# PHYSICAL METHODS OF STERILIZATION OF MICROÖRGANISMS

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CONTENTS



The means by which man fights microörganisms are ordinarily classified either as chemical means (commonly called disinfectants or antiseptics), or as physical means which include a number of very different agencies, such as heat, drying, grinding, pressure and others. While the contrast between the two types of agencies as such is sharp and fundamental, certain physical causes, e.g., radiations, may bring about chemical changes, and the ultimate cause of death may be a chemical reaction although brought about by a physical agent. Plainly physical even in its ultimate analysis is death by mechanical destruction, as by grinding. Plainly chemical is the slow death of dry bacteria which is due to oxidation of some essential cell constituents and follows the laws of chemical disinfection. Between the two extremes stand heat and radiation which destroy life by denaturation of some important cell proteins. This denaturation may be considered a chemical or a physical process.

Facts can be used to greatest advantage when the reasons for the facts are completely understood. The object of this review is not an enumeration of facts, but an attempt to correlate the knowledge acquired about physical disinfection, and to understand how physical agents can kill bacteria, or, more generally, how they can bring about the death of any cell.

The applications in the home, in industry and medicine, although of widest use and inestimable value, cannot be considered here. Drying and freezing have been used since prehistoric times to preserve food, but these processes do not sterilize. They may be compared with antiseptics rather than with disinfectants, because they prevent bacterial action and may kill a large proportion of microörganisms, but cannot be relied upon to kill all of them. The use

of artificial ultraviolet light, to destroy bacteria is of rather recent date. But the application of heat antedates Pasteur's discovery that food spoilage is caused by microörganisms. Appert based his process of preserving foods by long-continued heating upon the theory that the air over the food in the container was "rendered to no effect by the action of heat." A considerable canning industry had developed long before Pasteur published his first papers.

## I. MECHANICAL CAUSES OF DEATH

*1. Death by grinding and shaking.* It is obvious that cells are dead when they are broken into many small pieces. Experiments with protozoa have shown that recovery is possible only when the nucleus has remained uninjured. With bacteria and yeasts, recovery after mechanical injury may also be expected if the damage is slight.

To what extent the smaller microörganisms might be broken up mechanically, was not known until Buchner's famous demonstration of cell-free fermentation (1897). Yeast ground with quartz sand was still capable of changing sugar to alcohol and carbon dioxide, but had lost the power to produce colonies on nutrient agar. This experiment calls attention to the bacteriologist's definition of death which differs from the definitions by all other biologists. A bacterium (or yeast) is considered dead when it has lost the power to reproduce.

Bacteria can also be killed by shaking, but vigorous agitation is necessary to bring about a noticeable decrease in the number of viable cells (Campbell-Renton, 1942b).

An interesting combination of grinding and shaking has been studied by Curran and Evans (1942) who shook bacterial cultures or spore suspensions with different kinds of abrasives, such as glass beads, sand, or carborundum. With uniform shaking on a mechanical shaker, the number of fatal hits per minute must be directly proportional to the number of cells present. With $n$ bacteria at the start, and the fraction $p$ killed per hour (or $100p$ % per hour), the number of dead bacteria after the first hour of shaking is $np$, and the number of survivors is $n(1\text{-}p)$. Of these survivors, the fraction $p$ dies again during the next hour, and the fraction $(1\text{-}p)$ survives, which makes the survivors after 2 hours of shaking $n(1\text{-}p)\ (1\text{-}p)$. After the third hour, the survivors number $n(1\text{-}p)^3$ and after $t$ hours, $n(1\text{-}p)^t$. If we call the number of survivors $b$, we have

$$\begin{aligned} b &= n(1\text{-}p)^t \\ \frac{b}{n} &= (1\text{-}p)^t \\ \log b - \log n &= t \log(1\text{-}p) \\ -t \log (1\text{-}p) &= \log n - \log b \end{aligned} \qquad \text{(a)}$$

In this equation, $p$ is a constant, and therefore also $\log (1\text{-}p)$; and we may substitute $-\log (1\text{-}p) = \mathrm{K}$. This simplifies equation (a) to

$$Kt = \log n - \log b = \log \frac{n}{b}$$

where $n$ is the original number of bacteria, $b$ the number of survivors after $t$ hours of shaking, and $K$ a constant measuring the rate of death.[1] According to this equation, the logarithms of survivors plotted against time must fall on a straight line. As figure 1 shows, this is actually the case.

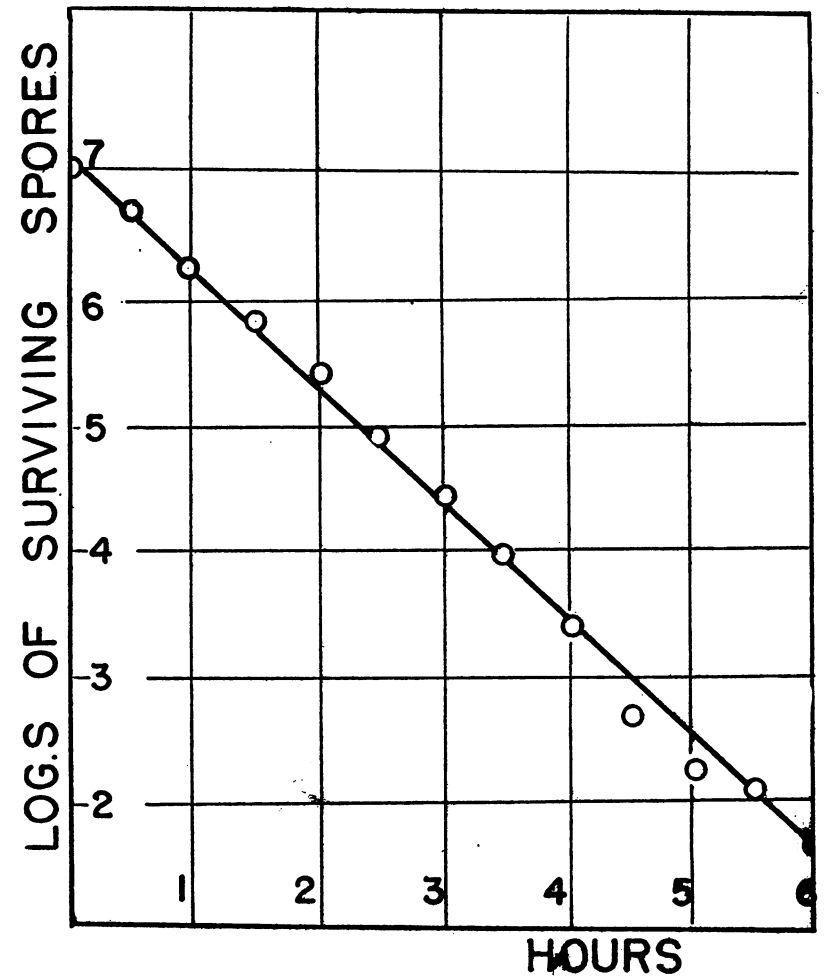


FIG. 1. SURVIVOR CURVE OF THE SPORES OF BACILLUS SUBTILIS SHAKEN WITH GLASS BEADS (FROM CURRAN AND EVANS, 1942)

The equation is identical with that claimed for the logarithmic order of death by disinfectants or heat. Such identity does not indicate any relation between the two causes of death. They are so entirely different that no conclusion can be drawn from one to the other. In the shaking experiments, the order of death

[1] The percentage of cells killed per hour (100 $p$) can be computed as follows:

$$\log (1-p) = -K$$
$$1-p = 10^{-K}$$
$$p = 1 - \frac{1}{10^{K}}$$

can be predicted, it must be logarithmic, and experiment has proved the reasoning to be correct. With chemical disinfection, this type of order was entirely unexpected, and its explanation and even its existence has been debated for more than 30 years. The equation has been considered in some detail here because radiation, according to the corpuscular theory of light, may be considered as a bombardment with electrons or photons or quanta, and this must result in a logarithmic order of death which will be discussed in a later chapter.

Table 1 shows some of the data obtained by Curran and Evans. The chemical nature of the abrasive, its degree of hardness and its specific gravity had no influence on the death rate. Spherical particles appeared more efficient than angular ones, perhaps because they produced larger surfaces of contact by slid-

TABLE 1

*Destruction of spores suspended in buffer solution (pH 7.0) when shaken with abrasives for 5 hours at 430 rpm*

| ABRASIVE (20 g IN 25 ml OF BUFFER) | | SPORES OF BACILLUS COHAERENS | | | SPORES OF BACILLUS MEGATHERIUM | | |
|---|---|---|---|---|---|---|---|
| | | Survivors per ml | Death rate K (per hour) | Per cent killed per hour | Survivors per ml | Death rate K (per hour) | Per cent killed per hour |
| At start | | 1,350,000 | — | — | 950,000 | — | — |
| Particles passed No. 20 sieve but not No. 40 | Sand | 70,000 | 0.293 | 49.1 | 7,300 | 0.423 | 62.2 |
| | Pyrex chips | 39,800 | 0.342 | 54.5 | 750 | 0.620 | 76.0 |
| | Carborundum | 95,500 | 0.231 | 41.2 | 8,050 | 0.414 | 61.4 |
| | Boron carbide | 44,000 | 0.297 | 49.5 | 5,400 | 0.449 | 64.4 |
| | Glass beads | 42,800 | 0.301 | 50.0 | 2,700 | 0.509 | 69.0 |
| Particles passed No. 80 sieve but not No. 100 | Alundum | 172,000 | 0.179 | 33.8 | 30,200 | 0.300 | 49.8 |
| | Emery | 173,000 | 0.178 | 33.6 | 20,800 | 0.332 | 53.4 |
| | Carborundum | 7,900 | 0.446 | 64.2 | 300 | 0.700 | 80.1 |
| | Boron carbide | 120,000 | 0.210 | 38.3 | 17,400 | 0.347 | 55.1 |
| | Glass beads | 30 | 0.931 | 88.3 | 3 | 1.100 | 92.1 |

ing or rolling past each other. The particle size was of great influence as may be seen from the table. Sand was found most efficient when the grain size was between sieve numbers 40 and 60, while glass beads destroyed bacteria most rapidly when they passed sieve No. 60, but not 80. An increase in size resulted in a lower death rate, and a decrease had the same effect.

Vegetative cells were more sensitive than spores, but the difference was not great. With the spores of *Bacillus cohaerens*, when shaken with glass beads in distilled water, the death rate constant was K = 0.8. *Escherichia coli* under identical conditions had the constant 1.8. The same *E. coli* shaken with the same glass beads in broth showed a death rate constant of only 0.8, the protection being probably due to the foaming. The spores of different species differ considerably in their sensitivity to mechanical destruction, as table I shows, and this difference is not correlated with the difference in heat resistance.

According to Campbell-Renton (1942b), bacteriophage is sensitive to shaking, but a great variation of sensitivity was observed with different phages. The shaking was carried out without addition of solid particles. With *Salmonella schottmuelleri*, the phage was inactivated to a much greater extent than the bacteria. It is possible to obtain apparently phage-free cultures of bacteria by shaking, provided that the culture is not too heavily infected with phage.

*2. Death by pressure.* It is not very probable, reasoning *a priori*, that pressure can affect bacteria suspended in a liquid. As there are no gas-filled spaces in the cell, the change in pressure can result only in slight differences in volume and the cells are not likely to be torn, unless the change is very sudden as with supersonic waves. Pressure cannot change the cell constituents greatly, nor can it alter their relative position. The theory of Johnson, Eyring and Williams (1942) that pressure changes the equilibrium between native and denatured protein does not apply in this case because in death, we are dealing with an irreversible process.

Chlopin and Tammann (1903) placed 24-hour broth cultures of many different microörganisms in sterile castor oil which was then subjected to pressures up to 2900 atmospheres, the pressure being increased in steps of 500 atmospheres every 15 minutes. A pressure of 2000 atmospheres for 4 hours at 36° killed the entire cultures of *Eberthella typhosa, Salmonella typhimurium, Vibrio cholerae* and *Micrococcus agilis, i.e.*, they had lost the power to reproduce, but the cells were still motile. Other species were weakened, but some cells survived. Virulence was considerably decreased, and remained decreased. An exposure to 2000 atmospheres for 4 days at 14–16° killed most of the species tested, but not *Bacillus anthracis, Oidium lactis, Corynebacterium pseudodiphthericum*, and beer yeast. All of these species were greatly weakened. Again, many of the "dead" bacteria remained motile. The increase to 2900 atmospheres did not change the result greatly. Rapid increase and decrease of pressure had little influence.

In 1914, Hite and associates attempted to preserve milk, vegetables and fruits by pressure in place of the customary application of heat in canning. They found that 100,000 pounds per square inch, at room temperature for seven days, did not destroy some of the milk enzymes, but no culture of bacteria could be obtained from such samples. "In old milk, an original count of 30 or 40 million bacteria per cc. may be cut down to a few hundred, or a few dozen, by an application of 100,000 pounds for 10 minutes."

Grape juice, cider, peaches and pears could be sterilized by 60,000 pounds pressure in 30 minutes, while blackberries and raspberries usually fermented after such treatment. Vegetables spoiled almost universally, and tomatoes began to sour after having been exposed to such pressure.

Experiments with pure cultures of different species showed certain differences. The logarithms of the death times plotted against the logarithms of the pressures fell on a straight line in most cases (fig. 2). Pressures less than 30,000 pounds or 2,000 atmospheres did not kill pure cultures in 3 hours. This agrees fairly well with Tammann's findings.

