Transient frailty induced by cell division. Observation, reasons and implications

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Abstract

We know that stress-factors, e.g. X-rays, have an effect on cells that is more lethal in rapid exponential growth than in stationary phase. It is this effect which makes radiotherapy effective in cancer treatment. This stress effect can be explained in two ways: (a) more vulnerability in the growth phase, (b) improved protection capacity and repair mechanisms in the stationary phase. Although the two explanations do not exclude each other, they are very different in the sense that (a) is a general mechanism whereas (b) is strain and stress-factor dependent.

In this paper we explore major facets of (a). Firstly, we emphasize that (a) can account for known experimental stress-factor evidence. Secondly, we observe that (a) rightly predicts that slow exponential growth (meaning with a doubling time of several hours) results in a lower death rate than fast exponential growth (doubling time of a fraction of one hour), an effect that cannot be explained in the (b) framework because both organisms are in the same phase. Thirdly, we conjecture that the stress-factor effect can be extended to situations of chronic stress due to non-optimal environmental conditions. If correct, this conjecture would imply that even in normal culture conditions the natural death rate is lower in the stationary phase than in the growth phase. Finally, the paper closes with the description of several open questions and of appropriate test-experiments meant to address them.

1 Introduction

To facilitate reading it can be observed that this paper was written in what can be called the “spirit of physics”. This means that it focuses on general causes rather than on the details of specific cases. As a parallel in physics one can mention the Navier-Stokes equations which describe the basic features of fluid flows, whether in air, water or any other fluid.

1.1 Vital shocks

It is a natural idea to suspect that when a living organism undergoes a major transition from a state $A$ to a state $B$ it faces elevated risks until the adjustment is completed successfully. Note that the change may affect the environment of the organism, or/and its internal state. An example which comes to mind is the process of moulding, e.g. when a cicada gets rid of its former exoskeleton. Although the new replacement exoskeleton has taken form under the old one, it remains soft for several hours (or days in case of larger species). During this short time the cicada is more vulnerable to predators.
In a series of previous papers (Richmond et al. 2016a,b) we have considered transient situations in human demography. Several cases were described in which an abrupt change in living conditions leads to a mortality spike. This effect was summarized in what we called the “Transient Shock conjecture”. It provides a qualitative model which leads to testable predictions. For instance, statistical evidence shows that persons who become widowed experience within months a mortality spike that is higher than their average long-term death rate either before or after becoming widowed. This case may not come as a surprise. More revealing is the marriage transition. Marriage certainly brings about a major change in personal and social conditions and according to our conjecture one would expect a mortality spike in the months following marriage. At first sight this may seem an unlikely proposition but it was shown that the existence of such a mortality spike is indeed supported by statistical evidence. (Richmond et al 2016b)

There is a noteworthy difference between widowhood and marriage in the sense that widowhood occurs usually in old age when there is already a situation of frailty. A similar case considered in the papers mentioned above concerns elderly persons who move from their home to a nursing home. The few data that are available for such cases often display a mortality shock in the months following admission. Here too, there is certainly a frailty effect. The biological phenomenon considered in the present paper will also involve these two components: (i) a shock in the form of an exogenous stress factor and (ii) a situation of frailty.

1.2 Births seen as transient shocks

Coming now to biological cases, in humans and in many other species, birth constitutes a major shock which, not surprisingly, results in a huge mortality spike (Berrut et al. 2016). This means that the death rate in the days following birth is much higher than the death rate in utero in the weeks preceding birth and also higher than the neonatal death rate (i.e. average death rate over the first 6 weeks). It can be observed that a large part (over 50%) of these early deaths are due to congenital malformations which were compatible with life in utero, but become lethal in the new environment; lung defects are a clear example. The congenital malformations may appear during embryogenesis but may also be triggered by defects already present in the initial female and male germ cells\(^1\), i.e. the oocytes and spermatozoons.

\(^1\)The extent of such defects is attested by the huge mortality peak in the initial phase of the embryogenesis (see Chen et al. 2020)
1.3 How the process of fast cell replication induces frailty

The question that we address in this contribution is whether or not cells experience a transient shock (similar to birth) in the process of division. We will propose reasons valid for both eukaryotic cells and bacteria for a state of fragility associated with cell division.

For a bacteria like *Escherichia coli*, the generation time is about 20mn. During this short time, many complicated processes have to be carried out (Fig.1). One can keep in mind (Soufi et al. 2015) that the proteome (i.e. the entire set of proteins expressed in the cell) of *E. coli* is comprised of some 2,300 different proteins with concentrations ranging from just a few copies to some 300,000 for the more abundant. Appendix A gives an exemple of the working of a protein enzyme.

