the DNA is represented by a dog chain. Each individual link of the chain is a reaction. This analogy is useful in illustrating the sequential nature of the process but it fails when it is necessary to take into account the properties of the links that are not always the same in nature and that may have a different resistance.

In the analogy of the chain, the entire chain is equivalent to the entire cell. The length of the chain is equivalent to the number of reactions that take place in the cell. The links that are alike are equivalent to the reactions that are alike. However, the properties of the links may be different, and this can lead to complications. For example, if one link is broken, the entire chain is disrupted. This is analogous to the situation in which one reaction goes wrong, and the entire process is disrupted.

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sheet music and the actual cell processes by the orchestra’s performance. One should realize that this analogy misses one very important point. An incident in a performance, e.g. when one of the musicians momentarily is out of tune, is of no consequence for the remaining part of the program. On the contrary, in the construction of a building if its foundation or one of the load-bearing walls are faulty this will jeopardise the whole structure. As biochemical reactions often work in sequences where the products of one serve as reactants for the next, a flaw at the beginning of the chain will have more serious consequences than one which occurs toward the end of the chain. The same argument also suggests that on average the frequency of mishaps increases with the length of reaction chains.

- **Greater accuracy requirements make faults more frequent and more consequential.** As already mentioned, the production of an egg shell is a fairly critical process, far more critical than for instance the production of the albumen content of the egg. In the same line of thought it can be observed that strabismus is one of the most common congenital defects. More generally, coordinated movements are a complex processes that involve many different muscles, nerves and parts of the brain. Any problem in this process may lead to difficulties that will be more consequential (and visible) than defects in processes which do not require the same level of coordination. In short, the higher the complexity of a manufacturing process, the higher the risk of error propagation.

- **Is a faster division time an aggravating factor?** In industrial production when the time allocated to a given task is reduced less controls are performed which in turn may result in more defects going undetected. Something similar can be conjectured in the process of bacterial replication. To put numbers on this issue let us mention two populations studied by Kelly and Rahn (1932). They observed divisions of *Bacterium aerogenes* whose average division time was 30mn and budings of *Saccharomyces ellipsoideus* whose average budding times was 105mn, i.e. 3.5 times longer. If our conjecture holds the fastest process should have a higher death rate.

- **Are volatility and deaths correlated?** As a characteristic easier to identify than death, one is tempted to consider volatility in daughter’s features. After all, occurrences of deaths may be seen as cases of volatility becoming so excessive that stretched features become incompatible with the continuation of life.

It is well known that at the beginning of the exponential phase the reproduction process is so fast that replication starts even before separation is completed. If our previous conjecture is correct, one should see in this phase a volatility that is higher than in the subsequent, slower part of the exponential growth.

### 1.5 Perspectives opened by the investigation of congenital defects

The previous points can be summarized by saying that any information about a process (e.g. length of passways, accuracy requirements) will allow us to propose conjectures and predictions concerning congenital anomalies.

Moreover, observation of manufacturing mishaps in the exponential growth phase is the simplest case one can think of. There are two reasons.

(i) The fact that all cells are clones limits genetic effects.

(ii) It has been emphasized (see Sezonov et al. 2007) that during the exponential phase all intrinsic parameters of the cells (e.g. their macromolecular composition) remain constant.

In other words, through its simplicity, this system should provide a valuable opening, just as the study of the hydrogen atom opened the way to atomic spectroscopy.

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2 The medical term for this condition is “developmental coordination disorder” (DCD) or more shortly dyspraxia. In this framework strabismus would be called oculo-motor dyspraxia.

3 The term “volatility” is understood here with the same meaning that it has in finance, i.e. it includes all statistical estimates of variability.
The rest of the paper proceeds as follows.

- In section 2 we draw on the mechanisms described above to predict possible regularities to be observed in infant mortality rates.
- Section 3 gives accounts of some former attempts at measuring post-fission death rates. It appears that this issue already attracted attention a long time ago.
- In the penultimate section prior to the conclusion we suggest a possible protocol based on modern techniques.

2 Expected regularities of infant mortality rates

First, we wish to explain the exact meaning given here to the expression “infant mortality”.

2.1 Meaning of infant mortality

In medical terminology “infant mortality” means mortality during the first year after birth. Conceptually this definition is not satisfactory however.