A different picture is obtained when the cultures are exposed to gas pressures, because this causes a chemical change of the environment. Compressing the air above the culture to 100 atmospheres would mean approximately 100 times as much dissolved oxygen in the medium, and this might kill even many aerobic species. The effect of such compressed gases is really chemical disinfection. It becomes physical only when the sudden release of the gas pressure tears the cell. This latter possibility will be treated at the end of this chapter.

*Oxygen.* That high concentrations of oxygen are toxic to bacteria, has been known for a very long time. In his review on inhibition of bacteria by oxygen, Rodenkirchen (1937) mentions experiments as early as 1873. Precise experiments with measured oxygen pressures and pure cultures were made by Porodko

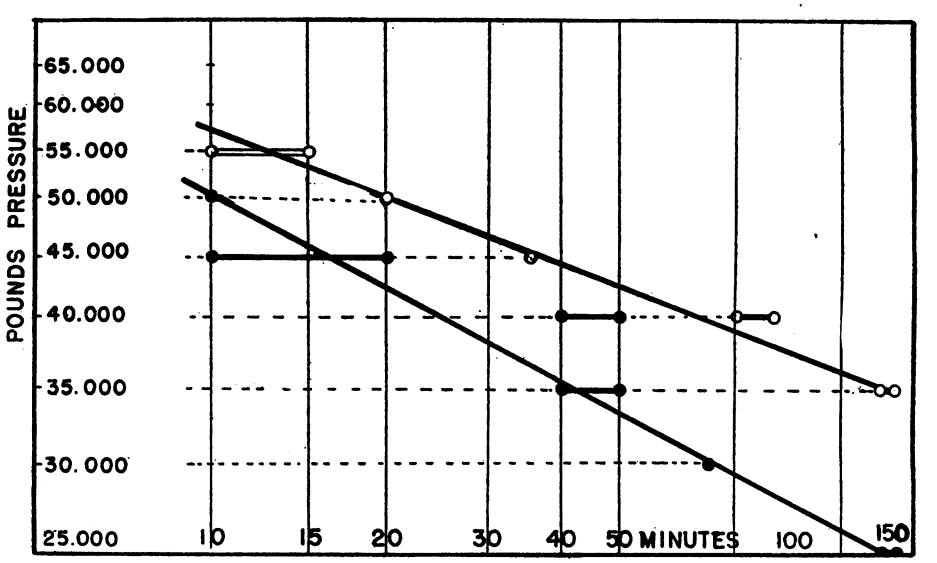


FIG. 2. THE DOUBLE-LOGARITHMIC RELATION BETWEEN PRESSURE AND DEATH TIME. ABOVE: SERRATIA MARCESCENS. BELOW: SACCHAROMYCES CEREVISIAE. (DATA OF HITE, GIDDINGS AND WEAKLY, 1914)

(1905) and Berghaus (1907). Inhibition of growth for 4 days was obtained by 2 atmospheres of pure oxygen with a pink yeast, *Bacterium cyanogenum, Bacterium bruneum, Alcaligenes faecalis, Pseudomonas aeruginosa, Vibrio cholerae,* and *Bacillus anthracis;* but they were not killed completely, and they multiplied when the oxygen pressure was released. Other species like *Pseudomonas fluorescens, Bacillus mycoides, Proteus vulgaris* and *Eberthella typhosa* were inhibited by 2.5 atmospheres of oxygen, *Escherichia coli* and *Salmonella enteritidis* required 3 atmospheres, *Sarcina lutea, Vibrio albensis, Bacillus subtilis,* and *Staphylococcus aureus* 4 atmospheres, and another strain of *E. coli* as well as *Serratia marcescens* 6.3 atmospheres. One species of micrococcus required about 10 atmospheres.

The oxygen concentrations for complete sterilization are much higher. Berghaus found *B. anthracis, Alcaligenes faecalis* and *Vibrio cholerae* dead after 24 hours' exposure to 2–4 atmospheres of oxygen pressure, but 10 representatives of

the colon-typhoid group, as well as *Pseudomonas aeruginosa* and *Staphylococcus aureus* required 60 to 75 atmospheres.

This inhibition of bacterial development by oxygen has been used in the Hofius process of milk preservation which consists in keeping the milk under 8 atmospheres of oxygen pressure (120 pounds) at low temperature. Such milk is claimed to keep 2 to 3 weeks (Müller, 1936, Rodenkirchen, 1937).

*Hydrogen.* Very little effect of hydrogen pressure was observed by Larson, Hartzell and Diehl (1918). A pressure of 120 atmospheres never produced sterile cultures. Of *E. coli*, only 10 to 40% of the cells had been killed in 24 hours, and the microscope showed many cells to be broken up. Unexpected was the result that "gram-positive bacteria would often become gram-negative, and even the acid-fast character of the tubercle bacteria was impaired."

*Nitrogen* at 120 atmospheres pressure did not kill the bacteria, nor did it change their morphological characters.

*Carbon dioxide* has been tested by many authors. Larson *et al.* (1918) could kill non-sporulating bacteria by 50 atmospheres in about 1½ hours, but 40 atmospheres had no effect whatever. Death was not due to the low pH of 3.15 because the bacteria could tolerate this acidity for 48 hours when it was produced by other acids, without the pressure of $CO_2$. Yeast cells survived the same pressure treatment for 48 hours. These authors consider the death to be due to the "sudden change of osmotic tension."

"Gram-negative bacilli could be brought to a marked degree of disintegration, although disintegration of all the bacteria in suspension was never attained. The gram-positive cocci suffered little morphologic change aside from slight irregularity in size, and often a tendency to lose their gram-positive character." Very little disintegration was noticed when the bacteria were suspended in broth or saline instead of distilled water.

Swearingen and Lewis (1933) assumed that the death of bacteria under high $CO_2$ pressure was due to the formation of gas bubbles within the cell which would make the cell explode when pressure was released. According to their calculations, about 40 pounds (2.6 atmospheres) of surface tension pressure must be overcome to form a gas bubble of the size of a bacterium. Therefore, death by explosion could not occur with low $CO_2$ pressures. However, with pressures less than 40 pounds, they observed a slow rate of death which they ascribed to the precipitation of certain colloid systems.

The pressures used in carbonated drinks are not sufficient to produce sterility, even after several months. Milk under 60 pounds of $CO_2$ will sour slowly. The results of attempts to use $CO_2$ in food preservation are summarized in McCulloch's book (1936).

*3. Death by sonic and supersonic waves.* Death by such waves is fundamentally not different from death by mechanical agitation. It is generally assumed that the waves produce a very rapid succession of compressions and releases of the liquid, which tear the suspended cells to pieces. It has been suggested that the rarefaction during release might go so far as to produce microscopically small areas of vacuum. While bacteria can withstand slowly rising or decreasing

pressures, the very rapid alternation injures them. The death rate rises with the frequency of the waves, which indicates that the suddenness of change between compression and release is an important factor.

Harvey and Loomis (1929) proved that luminescent bacteria could be killed by supersonic waves of approximately 375,000 cycles per second. By exposure for one hour or longer, complete sterility could sometimes be obtained. Williams and Gaines (1930) treated cells of *Escherichia coli* with waves of only 8,800 cycles, and observed a very slow decrease of about 60 to 70% of all cells per hour. The order of death was logarithmic. Chambers and Gaines (1932) found a logarithmic order for *E. coli* only with young cultures. The 5-day culture contained many cells of greater resistance, and their percentage as well as their resistance increased up to 14 days. *Streptococcus lactis* showed a strictly logarithmic order even with an 8-day culture.

More extensive were the experiments by Beckwith and Weaver (1936) who used equipment similar to that of Harvey and Loomis, i.e., one that yielded frequencies of about 400,000 c.p.s. With such high frequencies, heat is generated by the waves, and the cultures under test must be cooled. Aqueous suspensions of cells from a 24-hour culture of *E. coli* were always sterilized by a 5-minute exposure to these ultrasonic waves while a 6-hour culture of *Eberthella typhosa* in broth had 1 to 10% survivors after 10 minutes' treatment. Spores of *Bacillus subtilis* suspended in water, after 15 minutes, had decreased only about 50%. With the spores of thermophilic bacteria, 98 to 99% were killed when exposed in 5% sucrose solution, and only 75% died in 20% sucrose solution. Yeast in grapejuice varied enormously; sometimes 3 minutes sufficed to sterilize the culture; at other times, a few cells survived even after 15 minutes.

Equally fluctuating results were obtained with the mixed flora of milk. The efficiency of the method varied from 20% to 99% in terms of fatality. Systematic experiments showed that the great protection exerted by milk was due not to the fat or the lactose, but to the casein. The great retardation of death in the presence of proteins has spoiled all hopes that this would be an efficient method of sterilizing milk and other foods without heat.

This method of disintegrating cells can be used to obtain certain cell constituents from cell suspensions because heat-labile proteins are not denatured by sound waves. Chambers and Flosdorf (1936) produced cell-free antigens of *E. typhosa* and *Streptococcus hemolyticus* in this way.

## II. DEATH BY IRRADIATION

Of the wide range of radiations existing on earth, only two regions have a pronounced lethal effect on bacteria, namely, the ultraviolet range and the cathode to X-ray range. Visible rays do not affect bacteria appreciably. There is a possibility of a very slight effect according to Duggar (1936, p. 1127).

Radiations can produce chemical or physical changes only when they are absorbed. That is the reason why X-rays which have the power to penetrate organic tissues without being absorbed are not used in practical disinfection while ultraviolet rays, which are noticeably absorbed even by such transparent substances as glass and water, are applied in many ways for sterilization.

Color is produced by the absorption of selected wave lengths of visible light. The color of ultraviolet light is invisible to us, but its absorption by chemical compounds can be measured quantitatively by the absorption spectrum. Gates (1934) has shown that "the destruction spectrum of pepsin by ultraviolet agrees essentially with its absorption spectrum." This verifies the above statement that only the absorbed rays can produce chemical changes.

Details of the effects induced by different radiations can be more readily interpreted after a discussion of the fundamental cause of death by such rays.

*1. The cause of death* by irradiation is the inactivation of some essential cell constituents by the energy of the absorbed rays. X-rays have been used for many years to produce mutations in plants and animals (see review by Duggar, 1936). It is assumed that a quantum absorbed by a chromosome either destroys one or several genes, or disturbs their arrangement. If the destroyed gene is essential for multiplication, the cell may remain alive, but cannot reproduce; it becomes sterile. Microörganisms are no exception. Mutations by means of X-rays have been produced in bacteria, (Haberman and Ellsworth, 1939; Lincoln and Gowen, 1942) in yeasts, (Oster, 1934; Lacassagne *et al.*, 1939) and in molds (Beadle and Tatum, 1941–2). Mutations of molds (Stevens, 1930) and of bacteria (Sharp, 1940) have also been produced by ultraviolet light; and death by ultraviolet may well be considered to be a lethal mutation. Lea and Haines (1940) used this very term, apparently without knowledge of Rahn's (1929, 1934) explanations of death and Jordan's (1940) identical definition.

The corpuscular theory of light assumes that rays are minute energy projectiles moving with an enormous speed. They differ greatly in their energy content which is released upon absorption and causes changes which may lead to death. The physicist frequently speaks of a "quantum hit" when he means absorption of a quantum. Therefore, death by radiation is death by bombardment and comparable to death by shaking with glass beads, and we must expect a strictly logarithmic order of death. Most data with cathode rays (Wyckoff and Rivers, 1930), X-rays (Wyckoff, 1930) and ultraviolet (Wyckoff, 1932; Sharp, 1939) show this. Of the survivor curves for 10 bacteria given by Sharp (1939) seven are straight, while three are concave downwards resembling the survivor curves of multicellular organisms. The exceptions are represented by two staphylococci (which form clumps) and by the thread-forming anthrax bacillus. These bacteria would be expected to produce exceptional curves (Rahn, 1930). The survivor curves of mold spores are concave downwards (Whelden *et al.*, 1940; Zahl *et al.*, 1939). This is typical for mold spores by all causes of death (Rahn, 1943). The data of Gates (1929) for staphylococci are also concave downwards. The one unexplained exception is a curve of the same shape for *E. coli* suspended in air, observed by Sharp (1940).

The simplest case is the death by cathode rays which can be considered as a bombardment with electrons. According to Wyckoff and Rivers (1930), "for the two motile bacilli, *B. coli* and *B. aertrycke*, the absorption of a single 155 K.V. electron is sufficient to cause death. The same is undoubtedly true of *Staphylococcus aureus*. Furthermore, all, or nearly all, the electrons absorbed

are lethal.... The differences in sensitivity to cathode rays shown by the bacteria studied can be explained by the purely physical factor of size."