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**Fig.1 Replication and division of *E. coli*.** The first phase is marked by intensive protein production and replication of the genome and other components. It is the analog of the interphase in eukaryote cells. The replication of all the components of the cell is certainly a major task of information processing. On the right-hand side of the diagram it was assumed that, due to a defect in the replication procedure, a key-protein $P_1$ was not produced in appropriate amount in the upper cell. Despite that, the situation remained manageable as long as exchanges were possible with the mother cell. After separation the lack of $P_1$ may prove lethal. This fragility induced by division will be called the “replication frailty”.

In the manufacturing of an airliner accuracy requirements differ from one component to the other. If millimetric accuracy may be acceptable for the airframe, there are tighter requirements for the engines. There are good reasons to think that in the process of Fig.1 the most critical part is the replication phase.

- Any fault in the production of the proteins which function as the bricks and tools of the replication process may subsequently lead to fatal failures of the kind shown in the figure.
It is true that there are control and reparation mechanisms. For instance if a gap appears in the replication of the DNA the two ends may be joined again thanks to the cohesion forces which exist between consecutive elements. The problem is that control and reparations take time whereas in fact the whole process is designed to achieve high speed. For instance, instead of starting from one location of the DNA molecule, replication starts simultaneously from different locations. This speed requirement is a major cause of fragility. It will be seen shortly that bacteria having a doubling time of 20mn are much more vulnerable to stress factors than others whose doubling time is 5 hours.

Another crucial moment is the separation. However, what makes it risky are the mishaps that occured in the replication. To work well, *E. coli* cells need thousands of proteins. Now, suppose that something went wrong in the replication with the result that an essential enzyme-protein $P_1$ is not produced in appropriate quantity. As long as exchanges remain possible, the daughter cell can rely on the $P_1$ produced by the mother cell. This kind of sharing becomes impossible after separation. In other words, the death rate of daughter cells will reflect the replication defects, much in the same way as post-birth human mortality reflects the manufacturing anomalies that occurred during embryogenesis. Naturally, not all defects are lethal immediately after separation. In humans it is well known that some congenital defects (e.g. heart valve anomalies) become life-threatening only in old age when compounded with other factors, e.g. increased membrane rigidity. Whenever they occur, non lethal replication anomalies leave daughter cells in a state of fragility. If in addition the cells are subjected to adverse environmental conditions (e.g. X-rays, non optimal temperature, pressure or acidity) this fragility will bring about a death rate spike.

### 1.4 How the paper proceeds

So far we have explained the mechanisms on which our understanding relies. In the rest of the paper we adress two questions.

1. How compatible are the data on death rates in bacteria with the existence of a transient state of frailty during cell division. Many experimental results are available which describe the response of bacteria to harmful exogenous factors. It appears that the populations with high growth rates are much more sensitive to those harmful conditions than those with slow growth rates. Illustrative evidence will be presented and discussed.

2. Has the transient shock model predictive implications not yet considered and which may suggest new experimental tests? Can the tests on bacteria be extended to

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$^2$More precisely, (i) the replication starts in two directions and (ii) once the specific starting point has been duplicated, replication may also start from this point.
other systems of growing organisms, e.g. replication of viruses in cells, replication of eukaryote cells, cleavage of first cell in an embryo?

## 2 Response of bacteria to adverse exogenous factors

Adverse exogenous factors can be of many kinds:
- Ionizing radiations (e.g. X or gamma rays)
- Low or high temperature
- High pressure
- Inadequate acidity
- Disinfectants and antibiotics
- Pulsed electromagnetic fields
- Ultra violet light

Here we wish to focus on parameters which modify the division rate. The following can be considered.
- Normal cells versus cancer cells will be considered in the next subsection. As is well known cancer cells are more vulnerable to X-rays than normal cells.
- Different strains of bacteria have generation times (also referred to as doubling times) ranging from 20mn to several hours. Those with the fastest growth are the most vulnerable. An illustrative example will be given.
- Any new cultures of bacteria starts with a period of fast exponential growth but after a while it enters a stationary phase in which its birth rate converges toward zero. It will be seen that bacteria taken from the rapid growth regime are much more vulnerable to stress factors than those from the stationary phase. An illustrative example will be described.

Among the many publications on these effects a good introduction is provided by a series of three papers which emphasize the role of the growth rate, namely Ihssen et al. 2004, Berney et al. 2006, Lindqvist et al. 2014. They consider the cases of temperature jumps, ultra violet light and acidity. The impact of very high pressures of over 1,000 bars has been studied in Benito et al. 1999 and Manas et al. 2004.

### 2.1 Early observation of the division effect

One of the first observations of the replication effect was made in the early 20th century when it was discovered that ionizing radiations can destroy cancer cells without too much affecting surrounding cells. After coming about through the activation of germ-like stem cells, normal cells multiply exponentially until the required number is reached. Then, they enter a stationary phase in which their growth rate is almost
zero. On the contrary, cancer cells can grow exponentially without limitation. As an example, in the case of small cell lung cancer the doubling time of the cells can be as short as 30 days.