![Graph showing infant and adult mortality in humans and rotifers](image)

**Fig.1a Infant and adult mortality in humans and rotifers.** For human newborns very accurate mortality data are available which extend from a few hours after birth to late childhood. In this phase the infant mortality decreases as a power law of the form: \( y = 1/x^\alpha \), where the exponent \( \alpha \) is close to 1. Whereas for humans the phase of infant mortality lasts about 10 years for rotifers (swimming animals of a size of about 50 micrometers) it lasts only 10 hours. **Sources:** The statistical data are for the United States for the period 1999-2016 and are taken from the CDC data base for detailed mortality. The rotifer data are from an experiment conducted in 2019 and described in Bois et al. (2019).

What distinguishes newborn mortality from adult mortality is the fact that the first decreases with age whereas the second increases with age in conformity with Gompertz’s law (see Fig.1a). However, the decrease phase does not end after the first year, it continues until through the age of 10. A coherent definition of “infant mortality” should include the whole age interval during which the mortality rate
Fig.1b Schematic representation of the replication and division process of E. coli. At first sight reproduction by fission may seem very different from reproduction in multicellular organisms. However, some parallels can be drawn. The phase between replication of the DNA and separation of the two cells can be seen as the combination of two processes. First, the production of an oocyte followed by a process of embryogenesis and then finally the transformation of the embryo into a newborn by hatching of eggs or otherwise. Needless to say, this is a very schematic representation. For instance the DNA is shown concentrated whereas it is rather spread throughout the whole cytoplasm. Fig.2a shows again these steps (though even more schematically) because they are the starting point of our experiment. The periods B, C and D are a standard periodisation of the whole reproduction process.

Actually, the fact that after the age of 10 the mortality rate starts to increase does not mean that the deaths due to congenital factors come to an end, it rather means that adult mortality becomes dominant. Seemingly minor congenital defects can become a cause of death much later in life. We return to this point later.

2.2 Birth ends “compensation effects”

What is meant by the expression “compensation effect”?
In a multicellular organism each organ involves many cells, as a result the death of one (or even a small number) of them will have no serious consequences because the remaining cells will try to compensate the lost capacity. When there are only two cells, a shortage in one may still be compensated (at least partially) by the other. This effect is illustrated in Fig.2a. A shortage of, say protein A is compensated through transfer from the other cell. However, separation ends such compensation mechanisms. After birth, human newborns must be able to breathe; similarly after division, the daughter cell must be able to produce all proteins it needs.

2.3 Accumulation of defects

The accumulation of defects is a phenomenon which may be seen as specific to organisms which reproduce by division. The reason derives immediately from the argument just given. If one sup-
poses that the shortage of $A$ is not lethal and does not prevent reproduction, this defect will remain in existence on the mother side in subsequent generations. Such a scenario is consistent with the phenomenon of aging demonstrated in Stewart et al. (2005).

Although somewhat different in its occurrence, the phenomenon of defect accumulation is akin to the gradual wear-out that occurs in the aging process of multicellular organisms. As an illustration, consider osteoporosis; although it becomes visible only in old age, in fact it starts around the age of 25. There are many other examples, e.g. the ability to hear high frequencies or maximum heart rate capability, or progression of stiffness in heart valves. What distinguishes a heart valve problem in old age from a neonatal heart valve problem is not the organ involved which is the same but the fact that the first comes gradually whereas the second is triggered by a massive defect and leads to death within a few days.

One of the most fragile components of bacteria seems to be their membrane. In this case it is easy to imagine the same two regimes: (i) a gradual erosion of the membrane due to growing inability to produce the required proteins versus (ii) a dramatic failure in the form of a breach due to a lethal manufacturing defect. If massive enough, it could kill both daughter and mother cells if it occurs during the short time interval between “production” of the daughter cell (by its own DNA) and separation of the two cells.

The fact that membrane failure is a major cause of death is demonstrated by the data of Table 1, namely the near equivalence of deaths identified by stains (i.e. resulting from breached membranes) and the deaths identified by immobility and inability to divide further.

The process leading to a cell death is schematized in Fig.2a. Incidentally, human infant mortality strongly suggests that non-lethal defects largely outnumber lethal defects. However, for bacteria it is very difficult to identify non-lethal defects. The death rate gives only a crude estimate of the most serious manufacturing defects but at least it can be measured.