The explanation is to be sought in the great energy contained in the electron. The same authors state that "a 150 K.V. electron will liberate about $10^4$ ions within less than 0.001 $mm^3$. Together with this ionic shower, X-rays are emitted as a consequence of electron absorption." Since 0.001 $mm^3$ is the same as 1 cubic micron, and approximately the volume of an average bacterium, one absorbed electron can ionize the entire cell to complete destruction. The energy per quantum decreases as the wave length increases. With X-rays, Holweck (1929) and Lacassagne (1928) observed that a cell of *Pseudomonas aeruginosa* can be killed by absorption of a single quantum, but only if it is absorbed by a definite part of the cell which they call the "sensitive zone." Wyckoff (1930) measured the average number of quantum hits required to kill a cell with X-rays

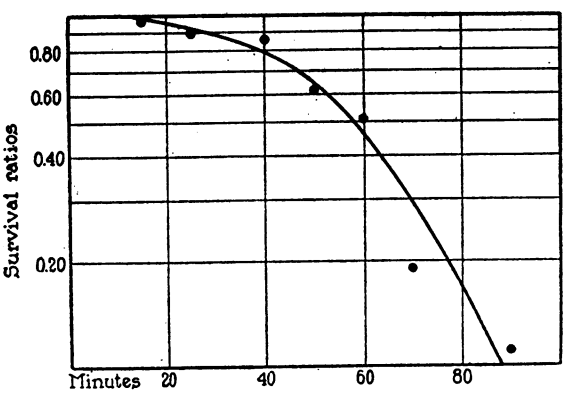


FIG. 3. THE SURVIVAL RATIOS OF THE SPORES OF RHIZOPUS NIGRICANS UNDER THE ACTION OF Kα X-RAYS (FROM LUYET, 1932)

of different wave lengths. From these, the size of the sensitive zone could be estimated. The results were:

| | | | | | |
|---|---|---|---|---|---|
| Wave length, A..... | 0.56 | 0.71 | 1.5 | 2.3 | 4.0 |
| Quanta required to kill..... | 4.54 | 6.46 | 14.2 | 29.5 | 69.7 |
| Sensitive volume (cell = unit)..... | 0.22 | 0.16 | 0.07 | 0.034 | 0.014 |

Yeast cells are larger, their volume is of the order of magnitude of 100 $\mu^3$, and it cannot be expected that every electron hit will strike a vital cell constituent. Consequently several quanta are needed to kill a yeast cell (Wyckoff and Luyet, 1931). Mold spores are larger than yeast cells, and the logarithmic survivor curve of the spores of *Rhizopus nigricans*, figure 3, shows plainly that many quantum hits are necessary to kill the spore.

In the case of ultraviolet radiation, several million quanta must be absorbed before a cell is killed, and Wyckoff's (1932) calculation of the "sensitive zone" proved it to be only about the size of a protein molecule. Wyckoff believed that to be impossible, and considered death by ultraviolet to be quite different from that by other rays. But Gowen (cf. Duggar, 1936, p. 1323) estimated that the

sensitive zone in *Drosophila*, which must be hit in order to produce a mutation, is about $10^{-18}$ cm$^3$, or a cube with sides of 0.01 $\mu$. This is the volume of a fairly small protein molecule. Fricke and Demerec (1937) estimated the average diameter of a gene to be about 25 A = 0.0025 $\mu$. Haskins and Enzmann (1936) obtained the same value. Since death of bacteria can be considered as a lethal mutation, the measurement by Wyckoff supports this viewpoint very well. The energy in a single quantum of ultraviolet radiation seems just sufficient to inactivate the protein molecule which absorbs it, but not sufficient to cause further effects. Thus, death occurs only when an indispensable and irreplaceable protein molecule is hit by the quantum. Quanta of visible light have less energy, and cannot inactivate the protein molecule even with a direct hit, and therefore cause no death.

This simple theory of death has been questioned by Rentschler *et al.* (1941) who believe that "the relation between the amount of ultraviolet radiation and the per cent of bacteria killed is determined by the distribution of bacteria of different resistivity to the radiation and is not due to the probability of hitting a vital spot in a given organism by a photon." They prove quite conclusively that bacteria at the stage of rapid cell division are much more sensitive than resting bacteria, at least 5 times as sensitive according to the method of calculation used. However, that does not disprove other experiments which were almost always made with resting cells. A graded resistance cannot explain the logarithmic order of death as Rahn (1943) has shown.

These authors claim further that the single photon-hit theory can hardly explain the fact that a sublethal dose retards the rate at which colonies develop after irradiation. However, this really should be expected. A photon hit, i.e. the absorption of a quantum of ultraviolet radiation, ionizes the immediate environment of the place of absorption. If no life-important gene is destroyed, there is likely to be other injury which, although reparable, may cause considerable delay of development.

Disturbances of the mechanism of cell division and growth coördination by rays have been frequently recorded. Luyet (1932) estimated the amount of injury by various rays upon the spores of *Rhizopus nigricans* by measuring the average length of mycelium per spore produced within 24 hours after exposure. He also observed spores which swelled to nearly 5 times their diameter, but never produced a mycelium. Oster (1934) reported giant cells of yeasts and two-cell groups from 3 to 8 times the size of normal two-cell groups, after exposure to ultraviolet. Gates (1933) described a loss of cell division, but continuance of growth by *E. coli* after ultraviolet irradiation. Some cells continued to increase in size, especially in length, but did not divide, and produced filaments, sometimes 50 to 150 $\mu$ in length, with a diameter occasionally three times normal. These cells finally degenerated, or began suddenly to divide.

The mechanism of cell division and coördination seems to be more sensitive than that of growth as such, i.e., of organic synthesis, and this again is more sensitive than that of catabolism, of enzyme action and energy provision. Yeast cells exposed to a mercury vapor lamp lost the ability to produce colonies on agar

more rapidly than the ability to ferment sugar to alcohol and carbon dioxide (Rahn and Barnes, 1933). The cells retained: after 20 minutes' exposure, 1.8% of viability, 60.0% fermenting capacity; after 40 minutes' exposure, 0.7% of viability, 39.0% fermenting capacity.

*2. Effect of temperature.* A single quantum of ultraviolet rays or of rays of shorter wave length, if absorbed at a specified location in the cell, destroys that cell's capacity to reproduce. An increase in temperature does not increase the energy liberated by absorption. It may, however, increase slightly the radius of the ionization zone around the absorbed quantum. Thereby, a slight increase in deaths may be observed at higher temperatures for such cases where the quantum hit was not close enough to the sensitive zone to cause inactivation at low temperature, but is just sufficient at the higher temperature. This leads to the assumption of a temperature coefficient analogous to that of photochemical reactions, which amounts to an almost negligible increment.

All measurements have confirmed this assumption. Hercik (1936) reported a $Q_{10}$ of 1 for the $\alpha$-particles of Polonium. For ultraviolet light, Bayne-Jones and Lingen (1923) found the value 1.15, Gates (1929) found an average of 1.1, E. Smith (1935) observed with Fusarium spores between 0 and 40 C a temperature coefficient of 1.13. This is definite proof that death is not caused indirectly, e.g., through formation of toxic peroxides. The lethal effect of peroxides would have a much higher temperature coefficient.

*3. Effect of wave length.* The death rate depends upon the number of quanta absorbed as well as upon the energy per quantum. In the range of cathode rays and X-rays, no preferential absorption of certain wave lengths has ever been observed, and death depends only upon the amount of incident energy.

In the ultraviolet range, different organic compounds are characterized by their preferential absorption of certain wave lengths, and we must expect the strongly absorbed wave lengths to cause more damage per erg per mm$^2$ of incident energy than those wave lengths which are but slightly absorbed. Gates (1930) determined the absorption curve for ultraviolet with *Staphylococcus aureus* and *E. coli* and found important points of similarity and of difference with the bactericidal curves. Ehrismann (1930) obtained essentially the same results. The difference begins with wave lengths longer than 2800 A. There, the great absorption is not accompanied by a corresponding death rate, probably because of the low energy per photon.

In practically all species investigated by Ehrismann, Coblentz and Fulton, Duggar and Hollaender, Gates, and Wyckoff, the greatest absorption takes place around 2650 A, and at this wave length, the largest number of cells per erg of incident energy is killed. With longer and with shorter wave lengths, the percentage of killed individuals decreases. At 3300 A, the deaths per erg are less than 1% of that obtained near 2650 A, and at 2400 A, about 50% of this maximum (see fig. 4).

In his study of the effect of the entire range of electromagnetic waves on three fungi, Johnson (1932) found that the only effect of the visible light rays was an increase in pigment in *Fusarium batatatis* after exposure for a week or more. No

effect was observed after exposure to infra-red rays above 7,000 A, or to Hertzian waves of 50 and 100 m.

*4. Sensitivity of different species.* The differences between different species in their resistance to X-rays or cathode rays have been explained by Wyckoff (1930) to be due simply to differences in size. Almost any absorbed quantum of these rays is lethal, and there seems to be no significant species difference of absorption.

This is also true for ultraviolet radiation. All investigations have shown that the sensitivity of different species of bacteria varies but little. Sharp (1939) working with 10 species reports, as extreme variations of energy required to kill, 168 ergs per mm$^2$ for dysentery bacteria, and 337 ergs for diphtheria bacteria. Even bacterial spores are easily killed. Sharp found in 1939 that a culture of *B. anthracis* with spores required 452 ergs. In 1940, he observed that a spore-containing culture of *B. subtilis* sprayed into air had to be exposed 2 to 3 times as long as *E. coli* to obtain the same killing effect. Duggar and Hollaender

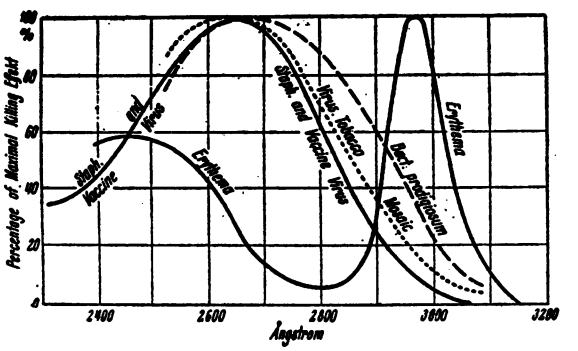


FIG. 4. COMPARATIVE INTENSITIES OF THE KILLING EFFECT OF DIFFERENT WAVELENGTHS ACTING ON DIFFERENT ORGANISMS (FROM RAHN, 1936)

(1934) could kill 85% of the vegetative cells of *B. subtilis* and *B. megatherium* with 165 ergs per mm$^2$ while the spores needed 182 ergs. Lea and Haines (1940) found the spores of *B. mesentericus* to require 5 times as much energy as *E. coli*.

The similarity of the death curves suggests that the molecules, whose inactivation by ultraviolet causes death, are similar in the different species. This agrees with the conception of death as a lethal mutation, because genes must be considered as nucleoproteins, and though they may differ specifically, their absorption spectra are probably quite similar.

The spores of molds are more resistant, and the resistance varies greatly with the species. Fulton and Coblentz (1929) studied the lethal action of ultraviolet radiation upon the spores of 27 widely different species. Sixteen of these could be killed by a 1-minute exposure to a mercury-tungsten lamp; with 4 others, less than 1% survived; but the two most resistant species had between 40 and 50% survivors after a 4-minute exposure. The authors explain this by "the difficulty in ray penetration of the spore walls due to their protective coloration or to their composition." The mycelium is more easily killed than

the spores. The spores of *Penicillium digitatum* required 9 times as long an exposure as *E. coli*. This penicillium belonged to the 16 easily killed species; and the observation by Koller (1939) that spores of *Aspergillus niger* require 50 to 100 times as much energy as *E. coli* is not contradictory.

The virus of tobacco mosaic was far more resistant than the spores of *B. subtilis* or of *B. megatherium*, but responded essentially to the same wave lengths (Duggar and Hollaender, 1934).

Ultraviolet rays are widely used for the sterilization or air, especially in hospitals and operating rooms (see review by Hart and Sharp in Glasser's Medical Physics, 1944). They are also employed to decrease the contamination from the air of breweries, bakeries, meat and vegetable storage rooms (see review by Porter, 1940).

Ultraviolet radiation has been used for the sterilization of the water supplies of a few cities in France. The process is efficient, but expensive. Many laboratory attempts have been made to sterilize milk, but this method has not been used as yet by the dairy industry (Supplee *et al.*, 1941). Sterilization of solid objects must necessarily be limited to the very surface, and while fair success is claimed for meat in storage (Porter 1940), Fulton and Coblentz obtained discouraging results in trying to sterilize oranges. Hall and Keane (1939) could kill all the spores of thermophilic bacteria in sugar in laboratory experiments, but in large-scale manufacturing, ultraviolet radiation destroyed only half of the spores, on account of the absorption of the rays by the sugar crystals.

### III. DEATH DURING AND AFTER DESICCATION

Two different effects must be considered separately when the desiccation of bacteria is concerned, namely, the number of fatalities due to the removal of moisture, and the gradual death of those bacteria which survive the change from the moist to the dry state. The two causes of death are quite different, and are independent of each other.

*1. Death during desiccation.* The earlier theories referred only to the death of bacteria spread in thin layers on some surface, and it was believed that bacteria could not survive complete drying, but were protected against this occurrence more or less completely by the capsule of the dry medium around them, and only those cells could survive which kept their natural moisture content. Modern drying methods, especially the spray-drying, leave only very thin protective layers around the bacteria, and yet many survive. The percentage of survivors may vary from 0 to nearly 100, depending not only upon the species and age of the culture, but upon the kind of medium in which the cells are suspended, the surface on which they are dried, and the rate and temperature of drying.

Most important is the medium surrounding the bacteria during the act of desiccation. Bacteria dried with their culture medium such as broth or milk survive fairly well while suspensions of bacteria from agar surfaces or of washed bacteria have only a very small percentage of survivors.