Why were cancer cells more affected than normal cells? There are (at least) three possible interpretations.

1. One explanation is to say that the radiations disrupt the DNA to the point of making any subsequent division impossible. Yet, taken alone, this reason cannot explain why normal cells are less affected.

2. Another explanation is to say that in the stationary phase the cells develop a number of protections which shields them from the radiations.

3. The third explanation relies on the fact that, as described above, cell divisions bring about a state of fragility which makes them vulnerable, not just to radiations but to any adverse factor. In this interpretation the more divisions cells undergo, the more vulnerable they are.

One great difference between interpretations (2) and (3) is that (3) not only explains the radiation effect but has also predictive implications for cell responses to other harmful factors. Actually, (2) and (3) do not exclude one another but are rather complementary. It is true that in the stationary phase cells develop means for long term preservation. The transformation of cells into spores is a clear illustration of this kind of mechanism. Yet, explanation (2) has no predictive power; it gives a description of the sporulation process whenever there is one, but it cannot predict what cells have the ability to form spores or what other preservation mechanisms can be activated.

Quite surprisingly, despite its early observation, the fragility mechanism has been all but forgotten in subsequent decades. A 343-page manual on how bacteria are affected by radiations, published in 1973 by the “International Atomic Energy Agency” (IAEA) does not even mention this effect. In contrast, there have been numerous papers describing various protection mechanisms of type (2). As such mechanisms are species-dependent, it may be a case of trees concealing the forest. We hope that by highlighting the frailty explanation the present paper will bring it back to the attention of researchers.

### 2.2 Prokaryotic versus eukaryotic cells

Why should replication and division put bacteria (that is to say prokaryotic cells) like *E. coli* in a particularly vulnerable situation? The fact that in bacteria the replication of the DNA progresses at the impressive speed of 1,000 nucleotides per second, compared to 100 per second for eukaryotes suggests that such high speed processes
are probably hypersensitive even to small perturbations. In the present paper we will
not discuss this point in more detail; our plan is to accept it as a fact and analyze its
implications.

Radiotherapy relies on the replication effect in eukaryotic cells and this makes us
expect an even stronger division effect in bacteria. In the following subsections we
give examples which quantify this effect. It will be shown to what extent the death
rate occasioned by adverse conditions is conditioned by the frequency of replications.

2.3 Effect of ionizing radiations

We start with this case because, as described above, it was historically the first to
attract the attention of researchers.

The following observation conveys very clearly the idea of the frailty effect.
It has been shown (see Lacassagne 1930 and Lea 1947, p.307) that when a cell is
irradiated death does not occur immediately but at (or following) the next division.
For instance, 10,000 roentgens delivered to yeast allows the cells to divide but their
daughter cells are usually unable to divide further and eventually die. A much larger
dose is required to kill the cells immediately; thus, a dose of 30,000 roentgens will
kill only 50%.

The same effect can be seen for eukaryote cells. When chick tissue growing in cul-
ture is irradiated, a dose of 100 r suffices to cause the death of the cells which attempt
to divide, but 2,500 r are required to cause the death of an appreciable proportion of
resting cells, i.e. those which do not divide.

In an experiment described in the aforementioned report of IAEA (1973, p.41) differ-
ent populations of bacteria were subjected to increasing doses of radiations until only
0.1% survived (Fig.2). This was done successively with two species. For Micrococcus radiodurans \((G = 5h)\) this result is obtained with a dose of 1.20 Megarad (rad
means “radiation absorbed dose”, this old unit is related to the SI unit called Gray by
the relation: 1 Gray=100 rad). In contrast, for Salmonella typhimurium \((G = 20mn)\),
only 0.05 Megarad were required to get the same result. In other words, division of
\(G\) by 15 resulted in a reduction of the dose by 24.

An alternative approach (see Appendix B) could be conducted by comparing the
required dose in the exponential phase of \(M.\ \text{radiodurans}\) to the dose for the same
bacteria but in the stationary phase (Serianni et al. 1968, p.198). One finds that a
dose about 3 times larger is required in the stationary phase. In short, the faster the
cells divide the more sensitive they are to radiation.

\(^3\)It has the additional advantage that radiations are always harmful. In contrast, for assessing the effect of a surge in
temperature, one needs to know what is the cells’ optimal temperature.
Fig. 2  Sensitivity of bacteria to X-rays. The sensitivity to X-ray radiations is strongly conditioned by the generation time $G$ of the bacterial strain. The faster the bacteria divide, the more sensitive they are to radiation.

Source: The data are from IAEA (1973, p. 41)

2.4 Effect of cold

Among numerous papers devoted to studying the effect of stress factors, the experiments done by James Sherman and William Albus (1923) are of particular interest for (at least) two reasons.

- Whereas most papers study the effect of temperature rises, this one investigates the effect of cold.
- In contrast with many papers in which the protocol is neither well designed nor well explained (see Appendix B), here the methodology is carefully designed and well explained.