One obstacle to sound measurement is of course the well-known inherent variability (of the order of 30%) across generations in the lengths of $E. coli$ at same stage of their life cycle (for instance at birth). As this variability seems to be truly random (see. Adicptainingrum 2015) it is possible to get rid of it through the time-honored method of taking averages over a large number of repeated events.

2.4 How can ages at death be estimated

Once the magnitude of the death effect has been asserted the second objective is to estimate the age at death. As in $E. coli$ age is strongly correlated with size, one can use the latter to get age estimates. For this purpose rod-shaped organisms are particularly convenient.

2.5 Conjecture for the post-birth death rate

In all species studied in Bois et al. (2019) the post-birth age-specific death rate was found to be a decreasing function of age. More precisely, it was found to be an hyperbolic fall. We conjecture that it will be the same here.

The experiment which should allow us to observe the age-specific death rate and check this conjecture is schematically summarized in Fig.2b,c. Let us explain how the hyperbolic fall conjecture can be tested. Assume that in 100,000 daughter cells which emerge from the fission process there are some 100 with potentially lethal defects that can be graded from very severe to somewhat less severe (the analog in human newborns would be heart defects ranging from misshapen hearts to only malformations of
**A** From DNA replication to death of daughter cell

![Diagram](image)

**Synthesis of a protein in the course of cell replication**

**Defect appears in daughter**
**Division makes defect potentially lethal**
**Death occurs after deferment time lag**

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**Fig. 2a Occurrence of a defect in a daughter cell.** The main purpose of the picture on the left hand side is to suggest that the synthesis of a single protein macromolecule is a process which involves many successive steps each of which comprises a chain of appropriate biochemical reactions. Reactions occurring in non-optimal conditions (e.g. inappropriate temperature or pH level) may affect the structure of proteins, enzymes and other important molecules. Eventually, this may lead to a default (schematized by an empty circle). At first the default may not be of great consequence for as long as the two cells are tied together a default in one can be compensated by the other. After division each cell must be able to live on its own resources which is likely to make the consequences of any defect much more serious (there is a similar situation for newborns immediately after birth). It is for the purpose of clarity that the nucleus are shown as black circles. *Source for protein synthesis picture: Slideshare website (public resource).*

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the aortic valve). The cells with first grade defects will die immediately, those with grade 2 severity somewhat later, and so on. As more and more cells die, those which remain will experience a smaller death rate.

Let us divide the age interval following fission into subintervals in the following way (time units are minutes):

\[ I_1 = (1, 2), I_2 = (2, 4), I_3 = (4, 8), I_4 = (8, 16) \]

It was found in Bois et al. (2019) that the numbers of deaths in each of these subintervals are nearly the same. This rule relies on an age-specific death rate curve of the form: \( y = C/x^\alpha \) where \( x \) is age, \( y \) the number of deaths and \( \alpha \) an exponent that is close to 1. This rule means that, in infant mortality, if age is multiplied by 2, \( y \) is divided by 2.

If, as suggested earlier, age is estimated by the length of the bacteria, the previous statement can be easily reformulated in terms of size. If the pattern described above can be observed it would be a further confirmation of a regularity already observed in other organisms.

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### 3 Toward accurate measurements of post-fission death rates

Attempts to measure the death rate of individual bacterial cells go back at least to the beginning of the 20th century. Rather than to provide a historical review, our goal is to show that this problem has
From death of daughter cell to flow cytometry

**Fig.2b: Identification of dead cells by life/death stain.** Staining and cell counting by flow cytometry (FC) work hand in hand. As the stain can diffuse into the cell only when the cell membrane is breached, the measurement is likely to provide a lower bound of the death rate. A clear advantage of the FC technique consists in the size of the samples that can be treated. Whereas in the observation of individual cells the sample-size in each assay is limited to a few hundreds, in FC samples of one million can be tested in each run. SG which means Sytox Green is the commercial name of a life/death stain. It can diffuse into a cell whose membrane is breached. Source: The FC picture is from Internet (public resource)

been recognized as important for a long time.

### 3.1 Paper of Kelly and Rahn (1932)

This is by no means the first paper on this problem but it provides the clearest statement of why it is a key-issue. The first lines of the paper are worth being quoted verbatim.