Paul and Prall (1907) dried the staphylococci from agar surface growths, after

suspension in water, on small stones (garnets) of uniform size in order to have bacteria free from the organic matter of the medium. A decrease of about 60% was observed in the first 24 hours, but after that, the number of survivors remained constant if they were kept in a vacuum at very low temperatures. The object of Paul and Prall's procedure was to obtain uniform bacterial suspensions for testing disinfectants in the absence of organic matter. However, the dead bacteria were actually merely organic matter. This becomes very evident from the study by Otten (1930) who made thick suspensions of bacteria from agar surface growths in saline solutions, and dried small quantities, a few drops or ½ to 1 ml, in tiny vials at room temperature in a vacuum over concentrated $H_2SO_4$. He obtained a survival rate of 2 to 5% with typhoid bacteria, 0.05 to 0.005% with dysentery bacteria, and only one survivor out of 10,000, sometimes less than one out of a million, with the cholera vibrio. Otten varied the conditions of drying, and observed that slow drying kills more bacteria than rapid drying. Quite important was the depth of the layer of dried cells. While 1 ml of a suspension of typhoid bacteria resulted in 0.04% survivors after drying, 0.1 ml of the same suspension dried on the same surface gave only 0.008% survivors. The same amount of suspension, dried in different containers of which one had an exposed surface ten times as large as the other, gave survival ratios 5 to 23 times as large with the smaller exposed surface. Otten then mixed the bacteria to be dried with a suspension of dead bacteria, and obtained far better survival; for instance:

| | | |
|---|---|---|
| 1 ml of the concentrated suspension yielded | 2.6% | survivors |
| 1 ml of a dilution (1:10) with saline yielded | 0.08% | " |
| | 0.03% | " |
| 1 ml of a dilution (1:10) with a suspension of dead bacteria yielded | 2.3% | " |
| | 1.8% | " |

Otten emphasizes that the bacterial proteins protect the cells not by forming a cover which prevents complete desiccation, but by acting as protective colloid which makes the drying process more gentle and less abrupt. In 1933, Otten applied this discovery to the drying of very sensitive species, such as the bacteria of meningitis or whooping cough, by the addition of dead staphylococci. He also showed that dried smallpox vaccine, was protected by the lymph proteins and could be kept active at tropical temperature for as long as 5 years by preservation *in vacuo* (fig. 5b).

The majority of experiments on desiccation refer to bacteria dried with the culture medium which is practically always of colloidal nature. Consequently, the survivor ratio is high. Rogers (1914), in his first experiments, dried freshly curdled milk cultures of lactic streptococci by adding an equal amount of lactose, and blowing warm air of 43 C over the culture. About 80% of the cells died during this treatment (table 2), and the death rate was greatest when the moisture dropped from 10% to 5%.

Higher survival rates were obtained by spraying the cultures into a current of

dry warm air. Drying was almost instantaneous, and took place at fairly low temperatures because of the rapid evaporation. Initial bacterial counts are not given, but the powdered cultures contained from 657 million to 8,590 million viable cells. The best method, however, was the desiccation of frozen cultures. For laboratory experiments, the cultures were frozen in Petri dishes in $CO_2$-snow, placed in a cold desiccator with concentrated $H_2SO_4$, or $P_2O_5$, and evacuated to a very high vacuum which is absolutely essential for rapid drying. 10 ml of a milk culture can thus be dried in 3 to 4 hours. The frozen and dried cultures contained between 380 and 12,670 million bacteria per gram. Neutralization of the milk cultures resulted in higher bacterial counts before drying, but in lower counts after drying.

"The Bureau of Dairy Industry prepares, for distribution in the field, dried cultures of *Propionibacterium shermanii*, the organisms largely responsible for the characteristic flavor and eye formation in Swiss cheese. . . . The final product

TABLE 2

*Survival of lactic streptococci during drying of a milk culture with added lactose*

| TIME OF DRYING | MOISTURE CONTENT OF CULTURE | SURVIVORS PER GRAM | |
|---|---|---|---|
| | | Moist powder | Water-free basis |
| *hours* | % | | |
| 0 | 59.05 | 785,000,000 | 1,917,000,000 |
| 0.5 | 48.05 | 750,000,000 | 1,443,000,000 |
| 1.0 | 34.71 | 963,000,000 | 1,475,000,000 |
| 1.5 | 24.05 | 942,000,000 | 1,240,000,000 |
| 2.0 | 10.56 | 916,000,000 | 1,024,000,000 |
| 2.5 | 4.74 | 351,000,000 | 368,000,000 |
| | 3.25 | 385,000,000 | 393,000,000 |

Data of Rogers, 1914.

may contain as many as 700,000,000 viable bacteria per gram" (Fundamentals of Dairy Science, 1935, p. 432). Commercial yeast cultures are usually dried on some cereal constituents; lactic cultures for dairy starters are sometimes dried on lactose; the rapid absorption of 5% water of crystallization by anhydrous lactose may be of help in rapid drying. Bacteria in soil survive drying quite well. Rahn (1907) found that 36% of the original flora of a good farm soil was still alive after 56 days of slow drying.

The American Type Culture Collection uses drying quite extensively to preserve cultures, because it not only avoids the necessity of continual transfers, but also prevents the formation of variants, dissociants, mutants, etc. Dried cells cannot possibly change their morphological or physiological characters.

Different species exhibit quite different resistances to desiccation. Stark and Herrington (1931) found that streptococci could tolerate the sudden change from moist to dry state (when the bacteria were dried in their culture medium) much better than *E. coli* or *Lactobacillus acidophilus*, while yeast and staphylococci showed an intermediate tolerance.

The survival of bacteria during the process of desiccation and afterwards is of importance in public health as well as in food preservation. In the manufacture of milk powder, for instance, not all bacteria are killed. Even the severe treatment of the drum-drying method, where the milk flows onto rotating, steam-heated drums and is scraped off as a paper-thin, dry sheet, leaves some vegetative forms alive. According to Supplee and Ashbaugh (1922), usually only one out of 10,000 bacteria survives. The very rapid spray-drying process yields a much higher survival ratio. The book of Hunziker (1935) has compiled a large number of data, and the plate counts of drum-dried powder range from 45 to 600,000 per gram while the spray-dried powders have the much higher limits of 4,400 and 5,500,000. A more recent survey of 671 English milk powder samples by Crossley and Johnson (1942) shows the wide extremes of 200 and 19,500,000 bacteria per gram.

Bacteria dried on metal surfaces die when in direct contact. The fact that many bacteria can be obtained from coins means only that there is a layer of protective dirt preventing immediate contact between bacteria and metal. Ordinary glass contains free alkali, and bacteria dried on coverglasses usually do not survive long.

Campbell-Renton (1941) tested the resistance of bacteriophage to desiccation and found that the decrease is approximately logarithmic. Different phages vary greatly in tolerance; of 15 phages for the dysentery bacteria, 8 were reduced to less than 1% of their original activity after 24 hours of drying, while 6 had more than 50% of their activity left. Once in a dry state, the viability decreases very slowly if the vacuum is maintained. Even after $3\frac{1}{2}$ years of storage over $P_2O_5$, some phages had lost little of the activity which was left after the initial decrease by the drying process as such. Most resistant were the phage "Pasteur" for *Staphylococcus aureus* and the phage "D M Large" for *Salmonella schottmuelleri*.

*2. Death of dry bacteria.* The death rate of dry bacteria was first studied by Th. Paul (1909) and by Paul, Birstein and Reuss (1910). *Staphylococcus aureus* was dried on garnets, and the cells died slowly, and in logarithmic order, when kept at room or incubator temperatures. The actual cause of death was found to be oxidation. Table 3 illustrates the effect of the oxygen concentration on the death rates which were computed from frequent plate counts. The bacteria of Series A were kept in air and in commercial oxygen while in Series B, an intermediate oxygen concentration was used. The concentration exponents[2] average 0.44 which means that the death rate is approximately proportional to the square root of the oxygen concentration. Rogers (1914) also found a higher death rate in air or in oxygen than *in vacuo*, in hydrogen or carbon dioxide. However, bacteria die also in the complete absence of oxygen, though quite slowly. The cause of this death has never been studied.

[2] The concentration exponent $n$ is calculated from the ratio of two different concentrations, and from the ratio of the corresponding death rate constants, by the equation $\left(\frac{C_1}{C_2}\right)^n = \frac{K_1}{K_2}$

The order of death is essentially logarithmic as may be seen from fig. 5a which shows the average decrease of viable bacteria in 9 samples of milk powder kept at 5 different moistures. The break at about 500 bacteria per gram may

TABLE 3

*Death rate constants of dried staphylococci at different temperatures and oxygen concentrations*

| SERIES | OXYGEN CONCENTRATION | DEATH RATE CONSTANTS = $\frac{1}{t}$ LOG $\frac{\text{INITIAL NUMBER}}{\text{SURVIVORS}}$ | | | $Q_{10}$ |
|---|---|---|---|---|---|
| | | at 18.2 C | at 24.9 C | at 37.4 C | |
| | % | | | | |
| A | 20.8 | 0.0017 | — | 0.0157 | 3.3 |
| | 96.2 | 0.0034 | — | 0.0256 | 2.7 |
| Concentration exponent $n$.... | | 0.46 | | 0.32 | |
| B | 20.8 | — | 0.0107 | 0.0264 | 2.1 |
| | 54.6 | — | 0.0152 | 0.0369 | 2.0 |
| | 96.2 | — | 0.0200 | 0.0444 | 1.5 |
| Concentration exponent $n$..... | | | 0.36 | 0.56 | |
| | | | 0.50 | 0.46 | |

Data of Paul, Birstein and Reuss, 1910.

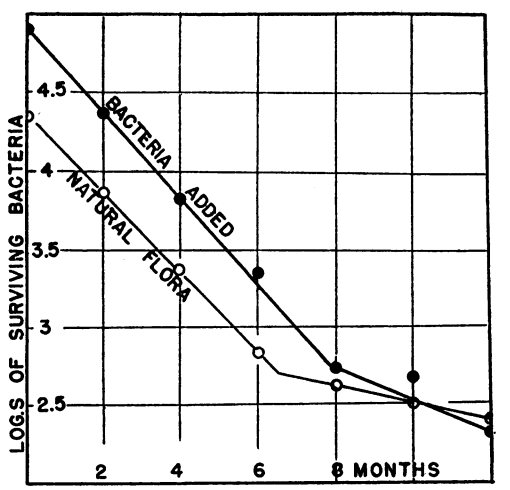


FIG. 5a

Decrease of bacteria in milk powder. (Data of Supplee and Ashbaugh, 1922)

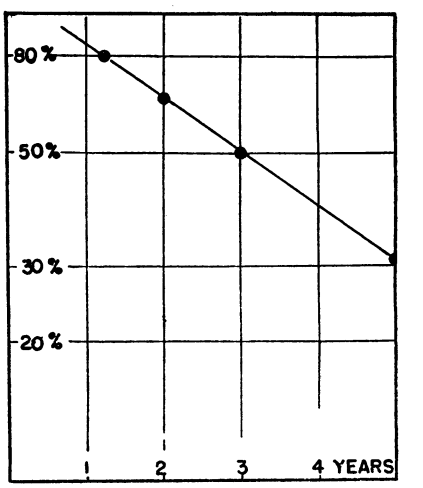


FIG. 5b

Decrease in activity of dry smallpox vaccine *in vacuo*. (Data of Otten, 1933)

be due to spores or very resistant species. Fig. 5b shows the decrease of viability of dried smallpox vaccine kept in high vacuum at room temperature in the tropics, according to Otten (1933).

The temperature coefficients of the death of dry bacteria are those to be expected for an oxidation process. The data of table 3 give an average $Q_{10}$ of

about 3 for series A, and of 2 for series B. Rogers' (1914) experiments with dried lactic cultures show a $Q_{10}$ of 3.6 between 0 and 30 C.

The survival of pathogenic bacteria, e.g. from dried feces, or spray-dried by coughing and sneezing, must be known in order to prevent epidemics. The bacteria remaining viable on dried vegetables and meats, on milk powder and egg powder decide how readily the food will spoil when moistened. Sugar contains usually the very resistant spores of thermophilic bacteria which spoil canned vegetables because the spores survive the canning process. While in some cases, we employ drying in order to kill as many bacteria as possible, we utilize, on the other hand, the longevity of dried bacteria as a great help in

TABLE 4

*Survivors of dried cholera vibrios, per million cells*

| STRAIN OF VIBRIO | SURVIVORS AFTER | | |
|---|---|---|---|
| | 24 hours | 5 months | 4 years |
| Shillong 653 | 1,950 | 284 | 26 |
| Shillong 1077 | 12,700 | 4,200 | 5.2 |
| Shillong 610 R | 4,630 | 2,600 | 0 |
| Shillong 610 R | 1,960 | 155 | 0 |
| Rangoon R | 14,200 | 450 | 12 |
| Inaba S | 1,950 | — | 97 |
| Inaba R IV | 16,000 | — | 7,000 |
| Rangoon S | 62 | — | 3.2 |
| El Tor D 12 | 11,000 | — | 50 |
| El Tor D 12 | 21 | — | 15 |
| El Tor D 31 | 4,100 | — | 30 |
| El Tor D 35 | 51,000 | — | 550 |
| El Tor D 35 | 100 | — | 12 |
| El Tor D 6 | 2,150 | — | 0 |
| El Tor D 33 | 4,700 | — | 0 |

Data of Campbell-Renton, 1942.

providing cultures for commercial purposes: magic yeast, starters for dairy purposes, and nodule bacteria dried on soil.

Bacteria are also dried to prevent them from changing their properties by mutation, dissociation or adaptation. Most of these cultures, if they are to remain alive for a very long time, are kept in a vacuum and at very low temperatures. How they gradually decrease, may be seen from Campbell-Renton's (1942a) experiences with the very sensitive cholera vibrios kept at room temperature in a high vacuum over $P_2O_5$ (table 4).