The authors start with the observation that it is well known that a brief exposure to cold (above the freezing point of the medium) causes almost no reduction in the number of viable \textit{E. coli} bacteria as counted by the CFU (Colony Forming Unit, see Appendix B) method, provided that the population is in the stationary phase. Then, the authors describe the following experiment.

\textbf{First experiment.} A culture of \textit{E. coli} in 1\% pepton was kept for 8 days at laboratory temperature (25 degree Celsius) before being diluted in distilled water at a temperature of 2\(^{\circ}\)C. The numbers of viable cells were counted on a small sample, immediately after dilution ($N_0$) and again after one hour exposure at this temperature ($N_1$). They found (average of two trials):

Old culture (8 days) $\implies \frac{N_1}{N_0} = 0.99 \pm 0.014$
Then, they repeated the same experiment with a culture which was only 4 hours old. This time they found the following numbers (again average of two experiments):

Young culture (4 hours) $\Rightarrow N_1/N_0 = 0.47 \pm 0.26$

They were tempted to conclude that there was an over-mortality among the young cells due to sudden cooling. However, the authors observe quite judiciously that it was not possible to conclude that it was the cooling which caused the mortality for in fact there were two changes: (i) temperature and (ii) transfer from a nutrient medium to distilled water. At this point the authors could have performed two tests:

1. Replace the water at 2°C by water at 25°C. This would show the water effect alone.
2. Replace the water at 2°C by pepton medium at 2°C. This would show the temperature effect alone.

The authors decided to do the second test. In a sense this was a logical decision because it is in the temperature effect that they were interested. However, just as a confirmation, it would also have been interesting to try the first test (see Appendix C).

**Second experiment.** A culture of *E. coli* in 1% pepton was kept for 12 days at laboratory temperature (25°C) before being diluted in pepton medium at a temperature of 2°C. The numbers of viable cells were counted immediately after dilution ($N_0$), and after 1 hour exposure at this temperature ($N_1$). They found (each measurement was done only once):

Old culture (12 days) $\Rightarrow N_1/N_0 = 0.96$

Young culture (3 hours) $\Rightarrow N_1/N_0 = 0.42$

The fact that the $N_1/N_0$ ratio for the young culture was almost the same as in the first experiment suggests that the pepton to water (both at 25°C) transition had almost no effect in terms of mortality. Naturally, in distilled water a cell cannot produce any daughter cell because the basic materials (e.g. carbon) are unavailable.

So, is this experiment the ultimate test? Not entirely. From the data given by the authors we observe that in the old culture, $N_0 = 970$ per cubic cm, whereas in the young culture: $N_0 = 3,420$ per cubic cm. In other words, the density of bacteria in the young solution is over 3 times higher than the density in the old culture. Can that be of importance? Probably not, but to be on the safe side it would have been easy to dilute the young culture so as to reach the same density as for the old culture.
The authors measured also the number of cells after 2 and 3 hours at 2C. There were only slight decreases:

\[ \frac{N_2}{N_1} = 0.80 \quad \frac{N_3}{N_2} = 0.85 \]

These numbers are consistent with the interpretation that the effect is mostly due to the cells which were in the process of division shortly before cooling. Such cells were very numerous in the young culture at the moment of its dilution in the medium at 2C. What happened to them? Their growth was certainly stopped. Were they killed or only inactivated in the sense of becoming unable to form colonies? We do not really know.

Novadays, measurements with a spectrophotometer would allow us to know whether or not some cells could successfully complete their division in the minutes after cooling. One would not be surprised that cells in the final phase might be able to terminate their division.

### 2.5 Effect of other stress factors

It can be noted that in the previous experiment by Sherman et al. (1923) the replication effect was rather weak in the sense that the number of viable cells was divided by only two whereas in the X-ray experiment we have seen much greater reductions. This is understandable because the cooling froze all new divisions; only the few cells whose division process was well advanced were affected.

This interpretation is confirmed by the next experiment in the same paper. In this case, the stress factor consisted in exposure in a solution of 2% NaCl during 1 hour. This factor stressed the cells but did not stop the divisions. As a result, the number of young cells was divided by 30.

In another experiment (in the same paper) the stress factor was exposure to a temperature of 53C during 20mn. In this case, the excess reduction of the young cells with respect to the old cells was a factor 75.

### 2.6 Internal changes when exponential growth slows down

We have already observed that there are internal changes. How important are they? In this respect the comparison between *M. radiodurans* and *S. typhimurium*, both of them considered in their exponential phase, has a clearer significance than experiments of type (2), defined in Appendix B. Why?