“It has been assumed by many bacteriologists that during the period of rapid growth, in a satisfactory culture medium, some bacteria will die in spite of good food and favorable environment. No doubt this assumption was derived from an analogy with populations of higher forms of life, of which a number of individuals are known to die before they reach the reproductive age even with good care.” (Kelly et al. 1932, p.147)

Using a method pioneered by J. Orskov (1922), the authors followed the replication of individual bacteria (and one yeast species) on a solid medium. They recorded the family trees spanning 4 generations. Altogether they observed 1,766 divisions. 977 of *Bacterium aerogenes*, 325 of *Bacillus cereus*, 464 *Saccharomyces ellipsoideus*. On average the time intervals between fissions of *Bacterium aerogenes* was 30 mn but with great inter-individual fluctuations (coefficient of variation $\sigma/m$ close to 100%).

The observations led the authors to the following conclusions.

- There was not a single instance where a cell ceased multiplying or became dormant.
- In the very words used by the author, there was not a single case of “infant mortality”, that is to say a cell which died after division.
Fig. 2c: Distribution of dead cells by age. The age-specific infant mortality has a characteristic shape (explained in the text). The droplets on the right-hand side contain the dead cells which will be investigated separately. The two other droplet releases contain the living cells and may be cells selected through a third criterion. Incidentally, most flow cytometers have the ability to measure the size of the cells at the same time as they identify the dead cells, but one would expect this capability to be more reliable for spherical bacteria than for rod-shaped bacteria. The later allow a better accuracy for the simple reason that for a given change of volume $\Delta V$ the change of the length is: $\Delta L \sim \Delta V$, whereas the change of the radius of a sphere is only: $\Delta R \sim [\Delta V]^{1/3}$. In the figure the acronyms FSC and SSC mean forward scattering and backward scattering respectively. The expression “stealth fluid” refers to a layer of fluid which ensures that the flow is laminar. This is particularly important for reliable measurement of the length of rod-shaped cells. The end step microscopic examination of the dead cells has a two-fold purpose: (i) to control the reliability of the length estimates (ii) to allow a close examination of the damaged cell membranes.

The first conclusion is reassuring because, even today, researchers often worry about the risk of mistaking dormant cells for dead cells (see the discussion at the beginning of Garvey et al. 2007).

The second conclusion is also interesting for it shows that if such an infant mortality exists (as is indeed observed in experiments done in the past two decades as reported below) then its rate is lower than: $1/1766 = 0.56$ per 1,000. (for a time interval of 30mn). This is a rough estimate obtained by bulking together all three species. Estimates detailed by species (at least for the two largest) are given in Table 1.
3.2 The Wilson paper (1922)

Ten years before the article by Kelly and Rahn, Wilson was already calling into question the dogma that in a young broth culture (up to 24 hours) all bacteria are living. Here is what he writes at the beginning of his paper.

"On looking up the literature it was found that of the many observers [the author cites 9 papers published between 1898 and 1920] who had made a comparison of the two counts [namely the total number of cells that can be counted in a culture, whether dead or alive on the one hand and the number of viable cells defined as cells that are able to fission on the other hand], the discrepancy was passed over with little comment. As in this stage all bacteria were assumed alive any discrepancy had to be due to errors."

Then, in his lengthy 41-page long paper the author examines one by one all successive operations required in a counting procedure. He tries to make them as rigorous as possible and he estimates the remaining error margin. For instance dilution before counting is an operation which requires great care but nevertheless cannot be made very precise. In fact, the author was facing an impossible task. It should be realized that despite being a standard counting technique in the 1920s, this was completely different from the observation of individual cells as pioneered by Kelly and Rahn (1932). Such global counting techniques were beset by too many uncertainties. Therefore, it is not surprising that Wilson arrives at vague and disappointing conclusions (p.444).

"It seems that in cultures of *Bact. suipestifer* there is a normal death rate even during the period of maximum growth. Its extend will vary from culture to culture. In some it is as high as 43%, in others it is only 20% or 10%, while finally in a few it is for a short period actually nil.

The Wilson paper makes us realize that the observation of individual cells pioneered by Kelly and Rahn represented a breakthrough. It is true that they were unable to see any death in the exponential phase but that was only because their sample of 1,766 cells was too small. Their methodology was sound and opened the door to further observations, possibly with larger samples. Yet, this did not happen until 73 years later. Indeed, in 2005 this investigation was resumed. Thanks to modern computerized counting techniques and a sample some 20 times larger namely 35,000 *E. coli* cells, it revealed some 16 deaths; see Stewart et al. 2005[4].