*3. Death by dry heat.* Dry cells display no life functions; the enzymes are not active in the absence of moisture; even endogenous catabolism has ceased. The cells die from oxidation, and when the temperature is raised above the maximal temperature of the species under test, death is still due only to oxidation. There is no coagulation of proteins because dry proteins do not coagulate when heated to 100 C, and dry enzymes retain their activity. All experiments show that at

higher temperatures, bacteria die more rapidly, but the gradual increase in the death rate is due merely to an increase in the rate of oxidation; there is no abrupt change in the death rate at the maximum temperature of growth, nor at any other point.

Otten (1930) found dried typhoid, dysentery and cholera bacteria able to survive 37 C for many months, 42 C for several weeks, 58 to 60 C for 7 to 10 days, and even 100 C for 1 to 2 hours. Boysen (see Rahn, 1932 p. 309) measured the death rate of yeast dried on infusorial earth and on sand. The temperature coefficient between 60 and 98 C fluctuated between 4.2 and 6.8 while between 30 and 50 C, it varied from 2.1 to 4.1.

Though dry heat is used in all laboratories to sterilize Petri dishes, pipettes and other equipment, there seems to be a surprising lack of a systematic study

TABLE 5

*Death times (in minutes) of the spores of Clostridium botulinum exposed to dry heat*

| TEMP. | I | II | III | IV | V |
|---|---|---|---|---|---|
| C | | | | | |
| 110° | >120 | >120 | >120 | >140 | 115–120 |
| 115° | >120 | >120 | >120 | >110 | 80– 85 |
| 120° | 110–115 | 95–100 | 110–115 | >120 | 100–105 |
| 125° | >60 | >60 | | 40–45 | 45– 50 |
| 130° | 55– 60 | 30– 35 | | 40–45 | 55– 60 |
| 135° | 35– 40 | 35– 40 | | >65 | >65 |
| 140° | 30– 35 | 40– 45 | 60– 65 | 15–20 | 15– 20 |
| 145° | 25– 30 | 15– 20 | 25– 30 | | 10– 15 |
| 150° | 20– 25 | 15– 20 | 25– 30 | | 10– 15 |
| 155° | 10– 15 | 10– 15 | 25– 30 | 5–10 | 10– 15 |
| 160° | 20– 25 | 15– 20 | 20– 25 | 10–15 | 10– 15 |
| 165° | | | 15– 20 | | |
| 170° | | | 10– 15 | | |
| 175° | | | 5– 10 | | |
| 180° | | | 5– 10 | | |

Data of Tanner and Dack, 1922.

of the death rates at high temperature. In 1921, Ayers and Mudge measured the death times of *E. coli* and of a heat-resistant lactic type by drying aqueous suspensions from agar slants on tin strips, and placing these in wide test tubes in an oil bath. They found that in order to be killed in 30 minutes, *E. coli* needed 60 C in milk, but 71–82 C in hot air; the lactic type needed 76 C in milk, but 110 C in hot air; a sporeformer needed more than 132 C in hot air.

All other experiments by these authors were made upon milk cans that were still wet when placed in hot air, so that no precise line between moist and dry heat could be drawn. In the experiments by Dahlberg and Marquardt (1932) the dairy utensils to be sterilized in dry heat were also placed into the heater while still wet.

The most detailed data that could be found are those of Tanner and Dack (1922) who swabbed sterile test tubes with cultures of *Clostridium botulinum*,

dried them, and determined the death times at temperatures ranging from 110 to 180 C. The results are given in table 5.

The temperature coefficients of death by dry heat are very low. From the data of table 5, the temperature coefficients for the entire range are 1.75, 1.85, 1.65, 1.59 and 1.83. This may seem contradictory to the $Q_{10}$ of about 3 shown in table 3. However, those coefficients refer to temperatures between 18 and 37 C. As will be explained in the section on Death by Moist Heat, temperature coefficients decrease slightly with increasing temperature. A reaction with a $Q_{10}$ of 3 at 20 to 30 C will display at 160 to 170 C a $Q_{10}$ of only 1.7 (see table 10). Therefore, the results of Tanner and Dack (table 5) and those by Paul *et al.* (table 3) are not in disagreement.

The obvious consequence of these low temperature coefficients is that an increase of 10 degrees does not reduce the heating time greatly. With a $Q_{10}$ of 1.7, the temperature must be raised 13 degrees in order to halve the sterilizing time. This low coefficient is the reason why the various laboratory manuals disagree widely on the times and temperatures necessary to sterilize dry glassware. There is no disagreement about the sterilization of media in the autoclave because the temperature coefficient in this case is so high that an increase of 2 to 3 C cuts the sterilizing time in half.

### IV. DEATH BY LOW TEMPERATURES

*1. Subminimal temperatures.* Most bacteria cease to grow at temperatures 5 to 10 C above the freezing point, and when they cannot grow, they die without being frozen, though very slowly. Hilliard and Davis (1918) suspended cells of *Escherichia coli* in glucose solutions and subjected them to temperatures as low as −6 C which did not freeze the solution. About 50% of the cells died in 3 hours while in parallel suspensions in water which crystallized, 93 to 99% of the cells were killed. *Streptococcus lactis* which cannot multiply at temperatures below 5 C was held by Rahn and Bigwood (1939) at 0 to 2 C. The original number of 227 million cells per ml of milk decreased in 114 days to

16,000 per ml when the culture was not treated;
34,000 per ml when the culture was neutralized at the start;
450,000 per ml when the air was replaced by nitrogen;
63,000,000 per ml when the culture was neutralized and kept under nitrogen.

Apparently, death is primarily due to a change of some essential cell constituent by oxidation which is prevented or repaired above the minimum temperature, i.e. as long as the temperature permits the synthetic mechanisms of the cell to function. At 0 C, the oxygen concentration is twice as high as at 30 C.

*2. Cold shock.* Bacteria may also die from cold shock. Sherman and Cameron (1934) could kill about 95% of very young cells of *E. coli* by cooling them very suddenly from 45 C to 10 C while gradual cooling during 30 minutes caused no injury. Some other species were less sensitive. In older cultures, only a small percentage of the cells died. The cause of death is not known. Bělehrádek (1935 p. 147) states: "Under the action of cold, the cellular content is some-

times displaced in an atypical way." Several examples are given. It seems probable that the suddenness of chilling is likely to enhance such displacement.

Two experiments on the effect of cold shock upon higher organisms are available for comparison. Kylin (1917) observed complete cessation of plasma streaming in the alga *Nitella clavata* after sudden cooling from 20 to 3 C. Here as with bacteria, the emphasis is on suddenness. It is imaginable that a very rapid temperature change disrupts the cell mechanisms either by upsetting chemical equilibria, or by spatially disconnecting some cell functions which depend upon each other. The observation by Plough (1942) that temperature shocks increase the mutation rate, seems less likely to explain the death of such a high percentage of bacteria although we have learned to look upon death of bacteria as a lethal mutation (see p. 9).

TABLE 6

*Death by continuous freezing and by alternate freezing and thawing*

(Numbers indicate plate counts per ml.)

| CONTINUOUS FREEZING | | ALTERNATE FREEZING | |
|---|---|---|---|
| *Eberthella typhosa* | | | |
| Inoculum | 40,896 | Inoculum | 40,896 |
| 24 hrs | 29,780 | Frozen 3 times | 90 |
| 3 days | 1,800 | Frozen 5 times | 0 |
| 4 days | 950 | Frozen 6 times | 0 |
| 5 days | 2,490 | | |
| *Serratia marcescens* | | | |
| Inoculum | 339,516 | Inoculum | 339,516 |
| 24 hrs | 36,410 | Refrozen once | 2,570 |
| 30 hrs | 41,580 | Refrozen 2 times | 275 |
| 48 hrs | 14,440 | Refrozen 3 times | 15 |
| 96 hrs | 4,850 | Refrozen 4 times | 0 |

Data of Hilliard and Davis, 1918.

*3. Freezing.* When the water surrounding the bacteria changes to ice, the water inside of the cell usually solidifies too, as its freezing point does not differ greatly from that of the medium. Solidification of the water prevents any kind of metabolic action, and there is some analogy between frozen bacteria and dry bacteria. In both cases, the act of transferring bacteria from the normal into the anhydrous state is a severe ordeal and kills many cells, but those which survive die at a very slow rate if kept anhydrous. Hilliard and Davis (see table 6) compared alternate freezing and thawing with the holding of frozen bacteria at −1 C. After freezing and thawing 5 successive times, all cells in cultures of *E. typhosa* and *Serratia marcescens* were dead while after remaining undisturbed in the frozen state for 4 days, several thousand cells remained alive, and most of those that were dead had been killed during the initial freezing.

The greatest injury by the act of freezing must be due to the change of water to ice which is accompanied by expansion, by crystal formation, and by colloidal changes. Expansion is not so likely to injure the rather elastic cell, but sharp-edged ice crystals may puncture the plasma membrane. Some colloidal solutions, after freezing and thawing, remain unchanged while others show a separation of the concentrated colloid from the liquid phase, the melted crystals. Such separation is commonly observed in frozen cells of plants and animals, and results in death of the cell.

Crystallization requires the presence of seed crystals or the formation of crystallization nuclei by a special collision of water molecules. The probability of such collisions is greatly reduced by colloids. According to Callow (1925), the addition of 3% gelatin to water reduces its velocity of crystallization to 1/350 of the normal rate. Ice formation in cells will therefore be slow. The number of nuclei per cell will also depend upon the volume involved, and ice formation in bacteria will be slower than in the much larger cells of plant leaves. This may account for the fact that as a rule, not all cells in a suspension of bacteria are killed by a single freezing.

Freezing involves several causes of death, and the most common cause, injury by ice crystals, is quantitatively unpredictable. Thus no order of death can be expected, and no order has been observed. The survivors of the freezing process die at a very slow rate when kept in the frozen state. Bacteria, yeast cells and mold spores have been known to survive for several years in the frozen condition. It is impossible to sterilize foods or even water by freezing.

A different picture is obtained when freezing is accomplished very rapidly to very low temperatures, e.g. by immersion in liquid air. Then, the water in the cells is not changed to ice crystals, but to a glass-like, amorphous mass. Luyet and Gehenio (1940) speak of this change as vitrification, and of the physical state as vitreous. Water in the vitreous state may change to the crystalline ice stage, and this happens more readily at higher temperatures, when the vitreous mass is warmed slowly to a temperature near the freezing point. If cells are successfully vitrified without formation of ice, they can be held at low temperatures for a very long time without danger of ice formation. This danger exists, however, during the thawing process. Bacteria which have survived vitrification, may thus be killed during the thawing. Rapid thawing will prevent this.

It is not surprising, therefore, that Kyes and Potter (1939) found tubercle bacteria to survive rapid freezing and thawing 20, 40, 80 and even 200 times when frozen in steel test tubes in liquid air, and thawed in hot water. Only one experiment was made with $CO_2$-ice in glass tubes, and no growth *in vitro* could be obtained after 25 alternations of slow freezing and thawing. Storage in the refrigerator for 6 years at $-3$ C killed all bacteria because the refrigerator was defrosted twice each year which meant twelve very slow freezings and thawings. What seemed offhand to be a most severe treatment, namely, the rapid change over 200 C in liquid air, proved to be rather harmless, thanks to vitrification. The literature on this point, and on freezing generally, has been reviewed critically by Luyet and Gehenio (1940).

## V. DEATH BY MOIST HEAT

Heat is applied in two different ways for the destruction of bacteria. Glassware and certain instruments and materials are sterilized with dry heat. The effect of high temperatures on dry bacteria has been discussed in the preceding pages. Other materials are heated when wet, e.g., foods in the canning process, milk, beer and wine during pasteurization, culture media for bacteria, and surgical dressings. For this process, the term "moist heat" is most commonly used although Chick, in the first detailed quantitative study, spoke of it as "death by hot water." The cause of death in moist heating is quite different from that in dry heating, and the rules applying to the one method do not fit the other. Death by dry heat is primarily an oxidation process; death by moist heat is due to coagulation of some protein in the cell.

*1. Thermal death point and thermal death time.* The standard of comparison of heat tolerance of different species was originally the Thermal Death Point, i.e., the lowest temperature at which a suspension of bacteria is killed in 10 minutes (see Descriptive Chart, Society of American Bacteriologists). This method cannot give comparable results unless the conditions are standardized as to age of culture, approximate number of cells, pH of suspension, dimensions of test tubes and thickness of glass in the test tubes.

Research workers in the canning industry found it more suitable for their purposes to keep the temperature constant and to vary the time. Thermal Death Time is the shortest time necessary to kill all bacteria in a given suspension at a given temperature. Bigelow and Esty (1920) suspended the bacteria or spores to be tested in clear juices from canned foods, distributed the suspension uniformly among a number of small narrow glass tubes, sealed these completely by fusing the glass, and dropped them into an oil bath of constant temperature. Tubes were removed at different times and incubated; survival of any bacteria became evident by clouding of the medium. A review of various slight alterations of technique is given by Beamer and Tanner (1939a). It is necessary to determine the initial number of cells or spores, because the thermal death time is longer with larger inocula.

*2. The order of death.* The order of death by heat is logarithmic. From the earliest quantitative measurements by Chick (1910) to the extensive investigations by Watkins and Winslow (1932), death of vegetative cells as well as death of spores has been found to be logarithmic. The investigations by Bigelow and others of the National Canners Association and the many studies of heat sterilization of spores of *Clostridium botulinum*, e.g., by Weiss (1921) and Esty and Meyer (1922) have confirmed this. A number of experiments by Beamer and Tanner (1939a, b) with vegetative cells of bacteria and with yeasts gave the same order.