When a culture gradually moves from the exponential phase to the stationary phase, many internal transformations take place. One of these, which is mentioned in the literature in connection with cell response to various stress factors, is the increase
in the RpoS factor\footnote{Although irrelevant for our argument, the origin of this cryptic name can be explained as follows. R stands for the first letter of RNA; po stands for polymerase, a catalyst which produces chained molecules and is used in DNA to RNA transcription; finally S refers to the fact that this factor is only active in the initiation of this transcription. Another often used protective technique against radiation and other stress factors is clustering.}. The production of this factor increases as the population enters the stationary phase and we are told that it may also be activated by a situation of stress. However, this can by no means explain the difference observed between \textit{M. radiodurans} and \textit{S. typhimurium} for we are talking here of bacteria in their exponential phase. In contrast, the explanation by vulnerability during divisions remains valid. In short, although we do not deny that there may be some specific defense mechanisms, we think that, because of its broad validity, the replication-frailty effect should be considered first.

\section{Conjecture and predictive implications}

\subsection{Statement of the conjecture}

We have shown that instead of relying on a multitude of protective mechanisms which are strain- and stress-dependent, all the experimental evidence about responses to stress can be explained by assuming that cell divisions make the cells particularly vulnerable to any pre-existing stress factor. Is it possible to extend this mechanism to situations where there is no specific stress factor? We believe so. This conjecture can be stated as follows.

\textbf{Frailty conjecture:} The death rate of a culture of bacteria grown under nearly ideal conditions should be correlated to its growth rate. Consequently, it should be lower in the stationary phase than in the exponential phase.

As all conjectures, this one relies on several assumptions. For instance, it is assumed that the natural stress factor due to non-perfect conditions does not increase as the growth rate of the culture decreases.

\subsection{Supportive argument}

A simple argument goes as follows.

A cell culture is never in a situation that is 100\% ideal. Nutrients, pH, supply of dissolved oxygen and so many other parameters are never all completely optimum for the simple reason that in fact we do not know exactly what the most perfect conditions are. The discrepancy between existing and perfect conditions are seen by the bacteria as a light form of stress. Thus, what we have said about stress factors
leads to the prediction that the natural death rate (usually denoted by: $\mu_n$) will be lower in the stationary phase than in the exponential phase.

It can be observed that the previous prediction is rather counter-intuitive. Why? Usually, the stationary phase is seen as resulting from adverse conditions in the form of insufficient nutrients and accumulation of residues. There is no doubt that such unfavorable conditions do exist. This means that in a batch experiment the two effects occur simultaneously and are in competition with one another. In the early stationary phase, the growth rate effect will probably dominate but it is certain that after a while the effect of bad conditions will prevail.

A graph published in Wilson (1921, p.430) suggests that for a population without external stress factor, if the stationary phase starts around time $T_s$ (in the case presented in the paper $T_s = 5$h), then the death rate begins to swell substantially after time $2T_s$ and the number of viable cells falls back to its initial level toward time $4T_s$.

One can get rid of these unwanted competing effects by using a chemostat in which nutrients are renewed and residues eliminated (see Appendix B).

3.3 Experimental tests of the conjecture

Although the previous argument seems reasonable we do not put too much faith in it. The real test will come from experiments.

However, one must realize that the required measurements will not be easy. Why? When there is a stress factor, the population falls very substantially, for instance it may be divided by two or three within one hour. Such big changes can easily be assessed even with fairly crude techniques such as the CFU counting technique.

On the contrary, for the natural death rate of E. coli in the exponential phase, one expects a death rate of the order of 1 per 1,000 living cells and per hour. If our conjecture is correct the death rate in the early stationary phase will be even lower. Needless to say, to get reasonable estimates for such low death rates one must be able to measure the number of dead cells with high accuracy.

3.4 Amplification of small differences in growth rates

Usually, growth rates are not easy to measure. The replication effect opens the possibility of replacing growth rate measurements by death rate measurements.

Translation of growth rate differences into death rate differences can be done by applying a standardized stress factor (e.g. X-rays or ultra-violet light). This may be useful in fields (e.g. oncology) where differences in growth rates are important.
4 Extensions to embryo and viruses

“Is it possible to express our conclusions concerning the effects that we have described in such a way that some general principles emerge from them?”

This is the question raised in one of his papers on this topic by Nobel prize winner André Lwoff (1959, p.120). To this end we state the replication effect in more precise form and then we will ask if its presumed applicability can be extended. This will make the testing game more risky but also more rewarding. It would be pointless to propose an explanation which cannot be disproved.

4.1 Enlarging and focusing

Whether for prokaryotes or eukaryotes, the division process goes through similar successives steps. Given that the overall objective is the same, it is hardly surprising that there are common features: production of a great number of proteins, replication of the DNA and transcription of the RNA, spatial separation of mother and daughter components at the two ends of the cell and finally separation. Actually, such steps are indispensable in all cases where a mother cell gives rise to a daughter cell. Apart from the two cases already considered, one can add to them two others not yet considered.

i The initial cleavage of a zygote, that is to say the first step in the process that will lead to an embryo and eventually to the birth of a new individual.

ii The replication of viruses in their host cell.