Whereas the measurement of Stewart et al. (2005) relies on the observation of individual cells, a paper of 2008 by Fontaine et al. relies on a global (not individual) observation. It is to the discussion of these modern investigations that we turn now.

3.3 The paper by Stewart et al. (2005)

In the Kelly and Rahn (1932) experiment successive divisions were followed over 4 generations, a process which from each single initial cell produced \(2^4 = 16\) cells. In the Stewart et al (2005) experiment (subsequently “Stewart 05”) up to 9 generations were followed, a process through which each initial cell gave rise to \(2^9 = 512\) *E. coli* cells. Time-lapse images were taken and analyzed automatically thanks to a dedicated software. As 94 colonies were analyzed this led to a total of 35,049 divisions.

The criterion used for the definition of death was immobility combined with no growth. Some 16 cell death were observed. Unfortunately their sizes were not included in the publication because the main purpose of the paper was in fact the detailed study of aging.

\(^4\)In fact, this was not the only objective of the investigation. Another purpose was to study the erosion of the fission ability in the course of successive generations. Such an erosion was seen as an indicator of aging.
Table 1: Bacterial death rates in the exponential phase

| Year | Paper | Method | Sample size | Number of deaths | Death rate (dr) per 1,000 and per hour |
|------|-------|--------|-------------|-----------------|---------------------------------------|
| 1 1922 | Wilson | Global | undefined | unreliable | unreliable |
| 2 1932 | Kelly (1) | Individual | 733 | < 1 | dr < 4.1 |
| 3 1932 | Kelly (2) | Individual | 420 | < 1 | dr < 1.4 |
| 4 2005 | Stewart | Individual | 35,049 | 16 | 1.5 |
| 5 2008 | Fontaine | Global | $10^6$ | 700 | 0.7 |

Notes: “Global” means measurement performed on a large number of cells in liquid medium. For this global measurement it is the technique of flow cytometry which brought about a breakthrough and allowed reliable measurements. The following bacteria and yeast were investigated (in parenthesis is the length in minutes of the reproduction cycle). 1: Bact. suipesifer and other species, 2: Bacterium aerogenes (30mn), 3: Saccharomyces ellipoideus (105mn), 4: E. coli (30mn), 5: E. coli (30mn). For case 5 it was assumed that the state having an optical density OD$_{600}$ around 0.2 lasted one hour.

Sources: Based on the papers cited in the third column.

3.4 Paper of 2008 by Fontaine et al. (FC measurement)

Beyond its specific purpose, this paper (thereafter “Fontaine 08”) can be seen as a continuation of the previous one as is indeed confirmed by the participation of two authors in both papers.

In the technique that is used here the cells are not monitored individually. Instead, the recourse to flow cytometry allows global estimates. Stained dead cells, or more precisely those cells whose breached membranes allow the stain to drift into the cytoplasm, are counted thanks to a flow cytometer (FC). In such a device the light of a laser is diffused by the cells when they move through the beam, then received by a sensor and amplified by a photomultiplier and finally analyzed by a computer software algorithm. Flow cytometry allows many characteristics of the cells to be identified and recorded. Here this technique is used to count stained or fluorescent dead cells.

Flow cytometry began to be used in the 1950s and was really a game changer. It replaced the successive manipulations that we mentioned in our account of the Wilson paper of 1922. Whereas improving their accuracy was an impossible task, FC provided a completely new approach which proved effective and reliable.

Although FC is the key of the measurement method, a number of additional verification tests are required to ensure that what is measured by the device is indeed the appropriate death rate.

3.5 Accumulation and amplification of mishaps

Here we come back to the question of the accumulation of defects in order to see how the shape of the age-specific death rate may be affected.

Assume that in generation $k$ a protein (call it $P$) has been produced in insufficient quantity, then in the next replication process there are two possibilities.

- The problem of the low level of $P$ is identified and corrected by an appropriate temporary over-production. Under this assumption generation $k + 1$ will have regained its nominate characteristics.
- Here we assume that the problem, either is not identified or, if identified, cannot be corrected because under current conditions increased production of $P$ is not possible.
In this case the default will not be corrected and may even be aggravated. Even though the initial mishap was not lethal it may become so in generation $k + 1$, $k + 2$ or later on.