The customary explanation that death is brought about by heat inactivation of the enzymes (see e.g., Isaacs, 1935) cannot be correct because, for mathematical reasons, a logarithmic order is possible only when death is due to the destruction of a single molecule in the cell (Rahn 1929, 1943). To be sure, Edwards and Rettger (1937) found that washed cells of bacteria, held for 24

hours between 40 and 60 C, lost all their respiratory enzymes at temperatures near the maximum for growth. However, enzyme deterioration of washed cells without food held for such a long time under very abnormal conditions permits no conclusions as to the behavior of the same cells in a suitable medium. Rahn and Schroeder (1941) repeated the experiment using the method of Edwards and Rettger for enzyme analysis, but they measured viable cells and enzyme content *in the same sample* of cell suspensions of *Bacillus cereus* suspended in phosphate buffer at 46 C and 50 C. The first line of data in table 7 shows that 99% of the cells were dead when only 14% of the peroxidase and 20% of the catalase had been inactivated. Enzyme coagulation could not possibly have been the cause of death. Similar results had been obtained with yeast by Rahn and Barnes (1933).

TABLE 7

*Death of cells and loss of enzyme activity of Bacillus cereus under the action of heat*

| TEMPERATURE | TIME OF EXPOSURE | VIABLE CELLS (PLATE COUNT) | CATALASE | DEHYDROGENASE |
|---|---|---|---|---|
| | | Percentage of cells or enzymes remaining | | |
| *C* | *minutes* | | | |
| 46 | 10 | 1.1 | 80 | 86 |
| | 20 | 0.04 | 65 | 86 |
| | 40 | 0.002 | 48 | 67 |
| | 80 | 0.00001 | 38 | 46 |
| 50 | 5 | 2.4 | — | 89 |
| | 10 | 0.006 | 56 | 57 |
| | 20 | <0.00002 | 56 | 9 |
| | 40 | <0.000002 | 48 | — |

Data of Rahn and Schroeder, 1941.

Microbial enzymes continue to function for a considerable time at temperatures above the maximum for growth. Table 8 shows that centrifuged cells of *Streptococcus lactis* suspended in buffer solution with glucose ferment rapidly at 42 C and even at higher temperatures. This strain multiplies most rapidly at 33 C and cannot multiply above 41 C, yet at 42 C, the energy available from fermentation is far higher than at 33 C. Multiplication at 42 C cannot be handicapped by the heat inactivation of the enzymes, but by the inactivation of the synthetic catalysts or the cell division mechanism. Similar data have been obtained with yeast which grows most rapidly at 29 C, ceases to grow at 35 C, but ferments at 44 C more rapidly than at 29 C (Rahn, 1932, p 132).

The mathematical necessity that death must be brought about by destruction of a single molecule brings us back to the definition given in the chapter on radiation that death is a lethal mutation. We may assume that heat coagulation of a single gene prevents reproduction. Such a cell is sterile, and according to bacteriological standards, it is dead, though its enzyme content may not be exhausted. However, the inactivation of the growth mechanism is likely to make

repair and replacement of inactivated molecules impossible, and the enzyme content of such sterile cells must gradually decrease and this is evident in table 8. The decreasing enzyme content is the *consequence* of inhibited growth, and not its *cause*.

*3. Death rates.* Regardless of whether we accept the one or the other explanation of the cause of the logarithmic order of death, its existence permits us to compute *death rates* and to draw conclusions from them which are independent of any explanation. Death rates make it possible to compare the heat resistance of different species at the same temperature, or the heat resistance of one species at different temperatures. It also enables us to describe in quantitative terms the effect of environmental factors, such as concentration of the medium or its pH, upon heat sterilization.

TABLE 8

*Per cent lactic acid produced in buffer plus glucose by centrifuged cells of Streptococcus lactis* (1,315,000,000 cells per ml.)

| TEMPERATURE | MINUTES | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | 60 | 90 | 120 | 150 | 180 | 240 |
| C | | | | | | | | | | | |
| 30 | | 0.036 | | 0.072 | | 0.108 | 0.144 | 0.189 | 0.234 | 0.279 | 0.360 |
| 33 | | 0.059 | | 0.107 | | 0.144 | 0.198 | 0.252 | 0.306 | 0.360 | 0.437 |
| 37 | | 0.072 | | 0.135 | | 0.189 | 0.261 | 0.315 | 0.369 | 0.405 | 0.481 |
| 40 | | 0.108 | | 0.180 | | 0.243 | 0.315 | 0.385 | 0.441 | 0.486 | 0.549 |
| 42 | | 0.099 | | 0.171 | | 0.243 | 0.315 | 0.385 | 0.437 | 0.473 | 0.495 |
| 45 | 0.054 | 0.099 | 0.144 | | 0.207 | 0.221 | 0.297 | | 0.351 | | 0.369 |
| 47 | 0.063 | 0.099 | 0.130 | | 0.198 | 0.216 | 0.270 | | 0.306 | | 0.306 |
| 50 | 0.036 | 0.063 | 0.090 | | 0.108 | 0.117 | 0.144 | | 0.171 | | 0.171 |

Data of Dorn and Rahn, 1939.

For the application of heat in the preservation of foods, the death rate constant is too cumbersome, and simpler constants have been introduced. Baker and McClung (1939) measured the time required, at a certain temperature, to reduce the bacteria to 0.01% of their original number. More elastic in its applications is the Decimal Reduction Time (D.R.T.) by Katzin, Sandholzer and Strong (1942) which is the time required to reduce the bacterial population to 10% of the original number. Accordingly, a doubling of D.R.T. must reduce the population to 1% and heating for 4× (D.R.T.) reduces it to 0.01%, so that the constant of Baker and McClung is identical with 4 times the Decimal Reduction Time.

Ayers and Johnson (1914), in their study of pasteurization of milk, observed that in cultures of *E. coli* and of lactic streptococci, a very few cells were sometimes found which were much more resistant than the great average. They introduced the term "majority thermal deathpoint" for the lowest temperature which kills the large majority of cells in 10 minutes. This phenomenon had been described in detail by Gage and Stoughton (1906) who worked with *E. coli*.

Beamer and Tanner (1939a, b) gave a clearer picture by drawing the logarithmic survivor curves which showed a sharp break, indicating that the remaining cells had a much greater, but uniform resistance. These experiments included, besides the colon-typhoid group, also several yeasts. Gage and Stoughton could find no evidence of spore formation, and they demonstrated that the greater heat tolerance was not inheritable. In all reported cases, the percentage of resistant cells has been less than 0.1. Whatever the explanation, the great practical importance of these very few highly resistant cells in commercial pasteurization of all kinds of foods is obvious. Chambers and Gaines (p. 8) had a similar experience when killing bacteria by sonic waves.

Very complicated is the mathematical treatment used by Ball (1923, 1928) in the study of the temperatures required in the canning of vegetables. This problem involves not only the heat sterilization of spores, but also the heat conductivity of the cans and their contents. The $z$ and $F$ factors which play an important role in these equations will be discussed later.

*4. The temperature coefficient of death by moist heat.* The death rate constants are either obtained from plate counts at certain exposure times, from the equation $Kt = \log \frac{\text{initial number}}{\text{survivors}}$ or they may be computed from the thermal death times. In this latter method, the initial inoculum for all tubes is the same for any series of experiments and the final number of survivors is also the same, namely less than 1, so that the quotient of initial number over survivors is constant.[3]

If the death time is determined at the temperatures $T_1$ and $T_2$ ($T_2$ being higher), we may call the corresponding death rate constants $K_1$ and $K_2$, and the death times $t_1$ and $t_2$. Since the order of death is logarithmic, we have the formula

$$K_1 t_1 = \log \frac{\text{initial number}}{\text{survivors}} = K_2 t_2$$

or

$$\frac{K_2}{K_1} = \frac{t_1}{t_2}$$

This quotient indicates how much more rapidly death proceeds at the higher temperature $T_2$. This is the temperature coefficient for the temperature increase $T_2 - T_1$. For comparative purposes, it is customary to give the coeffi-

[3] The thermal death times are not precise values. Between the last sample that showed viable bacteria and the first that showed none, some time has passed. During this interval, the number of survivors was reduced to less than 1 per sample. In all experiments, the number of survivors was identically the same at some moment between these two critical times, but the exact moment is not known. All death time data have a certain range of possible error, the magnitude of which depends upon the spacing of the time intervals. The number of survivors is never zero, but becomes very small, e.g., 1 in 100 liters, 1 in 1,000 liters, etc.

cient for an increse of 10 C which is designated as $Q_{10}$. The formula for conversion from $Q_n$ to $Q_{10}$ is indicated in the following relation:

$$Q_{10} = Q_n^{\frac{10}{n}} = \left(\frac{t_1}{t_2}\right)^{\frac{10}{n}}$$

A number of such temperature coefficients of heat disinfection have been compiled by Rahn (1932, pp. 320–323). For spores, they are fairly uniform, between 8 and 10 at temperatures from 100 to 135 C. With vegetative cells

TABLE 9

*Temperature coefficients of disinfection by moist heat*

| | $Q_{10}$ IN NUTRIENT BROTH | | $Q_{10}$ IN TOMATO JUICE | | | |
|---|---|---|---|---|---|---|
| | pH 7.05 | | pH 4.2 | | | |
| | Temperature interval | | | | | |
| | 55–60 C | 60–65 C | 55–60 C | 60–65 C | | |
| *Eberthella typhosa* | 28.9 | 26.1 | 20.2 | 2.7 | | |
| *Salmonella paratyphosa* | 37.7 | 10.3 | — | — | | |
| *Salmonella scottmuelleri* | 42.0 | 33.7 | 3.3 | — | | |
| *Salmonella aertrycke* | 23.7 | 9.0 | 5.2 | 27.7 | | |
| *Salmonella enteritidis* | 36.0 | 15.3 | 9.8 | — | | |
| *Staphylococcus aureus* | 28.9 | 59.2 | 10.1 | 3.9 | | |
| | $Q_{10}$ IN BROTH | | $Q_{10}$ IN BROTH | | $Q_{10}$ IN GRAPE JUICE | |
| | pH 6.8 | | pH 3.8 | | pH 2.6 | |
| | Temperature interval | | | | | |
| | 55–60 C | 60–65 C | 55–60 C | 60–65 C | 55–60 C | 60–65 C |
| *Debaryomyces globosus* | 4.0 | — | 12.2 | — | 6.5 | — |
| *Monilia candida* | 4.0 | — | 71.0 | — | 63.0 | — |
| *Saccharomyces ellipsoideus* | 11.6 | 12.2 | 15.4 | 9.0 | 21.3 | 19.6 |
| *Torula monosa* | 20.6 | 7.0 | 7.5 | 8.0 | 6.4 | 29.2 |

Data of Beamer and Tanner, 1939a, b.

at 50 to 80 C, they are usually higher. Some more recent data for vegetative cells are given in table 9.

The coefficients computed from the data by Beamer and Tanner fluctuate greatly, each value being the result of only one experiment. This fluctuation is due to the fact that the determination of death rates generally is subject to considerable experimental error; the possibility of error is greatly enlarged when the quotient of two such death rates is computed. Most of the coefficients are above 10, and where they are lower, it is due to the survival of a few individuals with much higher resistance, e.g., in the case of *Debaryomyces* and *Monilia* (Rahn, 1943). The high temperature coefficient makes it practically certain that death by heat is a coagulation (or denaturation) process, as such high temperature coefficients are very rare except with protein coagulation.

Temperature coefficients are not really constant. The thermodynamic definition of the temperature coefficient is

$$Q_{10} = \frac{\text{RATE at } T + 10^\circ}{\text{RATE at } T^\circ} = \frac{K_{T+10}}{K_T} = e^{\frac{\mu}{2}\frac{10}{T(T+10)}}$$

where $e$ is the base of the natural logarithms and $\mu$ is the temperature characteristic of the reaction. It is this value $\mu$ that is constant. As T, calculated

TABLE 10

*The temperature coefficient decreases with rising temperature while the temperature characteristic $\mu$ remains constant*

above: "normal" chemical reactions

below: denaturation of proteins

| TEMP. RANGE | $\mu$ = 12,286 | $\mu$ = 19,506 | $\mu$ = 24,610 |
|---|---|---|---|
| C | | | |
| betw. 0– 10 | 2.22 | 3.49 | 4.92 |
| 10– 20 | 2.10 | 3.20 | 4.42 |
| 20– 30 | **2.00** | **3.00** | **4.00** |
| 30– 40 | 1.91 | 2.76 | 3.66 |
| 40– 50 | 1.84 | 2.60 | 3.38 |
| 100–110 | 1.53 | 1.98 | 2.37 |
| 150–160 | 1.40 | 1.70 | 1.96 |
| 160–170 | 1.38 | 1.66 | 1.90 |
| 170–180 | 1.36 | 1.62 | 1.84 |
| | $\mu$ = 49,600 | $\mu$ = 64,600 | $\mu$ = 99,200 |
| betw. 50– 60 | **10.0** | **20.0** | **100.0** |
| 60– 70 | 8.8 | 17.0 | 76.7 |
| 70– 80 | 7.8 | 14.5 | 59.6 |
| 80– 90 | 7.0 | 12.4 | 47.9 |
| 90–100 | 6.3 | 10.9 | 38.9 |
| 100–110 | 5.6 | 9.6 | 32.0 |
| 110–120 | 5.2 | 8.6 | 27.0 |
| 120–130 | 4.8 | 7.7 | 23.0 |

as absolute temperature, increases, the value T (T + 10) becomes larger, and therefore the exponent of $e$ becomes smaller; consequently, $Q_{10}$ decreases with increasing temperature.