Having thus enlarged the question, we wish at the same time to make it more focused by asking what is the most crucial part of the division process as far as manufacturing mishaps are concerned. In fact, this question was already addressed earlier (see also Appendix A) In the framework of our paper, it is clearly the early phase of the division which is considered to be the most critical. Called interphase in the case of eukaryote cells, this phase (more precisely the G1-S phases of the interphase) comprises the synthesis of many proteins and the replication of the DNA. Our conjecture is that it is during this phase that the cell is the most vulnerable to external perturbations. The main question is whether the mechanism brought to light for bacteria and eukaryote cells remain valid for viruses and embryonic development. It will be addressed in the following subsections. However, our purpose is only to open new perspectives that can be considered more fully in subsequent studies.

Although the broad mechanisms are fairly clear many questions regarding their real implementation remain unanswered.
4.2 Frailty mechanism for viruses

Broadly speaking the existence of such a mechanism is both self-evident and yet still mysterious. It is self-evident because viruses are living entities only during the time when they get replicated inside an host cell. Naturally that does not mean that they cannot be destroyed outside of their host-cells. Any molecule, and particularly macromolecules, can be broken up into pieces by radiation or excessive temperature [6].

Once inside their host-cells the replication of the viruses happens to be highly temperature dependent (see Lwoff 1959, p. 111). Unlike chemical reactions which, according to Arrhenius’ law, become faster when the temperature is (moderately) increased, for viruses it is the opposite. As soon as the temperature becomes higher than their optimum temperature, their replication is reduced by several orders of magnitude. Lwoff gives measurements for the poliovirus which show that at 40°C their development is reduced by a factor of almost 1,000 with respect to what it is at 37°C. Such a high vulnerability to a fairly small temperature change is indeed coherent with the high growth rates achieved by some viruses. Here again, the fastest the division, the higher the vulnerability to stress factors.

4.3 Frailty mechanism in embryogenesis

The early steps of embryogenesis consist in a replication process. Here we have a limited goal. We wish to draw attention on just one point which may help us to better understand the bacteria case.

For most species of fish the fertilization of the eggs produced by the female occurs outside of the body which means that it can easily be observed from the very first moment on. In this way one can measure the death rate of the embryos during their whole development. It appears that the bulk of the deaths is concentrated toward the beginning of the development and that it can reach very substantial rates of the order of 10% (see Chen et al. 2020). This is 100 times more than the death rate for E. coli cells during the phase of exponential growth. Why do we have such high rates here? We believe there is a simple reason which is related to how the female and male germ cells are produced.

In the case of E. coli any cell can be considered as an initial cell that has been produced by a mother cell through a standard process of division that is known to work fairly well with about only one defect in 1,000 divisions. For the oocytes produced by the female it is a very different matter. They are produced through an intricate

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[6] Lwoff addresses this point by saying that mere “thermal inactivation of viral particles [i.e. outside of the host cell] in few hours in temperatures ranging from 37°C to 41°C is negligible.”
process which comprises many steps (including two divisions). Although there may be some control mechanisms along this production process there is no screening process that would eliminate the defective eggs. In fact, the actual screening takes place in the first divisions of the embryogenesis.

If a parallel can be helpful it would be with the launch of a rocket compared to a departing train in a station. The departure procedure (e.g. announcing imminent departure on the train’s loud speakers, closing the door, starting the engine) has been tested multiple times in previous stations. On the contrary, although the individual components (e.g. the engine, the navigation system) may have been controlled in many ways, the launch will be the first time for all components to work together. It is well known that despite multifold tests, the probability of failure remains usually of the order of 5%. Similarly, the division of the zygote is for the female and male components the first time to work together.

5 Perspectives: complementary experimental tests

In this section we describe a number of open questions. Experiments are proposed which should help to answer them.

Does cell concentration constitute a stress factor for a sample of cells? This is an important question that we did not consider so far. Two interrogations come to mind immediately.

- The term “concentration” can be understood in two ways: (i) with respect to the total volume of the liquid, $c_v$, (ii) with respect to the amount of nutritive elements, $c_f$, contained in the liquid. For the sake of brevity we call them “volume-concentration” and “food-concentration”, respectively. When a culture is growing, both $c_v$ and $c_f$ decrease. It would be of interest to know the effect of each factor separately. Clearly, if $c_f \to 0$, division will stop but we do not really know what happens when $c_v \to 0$ while $c_f$ is kept constant. The experiment summarized in Fig.3 should answer this question.

- The term “stress factor” can be understood in two ways: (i) a factor limiting growth (ii) A factor inducing higher mortality. For the sake of brevity these meanings will be referred to as “growth stress factor” and “life stress factor” respectively. In the papers reviewed above the reduction in the number of viable cells was well documented but there was little information about birth rate changes. Probably, it was assumed that a fall in viable cells would necessarily be paralleled by an interruption of the division process. However, that is by no means evident. Cases of

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7We did note mention possible defects of the sperm cell because it is about 1,000 times smaller than the egg, nevertheless defects cannot be excluded altogether.
human populations having high death rates coupled with high birth rates are well documented in developing countries.