Note that in generation $k + 1$ the default is likely to be shared equally by the mother and daughter which means that the compensation mechanism illustrated in Fig.2b cannot take place. Therefore, the separation of mother and daughter will not have the same filtering effect. Under this assumption there is no reason for death to occur specifically in the first moments after separation. This phenomenon would give a fairly uniform distribution of deaths with respect to age.

In short, according to the previous discussion, a fairly uniform distribution of cell deaths over their life time would suggest a mechanism of gradual amplification of mishaps. The occurrence of pair-wise deaths (meaning deaths of both mother and daughter at about the same age after division) would be an indication pointing in the same direction.

3.6 Summary of death rates measured in the exponential phase

Table 1 presents the death rates results found in the papers discussed. They cluster reasonably well around an average value of 1 per 1,000 and per hour.

For red blood cells, using data found on the Internet (30 trillion red blood cells in total, 2 million die per second) one gets a death rate of: 0.2 per 1,000 and per hour. These cells are somewhat special in the sense that they have no nucleus and therefore cannot make proteins to repair themselves. Their life span is known to be of the order of 120 days. Thus, one would expect their death rate to be a kind of upper bound.

3.7 Life-death separation based on buoyant density

The experiment described in Fig.2a,b,c comprises two successive steps, first the cytometric life-death separation and secondly the optical determination of the distribution of the sizes of the dead cells.

Instead of the cytometric separation, can one imagine a separation based on buoyant density? The idea is not altogether absurd. Several studies (Pierucci 1979 Table 1, Woldrigh et al. 1981 Table 1) have shown that the specific gravity of E. coli cells is about 1.1. Moreover, whereas the length of the cells is affected by the growth rate (the variabilty reaches 40%), the specific gravity is very stable (variability lower than 2%). This means that in water dead cells will sink to the bottom whereas, due to their mobility, living cells will remain distributed between bottom and surface. The key-question is how fast they will sink; it can be answered based on Stokes law. This law applies whenever the Reynolds number is smaller than 1 which is obviously true for objects as small as bacteria. A falling cell experiences three forces: (i) downward gravity force and two upward forces: (ii) buoyancy (iii) drag given by Stokes formula. Once the velocity has become stationary the sum of the three forces is zero and from this equation one can derive the stationary velocity $v$ in the following form.

$$v = \frac{d^2 g (\rho_c - \rho_w)}{18 \mu}$$

where:
- $d$: Equivalent spherical diameter of a cell rod; about 1 cubic micrometer.
- $g$: acceleration of gravity, $g = 9.81 \text{m/s}^2$
- $\rho_c$, $\rho_w$: specific gravity of cell and water respectively, $\rho_c = 1.1$, $\rho_w = 1.0$
- $\mu$: Dynamic viscosity of water, $\mu = 8.9 \times 10^{-4} \text{Pa}\times\text{s}$.

The specific gravity of an LB medium is almost 1.
One gets: \( v = 6 \) micrometer/minute, a speed which is of course too slow to be useful. In the present case one cannot use centrifugation for the centrifuge force would overpower the tiny upward sustaining force generated by the cells. The velocity \( v \) may be substantially increased by replacing water by a liquid of lower specific gravity and lower viscosity.

The previous calculation can tell us something else which may be of interest for the step following cytometry. When one uses centrifugation to increase the concentration in dead cells of the solution provided by the cytometer, the previous result shows that to get a velocity of 6mm/minute one must replace \( g \) by \( 1000g \). As, however, the shape and size of the cells may be affected by such a high acceleration, we see that it is certainly better to use only \( 10g \), yet applied during 100 mn. in order to get the same separation.

4 Conclusions

The main incentive for measuring age-specific death rates is to verify whether our understanding of infant mortality gained from the observation of multicellular organisms is correct. The conjectures proposed in the present note will offer instructive tests.

Human infant mortality helps us to understand congenital anomalies. In a similar way, the infant mortality of microorganisms reflects the mechanisms which rule their life cycle. Today new technologies provide a novel route to test our conjectures and give us a better understanding of questions which have challenged scientists for well over a century. Preliminary cytometric measurements (which require confirmation) suggest the existence of a mortality peak in the minutes prior to separation.

We have summarized the evidence already available and we have outlined a protocol in the hope that it will give our readers an incentive to carry out the experiment.

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Ethical statement

1 The authors did not receive any funding.
2 The authors do not have any conflict of interest.
3 The study does not involve any experiment with animals that would require ethical approval.
4 The study does not involve any participants that would have to give their informed consent.

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