Table 10 shows how the temperature coefficient of the same reaction changes when the temperature varies. The upper part of the table shows that reactions with a $Q_{10}$ of 2, 3 and 4 at 20 to 30 C will have at 170–180 C the much smaller coefficients of 1.36, 1.62 and 1.84. In sterilization by dry heat, where oxidation is the cause of death, a "normal" temperature coefficient of about 3 can be expected, but this value 3 refers to room temperature, and an increase from 170 to 180 C will increase the rate of death only 1.6 times.

The lower part of table 10 deals with protein reactions such as coagulation, denaturation, or inactivation, which have very high temperature coefficients. A coefficient of 100 at 50 C corresponds to a $Q_{10}$ of 23 at 120 C. The fact that the $Q_{10}$ of spore sterilization in canning averages about 9, while that of milk pasteurization is about 20, does not prove that we are dealing with different reactions. The same reaction with a $Q_{10}$ of 20 near 60 C has at 120 C a $Q_{10}$ of 8.

So long as temperature effects are studied within the narrow limits of life, i.e., between 10 and 50 C, the assumption of a constant $Q_{10}$ does not introduce a great error, but when the range becomes as wide as in the study of death by heat, the assumption of a constant temperature coefficient may be very misleading.

Two different methods of pasteurization of milk are permitted by the New York State Department of Health, namely the holding process which consists of holding the milk for 30 minutes at 143 F, and the short-time, high temperature process, usually called the flash heat process, which requires holding for 15 seconds at 160 F. The temperatures are 9.44 C apart (71.11–61.67) and the heating time of the one process is 120 times that of the other. This means, that the authorities assumed a $Q_{9.44}$ (which is practically $Q_{10}$) of 120, a very high temperature coefficient for this range.

It is obvious that the temperature coefficients of death cannot be extrapolated into the region of growth. If a bacterium can multiply, though very poorly, at 40 C, but dies at 50 C, the temperature coefficient will equal the death rate at 50 C divided by zero which means $Q_{10} = \infty$. A sharp drop from infinity to the actual temperature coefficient of inactivation of the life processes must be expected, and very high coefficients must be expected at the lowest lethal temperatures. This accounts for the high coefficients found for *Escherichia coli* near 50 C since its maximum temperature of growth is 47 C. Watkins (1933) computed the increase of death rate between 50 and 55 C to correspond to a $Q_{10}$ of 560. Baker and McClung (1939) found for the same bacterium in three experiments:

| | | | |
|---|---|---|---|
| between 51.7 and 54.5 ..... $Q_{10}$ = | 296.0 | 315.0 | 16.6 |
| between 54.5 and 57.3 C ..... | 22.7 | 21.6 | 408.0 |
| Average between 51.7 and 57.3 C ..... | 86.7 | 70.8 | 89.2 |

Henderson Smith (1923) computed from his experiments with the spores of *Botrytis cinerea* the following temperature coefficients:

| | |
|---|---|
| between 31 and 37 C | $Q_{10}$ = 690 |
| between 37 and 44.3 C | 132 |
| between 44.3 and 47.0 C | 92.8 |
| between 47.0 and 50.3 C | 29.5 |

The data on the death rates of spores show a far greater uniformity and constancy than those for vegetative cells, because all data on spores refer to temperatures which were at least 30 C higher than the maximal growth temperature,

while many of the experiments with vegetative cells were made within 10 or 15 C of the maximum for growth.

*5. Factors controlling death by heat.* Sensitivity to heat varies with the species. Pasteurization of milk by holding at 63 C for 30 minutes destroys all pathogenic bacteria except the spores, and kills many species of saprophytes, but not all. With some species such as lactobacilli, the death rate is low, and sometimes, thermophilic bacteria multiply during pasteurization. The spores of many species can survive a short period of boiling, but the spores of *Methanobacterium omelianskii* are killed at 80 C in 10 minutes (Barker 1940). The cause of the great resistance of bacterial spores will be discussed at the end of this chapter.

The medium in which bacteria are suspended can influence the death rate. The best-known example is the effect of the acidity of the medium. Bacteria die more rapidly in acid or alkaline media than in neutral suspensions. This suggests, of course, the assumption of chemical disinfection rather than physical coagulation, but two facts speak against this. In chemical disinfection, the temperature coefficients should be relatively low while table 9 shows that they are, on the average, higher in acid than in neutral media. Further, yeast which grows better in acid media is killed by heat more easily in acid than in neutral media (Beamer and Tanner, 1939b).

Extensive experiments on the effect of pH on the heat resistance of spores have been carried out by Weiss (1921), Esty and Meyer (1922) and others. The study by Townsend, Esty and Baselt (1938) may serve as a more recent example. These authors did not use death rates or temperature coefficients, but the factors $F$ and $z$ which Ball had introduced in 1923 for the computation of processing times in the canning industry. As these two values are frequently used in the canning industry which furnishes the largest amount of data for death by heat, the meaning of these factors will be discussed here.

The value $F$ is the thermal death time of the species at 121 C or 250 F. The number $z$ is the temperature increase, in degrees Fahrenheit, necessary to reduce the death time to one-tenth. We introduce the temperature coefficient for 1 C, $Q_1$, which can be transformed into $Q_{10}$ by the simple relation $Q_{10} = Q_1^{10}$. A few pages earlier, it has been shown that the ratio of the death rates at temperatures $T_2$ and $T_1$ can be measured by the corresponding death times, viz.,

$$\frac{K_2}{K_1} = \frac{t_1}{t_2}$$

This ratio is the temperature coefficient for $n° = T_2 - T_1$ degrees, hence

$$Q_n = Q_1^n = \frac{t_1}{t_2}$$

The definition of $z$ specifies that with an increase of $z$°F, or 0.555$z$°C, the death time $t_2$ is one-tenth of $t_1$. Therefore, $n = 0.555z$, and $\frac{t_1}{t_2} = 10$.

$$Q_1^n = Q_1^{0.555z} = \frac{t_1}{t_2} = 10$$

$$0.555z \log Q_1 = \log 10 = 1$$

We have seen that $Q_{10} = Q_1^{10}$, therefore

$$\log Q_{10} = 10 \log Q_1 = \frac{10}{0.555z} = \frac{18}{z}$$

$$z = \frac{18}{\log Q_{10}}$$

The value of $z$ or $Q_{10}$ indicates the slope of the straight line obtained by plotting the logarithms of death times against temperature. The value $F$ gives one point on this curve, and thus, $F$ and $z$ (or its corresponding $Q_{10}$) are sufficient to characterize the thermal resistance of the spores of a species at any temperature.

The authors studied in detail the heat resistance of the spores of two strains of *Clostridium botulinum*, and of a bacillus isolated from spoiled canned corn. The summarized results are given in table 11 where the $z$ values have been converted into $Q_{10}$. With the bacillus spores, the value $F$, the death time at

TABLE 11

*The heat resistance of the spores of three bacteria, expressed by the values F and $Q_{10}$*

| MEDIUM OF SPORE SUSPENSION | pH | BACILLUS 3679 | | C. BOTULINUM 62A | | C. BOTULINUM 213B | |
|---|---|---|---|---|---|---|---|
| | | $F^*$ | $Q_{10}$ | $F^*$ | $Q_{10}$ | $F^*$ | $Q_{10}$ |
| Phosphate buffer..... | 7.0 | 4.00 | 12.1 | 1.70 | 12.5 | 2.00 | 10.0 |
| Asparagus..... | 5.4 | 3.30 | 7.1 | 0.39 | 15.9 | 0.39 | 15.9 |
| Peas..... | 5.4 | 3.00 | 11.6 | 0.30 | 22.0 | 1.40 | 14.2 |
| Spinach..... | 5.4 | 2.60 | 9.8 | 0.65 | 14.5 | 0.68 | 14.5 |
| Milk..... | 6.3 | 2.60 | 7.8 | 0.45 | 16.8 | 0.50 | 18.2 |
| Average..... | | | 9.7 | | 16.3 | | 14.6 |

* Death time in minutes at 121 C.

Experiments of Townsend, Esty and Baselt, 1938.

121 C (250 F), was not greatly affected by the medium, but the spores of the two clostridia survived in the neutral phosphate buffer 2 to 4 times as long as in milk and in the vegetable juices. The differences cannot be explained by pH alone. It has been observed in commercial canning that the heat tolerance of spores is not the same in different vegetables, but the cause is not known. The temperature coefficient with the spores of the two clostridia was higher in the vegetable juices than in the buffer, whereas the spores of the bacillus showed the opposite. In the buffer, the three behaved nearly alike. Tanner (1944, p. 962) speaks of a "food factor" which is the ratio of death time in food juice to death time in neutral phosphate buffer.

Different from this chemical effect is the protection afforded by concentrated solutions. The well-known increase of the thermal resistance of bacteria through addition of sugar is usually explained by partial dehydration of the protoplasm. In concentrated solutions of sugar, proteins are not coagulated by heat (Beilinsson, 1929). According to Fay (1934), egg albumin which coagulated in 3 to 4 minutes at 60 C, required 23 and 31 minutes respectively in molar solutions

of glucose and sucrose, and 73 minutes and more than 24 hours, respectively, in twice molar solutions. Rennet enzyme dissolved in water was completely inactivated at 70 C in 15 minutes, but molar $CaCl_2$ protected it for 45 minutes; and in 4 molar sucrose it lost only half of its strength in 5 hours. K. von Angerer and Küster (1939) confirmed the assumption that bacteria become partly dehydrated by comparing the turbidities of bacterial suspensions in water and in concentrated solutions.

The time of contact is of importance. Fay obtained only 2.5% survivors of *Escherichia coli* upon heating immediately after suspending the cells in 50% sucrose, whereas the same heating left 79% of the cells unharmed if they had been in the sugar solution for 2 hours before heating.

TABLE 12

*The harmful effect of high temperature on liquids containing reducing sugars (in half-molar concentration)*

Survivors of *E. coli* after being heated for 8 minutes at 54 C in variously treated sugar solutions at pH 7.0.

| CONCENTRATION | SUGAR | PERCENTAGE SURVIVORS IN MEDIUM PREVIOUSLY STERILIZED BY | | | |
|---|---|---|---|---|---|
| | | Autoclaving | | Filtration | |
| | | In buffer | In broth | In buffer | In broth |
| % | | | | | |
| 0 | (control) | 1.3 | 7.2 | 1.9 | 3.9 |
| 9 | glucose | 0.08 | 2.7 | 5.3 | 7.9 |
| 9 | galactose | 0.04 | 1.8 | 4.3 | 8.0 |
| 17.3 | lactose | 0.09 | 1.6 | 18.0 | 11.8 |
| 17.3 | maltose | 0 | 3.1 | 5.0 | 7.3 |
| 17.3 | sucrose | 9.2 | 15.0 | 12.0 | 10.0 |
| 9.1 | mannitol | 4.5 | 10.8 | 5.5 | 8.9 |
| 4.1 | glycerol | 3.2 | 9.4 | 3.7 | 6.4 |

Data of Baumgartner, 1938.

Some inconsistencies in the results by various investigators are explained by Baumgartner (1938) as being caused by harmful decomposition products originating from reducing sugars during autoclaving. This is prevented by aseptic filtration. Table 12 shows that the percentages of survivors in the last three solutions, representing non-reducing compounds, are nearly the same in autoclaved and filtered media while the four media containing reducing sugars have become quite toxic by autoclaving.

The theory of dehydration of the protoplasm is too simple to fit the facts. Protection is not proportional to molarity (table 12), disaccharides protect more than monosaccharides but maltose seems to be an exception. Von Angerer and Küster (1939) measured the death rates of *Escherichia coli* and *Staphylococcus sp.* at 56 C in concentrated solutions of various carbohydrates and other easily soluble substances. It was found that starch, gum arabic, urea, pyruvate,

glycocoll, calcium chloride, or phosphate buffer offered no protection while glycol, glycerol, sorbitol and various mono- and di-saccharides retarded death very noticeably.

*The Unusual Resistance of Spores* is not limited to heat. It is very conspicuous with disinfectants, but does not extend to irradiation (see p. 13). It has been the cause of many investigations because bacterial spores are the most resistant organisms existing. The spores of molds, although more hardy than the mycelium, are killed readily by boiling, and the spores of yeasts are only slightly more resistant than the vegetative cells.

The oldest explanation, the assumption of a practically impermeable spore wall, can hardly be applied in the case of heat. A wall of only 0.1 $\mu$ thickness could not possibly insulate the cell contents against high temperature for several hours. Nor could such extreme lack of heat conduction protect spores e.g., for 440 minutes at 100 C, but for only 5 minutes at 120 C. Absence of moisture has been another explanation. According to early investigations, the specific gravity of spores seemed to be about 1.35 to 1.4 which indicated a low moisture content. Since dry proteins are not coagulated by heat, this explanation seemed plausible. However, newer measurements indicate that spores contain nearly as much moisture as vegetative cells (Virtanen and Pulkki, 1933; Henry and Friedman, 1937). K. von Angerer and Küster (1939) could extract water from spores by suspending them in concentrated sugar solutions. Virtanen and Pulkki assumed that the enzymes of the spores were in an inactive form, and therefore resistant. Friedman and Henry (1938) explained this resistance as due to the large amount of bound water in the spores. From measurements of the freezing points of spore suspensions in water and in sucrose solution, the bound water for three different species of bacilli could be calculated to 69, 63 and 59% of the weight of moist spores whereas the corresponding figures for vegetative cells were 0, 18 and 28%. The calculations are based on temperature differences of a few hundredths of one degree, and probably have a large experimental error.