5.1 Is the volume-concentration of cells a growth-limiting stress factor?

The experiment consists in three steps.

1. After the two samples have been extracted from the culture their optical density and their doubling time are measured.

2. Dead cells are added to sample $A$ and the same volume of medium but without cells is added to sample $B$.

3. The doubling time is measured again in order to see if (and by what amount) the growth of sample $A$ was slowed down with respect to the control sample $B$.

Fig. 3 Sensitivity of bacteria to volume concentration. In order to change the volume concentration without changing the food concentration dead cells are added to the culture.

5.2 Effect of: medium $\rightarrow$ water transition.

In the analysis of the experiments conducted by Sherman and Albus (1923), we came across the question of whether it is possible to define what can be called the momentum of inertia of a culture. Simply stated, this notion gives an estimate of how long a culture can continue to grow once it is put in distilled water. Here, we propose a simple experiment.

Two different growth surges can be expected.

1. The growth based on “endogenous” resources may occur shortly after the transfer into water. One knows that in the phase of rapid growth of $E. coli$ the replication
Test of the momentum of a culture

Fig. 4: What amount of building materials are stored in the cells during exponential growth? The graph at the top would be observed in case of an important stock of advance material. On the contrary, the bottom graph corresponds to the assumption of “just in time” production that is to say with the minimum of advance storage.

of the DNA destined to the grand-daughter starts before the separation of the daughter has occurred. This means that the cells certainly contain more materials than the amount strictly needed to produce a single daughter.

2 An exogenous resource is provided by dead cells which release some of their components. This is a fairly obvious effect in which we are not really interested. One hopes that the time lag between the first and second effects is large enough to allow a clear distinction.

This simple experiment should be able to tell us what is the amount of “building materials” that are stored in the cell at different moments of the growth cycle. One would expect that this amount dwindles when the speed of growth decreases.

This effect can also be used to probe the age distribution of the population. To make this point clearer let us assume that the population is synchronized. Then, if all cells are freshly divided they do not possess the materials necessary for replication and therefore will be unable to divide. Thus, one expects the bottom graph in Fig.4. On the contrary if all cells are mature and about to divide one expects the top graph in Fig.4.

In a mixed case, the length of the inertia phase will give an estimate of the proportion of mature cells.
A Appendix A: Manufacturing defects versus mutations

In this appendix we wish to explain the implications of a defective blue-print (as a result of mutations in the genome) as contrasted with mishaps in how the prescribed tasks are carried out. It is true that mutations of the genome may be increased by external factors such as X-rays or ultra violet light. However, even if the blue-print is correct there will be defects in the implementation process. This question is outside the scope of our paper but it is important to realize that mutations of the genome are not the only mechanism which leads to anomalies.

A.1 Defects and mishaps

Instead of the expression “manufacturing defects” we were at first tempted to use more specifically biological expressions such as “error of metabolism” or “enzymatic defects” but then we realized that in fact we have in mind few clear cases of enzymatic defects that would illustrate our argument. In contrast, examples of manufacturing defects can be cited easily. Consider for instance the construction of a building. Naturally it will proceed in accordance with the blueprint established by an architect. But in its implementation there may be many mishaps. In hot weather concrete may dry too fast thus impairing the mechanical properties of the walls. Freezing weather may also cause problems. In other words, at each step, external non-optimal conditions may exist. In some cases the effect may be light while in others they may lead to structural flaws.

In biology inappropriate temperature or pH conditions may lead to inordinate protein production: too little, not at the right location or not in the appropriate 3-d configuration (for instance a cis- instead of a trans-molecular structure), any of these defects may possibly lead to life threatening abnormalities. Naturally external parameters are never 100% optimum, but may nevertheless be “acceptable”. For a process which does not require high accuracy (e.g. the production of tissue) the acceptable bounds will be fairly wide. In contrast, for processes which require high accuracy they may be rather narrow.

Although we know of few specific examples of defect forming here is one. There is a mechanism called the “induced-fit process” but that we prefer to call the “hand-glove adjustment” in which a substrate must do more than simply fit into the already preformed shape of an enzyme’s active site. When the substrate approaches the enzyme surface, it induces a change in its shape that optimizes a correct placement. In short, there is a two-sided adjustment, hence the hand-glove expression. A case in point is the digestive enzyme carboxypeptidase in which the binding of the substrate causes a tyrosine molecule (a standard hydrophobic amino acid used by
cells to synthesize proteins) at the active site to move by as much as 15 angstroms which is about twice the size of the molecule itself. In fact, depending on the temperature, the real distance will differ. A large change of that kind may even disrupt the adjustment and therefore the production of the protein.

We see that although examples of mishaps can be given, we have little intuitive understanding of them because these phenomena occur in the microscopic world with which we are not familiar.

A.2 Mutations

In contrast, we have a fairly clear view of the effects of DNA mutations because these are illustrated in daily experiments in which one can describe side by side the mutation and its phenotypic consequences. In other words it is not surprising that we are much more familiar with mutations than with mishaps. By the way, the same holds in our building parallel. Whereas it is very easy to identify a flaw with respect to the blueprint, a defect in the structural properties of the walls is not easily detected.

A.3 Randomness versus semi-randomness: the case of strabism

Finally, one must keep in mind one major difference between mishaps and mutations: while mutations are completely random, mishaps are rather semi-random. In which sense do we mean that? Depending upon the degree of accuracy that is required, mishaps will be more or less frequent. Here is an illustration.

The control of the pupils of the eyes requires an excellent coordination of the surrounding muscles; this means that only small fluctuations are acceptable, which in turn imposes narrow bounds on the values of external parameters. Thus, one will not be surprised that this congenital malformation (called strabism) occurs with a fairly high frequency of the order of 3% according to US statistics. This is one of the highest malformation frequencies. Similarly, the making of heart valves requires strict manufacturing constraints; as a result heart valve defects have a relatively high likelihood.

B Appendix B. Comparison of experimental protocols

In reviewing the literature we found that the experimental conditions are often loosely chosen and described. As a particularly striking case one can mention the following (IAEA 1973, p. 55, Fig.10). The graph shows two curves (a) and (b) of sur-

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8Whereas the connection mutation → abnormality is easy, the converse connection is much more difficult. In fact the genetic basis of most abnormalities is unknown.
viving bacteria fractions as functions of the radiation dose. The caption says: “S. typhimurium irradiated in meat at -15C. (a) Following growth in the meat at 37C for two days. (b) Without pre-irradiation growth in the meat”. The growth rates before irradiatation are not given. In (a) the culture was brutally brought from a temperature of 37C to -15C. For (b) the pre-irradiation temperature is not indicated. In short, this graph mixes up temperature and radiation effects. Due to an unclear design and a sketchy description it is impossible to draw any conclusion.

This example shows that great care should be given to both the design and the description of the experimental conditions. It is the purpose of this appendix to emphasize some important distinctions in this respect.

### B.1 Three types of measurements

Before we come to the data we wish to describe how these experiments can be done. There are basically three methods.

**Type 1**  
The simplest way is to use bacteria which have different generation times $G$. This is the time interval between successive divisions as long as one is in the exponential phase. In this phase, the population doubles in time $G$ which for that reason is also called the doubling time. As examples, for the bacteria *Salmonella typhimurium* $G$ is equal to 20mn, whereas for the bacteria *Micrococcus radiodurans* $G$ is equal to 5 hours (i.e. 15 times more).

**Type 2**  
If one wishes to focus on a specific bacterium one can make a comparison between $G$ in the exponential phase and the very large value of $G$ observed in the stationary phase. Although many papers use the comparison exponential phase versus stationary phase one should observe that it is a methodology which is not very accurate for at least two reasons.

(i) The comparison relies on only two values. It is true that in addition $G$ can be changed by controlling the temperature, however we did not find any paper in which this possibility is used.

(ii) Actually $G$ is not really constant in the exponential phase; it increases slowly but steadily. In addition, for bacteria like *E. coli* which have a small $G$ the whole exponential phase lasts less than 3 hours which may be too short to fully observe the impact of the exogenous factor.

**Type 3**  
The best method is to use chemostat-cultivated bacteria. A chemostat is a device in which a flow is maintained so that the bacteria remain in the exponential phase indefinitely. In addition, it is possible to control the growth rate by changing the dilution rate. Unfortunately, the chemostat methodology requires an appropriate device and an initiation to learn how to control the various parameters. In the paper by Linqvist et al. (2014) there is a useful comparison, for the same stress factor,
between a batch and a chemostat experiment.

**B.2 Two key-variables and how to measure them**

These key-variables can be described as follows:

(a) The overall number of cells, whether dead or alive, is measured with a spectrophotometer which tests the turbidity of the medium. Increases in the optical density give estimates of the number of cell divisions, and therefore of the growth rate in the form \( \alpha = \frac{1}{\Delta t} \log\left[\frac{y(t)}{y_0}\right] \) which is constant for an exponential growth. Note that the doubling time is given by: \( \tau = \log 2/\alpha \).

(b) The second key-variable is the number of viable cells. It is estimated either with a cytometer or through the CFU (colony forming unit) method. When a cell can give rise to a colony on a Petri dish it is considered viable. Note that some living cells may become unable to develop into colonies. For this and other reasons the method is fairly inaccurate. Wilson (1921) gives many examples of possible pitfalls.

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