O. B. Williams (1929) approached the problem from a different angle. He showed that the ash content of spores is low, and as proteins do not coagulate readily in the absence of electrolytes, the low electrolyte content of the spores may be responsible for their heat resistance.

A different viewpoint was investigated by Rosel von Angerer (1939) who considered the fairly high fat content of spores as a possible means of protection. Long extraction with fat solvents decreased heat resistance while impregnation with paraffin (dissolved in carbon tetrachloride) doubled it. Presence or absence of oxygen did not affect the death time. She could not verify the theory of Sobernheim and Mündel (1938) that spores adsorbed on porous materials such as soil are much more resistant. Of all the materials tested, only granulated coal increased the death time significantly.

However, the most probable explanation is the assumption that the protein in spores is different from that of vegetative cells (Heim, 1938). The temperature of denaturation of different proteins is by no means the same. Among

enzymes, cytochrome oxidase is inactivated at 53 C, trypsin at 66 C, urease between 70 and 80 C, while ribonuclease and taka-diastase can tolerate a short period of boiling. It seems possible that during sporulation, the protoplasm of the cell is so altered that it is not easily denatured; during spore germination, this process would be reversed. It is imaginable that this change is somehow connected with changes in bound water.

TABLE 13

*Relative resistances of bacterial and mold spores, and of viruses, referred to the resistance of E. coli as unity*

| STERILIZING AGENT | ESCHERICHIA COLI | BACTERIAL SPORES | MOLD SPORES | VIRUSES AND BACTERIOPHAGE |
|---|---|---|---|---|
| Phenol | 1 | 100,000,000[a] | 1– 2[f] | 30[h] |
| Formaldehyde | 1 | 250[b] | | 2[i] |
| Dry heat | 1 | 1,000[c] | 2– 10[g] | ±1[j] |
| Moist heat | 1 | 3,000,000[d] | 2– 10[g] | 1– 5[k] |
| Ultraviolet | 1 | 2–5[e] | 5–100[e] | 5–10[e] |

[a] Chick (1908) gives death rate $k = 0.0466$ for anthrax spores with 5% phenol, and $k = 0.212$ for paratyphoid bacteria with 0.6% phenol. Assuming concentration exponent $n = 6$, the ratio is $\left(\frac{5}{0.6}\right)^6 \frac{0.212}{0.0466} = 10^8{:}1$.

[b] Formaldehyde, 5%, kills anthrax spores in 32 hours while *E. coli* is killed by 2% in 20 minutes (Chick, 1908). Assuming concentration exponent $n = 1$, the ratio is 250:1.

[c] No records could be found on death rates of dried *E. coli* and of dry bacterial spores at the same temperature. If we assume that dry spores at 160° die at about the same rate as dry *E. coli* at 60°, the death rate of *E. coli* at 160° (100° increase, $Q_{10}$ averaging 2) would be $2^{10} \cong 1000$ times as high as that of spores.

[d] No records could be found on death rates of *E. coli* and of bacterial spores in water at the same temperature. If we assume that moist spores at 120° die about as rapidly as *E. coli* at 60°, the death rate of *E. coli* at 120° would be obtained by multiplying the rate at 60° with the average temperature coefficient raised to the 6th power. From Table 10, the average $Q_{10}$, with $\mu = 64{,}000$, is about 12. The death rate of *E. coli* is therefore about $12^6 \cong 3{,}000{,}000$ times as great as that of spores.

[e] See p. 13.

[f] Woodward *et al.*, 1934.

[g] Own experience.

[h] From data in McCulloch, p. 353.

[i] From data in McCulloch, p. 373.

[j] See p. 19.

[k] From data in McCulloch, p. 118–119.

Table 13 shows how many times more resistant bacterial spores, mold spores, viruses and bacteriophages are than *E. coli*. A comparison of this kind can give only the order of magnitude, because the spores of different bacteria are not equally resistant, the spores of different molds differ widely in resistance to ultraviolet, and the phages and viruses do not react identically either. But the great contrast between bacterial spores and all the other forms is quite striking; and the absence of this contrast with ultraviolet and the differences within each group are suggestive of the different lethal reactions causing death.

Experimentation with the resistance of spores is complicated by a great variability of this property due to environmental conditions. A summary of the literature is given by Theophilus and Hammer (1938). Bacterial spores will occasionally remain dormant for a long time before germinating. This accounts for the difficulty encountered in the sterilization of food by intermittent heating on successive days. An extensive study has been made by Morrison and Rettger (1930). According to Evans and Curran (1943) incubation of spores at sublethal temperatures, e.g., 65 C, accelerates the rate of their germination.

## VI. SURFACE TENSION DEPRESSION

*1. Effects of surface tension depression.* Many compounds which lower the surface tension of water are good disinfectants. Best known among these are

TABLE 14

*Surface tension of solutions killing Staphylococcus aureus in 5 minutes*

| | CONC. | SURFACE TENSION IN DYNES | |
|---|---|---|---|
| | | In water | During test |
| | % | | |
| Phenol | 1.25 | 41.5 | 42.0 |
| o-Cresol | 1.00 | 37.5 | 37.5 |
| Hexyl resorcinol | 0.025 | 37.5 | 37.5 |
| Thymol | 0.0625 | 38.5 | 38.5 |
| Chlorothymol | 0.25 | 49.0 | 49.0 |
| Mercuric chloride | 0.025 | 70.0 | 68.5 |
| Merthiolate | 0.025 | 51.0 | 51.5 |
| Mercurochrome | 1 | 71.5 | 65.0 |
| Iodine | 0.02 | 59.5 | 53.5 |

Data of Gershenfeld and Witlin, 1941.

some of the fatty acids, the alcohols and phenols, the soaps, the dyes, the bile salts, and many of the large number of modern wetting agents and detergents.

If the decrease in surface tension were the fundamental cause of death, the bacteria should die when a certain tension is reached, regardless of the chemical structure of the compound used. This is not the case. Ayers, Rupp and Johnson (1923) found streptococci inhibited at different surface tensions when different depressants were used. According to Frobisher (1926), *Staphylococcus aureus* is completely inhibited at a surface tension of 44 dynes per cm when produced by sodium glycocholate, but multiplies readily at as low a tension as 34 dynes when produced by sodium oleate. Gershenfeld and Witlin (1941) determined the concentrations required to kill *Staphylococcus aureus* between 5 and 10 minutes, and measured the surface tensions of the killing solutions. Table 14 shows that there is no correlation.

It may be argued that no direct parallelism between lethal concentration and surface tension can be expected because the lethal effect takes place at the interface between medium and cell membrane, and this interfacial tension need

not be parallel to the tension between medium and air. The interfacial tension between cell and medium cannot be measured. Davis (1927) measured the drop size of oleic acid in chloride solutions, and compared this with the concentration of different chlorides necessary to inhibit bacterial growth. He believed that the toxic effects of the cations can be explained by interfacial tension "if bacteria are looked upon as analogous to oil drops." This analogy has not been generally accepted, and the data presented to support this view show considerable exceptions to the rule.

It must be concluded from these and many other experimental results that death is not caused by change in surface tension, but by some chemical reaction of the surface tension depressant upon some vital cell constituent. However, the subject cannot be dismissed entirely as chemical disinfection because of differences in the mode of action, and of interaction. The difference arises primarily from the concentration of surface-active substances at the surface and interfaces. They act upon the cell not according to the concentration in the liquid, but according to the much higher concentration at the cell surface. This explains perhaps the fact that so many surface-active substances are good disinfectants and antiseptics.

*2. Wetting agents.* The soaps which have been almost the only efficient detergents for more than a thousand years are gradually being replaced by synthetic detergents which decrease the surface tension greatly, but are efficient in acid as well as in alkaline media, and are not precipitated by hard water. These modern "wetting agents" can be divided into anionic, cationic, and undissociated detergents. The composition and origin of a number of these substances is given by Baker *et al.* (1941a, b). In the cationic detergents, the organophilic (or hydrophobic) group is positively charged, as in Emulsol 660 B = (lauryl pyridinium)$^+$ (chloride)$^-$; and in the anionic detergents, this group is negative as in (Na)$^+$ (lauryl sulfate)$^-$. The only neutral, non-ionized detergent mentioned is Demal, representing an organic ester.

Domagk (1935) called attention to the antiseptic properties of this new group of chemical products. According to Baker *et al.*, (1941b) the cationic detergents, as a group, are quite efficient disinfectants, capable of killing gram-positive and gram-negative bacteria in 10 minutes at a concentration of 0.015% (1:6000), or even 0.003%. A few preparations were less efficient. The anionic detergents did not kill these bacteria even after 90 minutes in concentration of 0.1%. No data on surface tension are given.

Cationic and anionic detergents neutralize and precipitate each other. The action of Zephiran is inhibited by decyl sulfate or sodium taurocholate (Baker *et al.*, 1941c) or by Aerosol O T (Gershenfeld and Perlstein, 1943). Different from this ionic reaction is the protection of bacteria against detergents by phospholipids, lecithin and related compounds, if they are added either before or simultaneously with the detergent (Baker *et al.*, 1941c). Addition after the detergent had no effect. Compounds which did not reduce surface tension did not counteract the bactericidal effect. On the other hand, phospholipids did not decrease the germicidal effect of several mercury compounds. Thus,

the germicidal power of detergents and its decrease by other substances is interlinked with a surface tension effect. The neutral compound Demal counteracted cationic as well as anionic detergents; it also counteracted gramicidin.

Valko and DuBois (1942) found that if a strongly germicidal detergent is mixed with a weak one of the same charge, the total efficiency is smaller than that of the stronger germicide, because the weaker one has occupied many places on the bacterial surface, and the surface concentration of the strong disinfectant is decreased. This is an indirect proof for the claim that surface tension as such is not the deciding lethal factor. The same authors (1944) also show that bacteria "killed" by a cationic detergent may be revived by addition of a high molecular anion if this is applied within a certain time of perhaps 10 to 30 minutes. They consider the reversible death as due to ion exchange in the cell, similar to the death by mercury salts, i.e., a chemical phenomenon.

The germicidal efficiency of these detergents depends largely upon the pH of the medium in which they act. The anionic detergents resemble benzoic or salicylic acid in their rapid increase of efficiency with acidification of the medium (Rahn and Conn, 1944). The analogy permits the conclusion that only the undissociated acid molecules of these compounds have bactericidal properties. Gershenfeld and Milanick (1941) found the following concentrations, in ppm, necessary to kill *Staphylococcus aureus* in 5 minutes:

| | At pH | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 |
| Aerosol OT | 29 | 33 | 250 | >10,000 | >10,000 | >10,000 |
| Tergitol 4 & 4 T | 140 | 250 | 500 | >10,000 | >10,000 | >10,000 |
| Triton K 12 | >10,000 | 10,000 | 3,300 | 1,250 | 110 | 55 |

The last compound is a cationic detergent, and in this case, the base in its undissociated state is probably the effective agent.

*3. Combination of disinfectants with surface tension depressants.* When surface-active substances are added to a disinfectant solution, they may decrease its germicidal efficiency. Hampil (1928) obtained such marked inhibition of the bactericidal action of various phenols by the addition of soap as to indicate the impossibility of producing a germicidal soap by incorporating small quantities of a phenolic compound. As an example may be given the concentration of butyl resorcinol required to kill *Staphylococcus aureus* between 1 and 2 minutes at different soap concentrations:

| % Na oleate | 0 | 0.2 | 0.5 | 1.0 | 3.0 | 5.0 |
|---|---|---|---|---|---|---|
| % disinfectant required | <0.05 | 0.067 | 0.1 | 0.2 | 0.5 | 0.5 |

The same has been found to be true with mixtures of mercury compounds and soap, unless the soap is very much diluted (Frobisher, 1927; Rettger *et al.*, 1929; Schaffer and Tilley, 1930; Tilley, 1939). The general explanation is that so much soap is concentrated at the surface that the other, stronger disinfectant cannot reach the cell as readily as without soap.

It is frequently stated in literature that a decreased surface tension of the medium must increase the efficiency of any antiseptic because the rate of diffusion through the cell membrane is increased. For example, Frobisher (1944) points out that aqueous iodine solutions do not spread easily on the skin while an alcoholic solution has a much greater wetting power. This leads him to the conclusion in the next paragraph that solutions of disinfectants in dilute alcohol or in similar solvents which have low surface tensions, are much more likely to be effective than when dissolved in water. However, Gershenfeld and Miller's data (1932) prove this to be a poor example because with iodine, the difference

TABLE 15

*Concentrations, in per cent, required to kill Staphylococcus aureus in 10, but not in 5 minutes*

| | pH | | | |
|---|---|---|---|---|
| | 7 | 6 | 5 | 4 |
| | Aerosol concentration, when added | | | |
| | 0.02 | 0.008 | 0.0017 | 0.0011 |
| Phenol | 1.25 | 1.17 | 1.11 | 1–1.05 |
| same + aerosol | 0.56 | 0.42 –0.50 | 0.33 | 0.26 |
| Mercuric chloride | 0.0067 | 0.004–0.005 | 0.004–0.005 | 0.0025–0.00275 |
| same + aerosol | 0.005–0.0067 | 0.0033–0.004 | 0.00275 | 0.0020–0.0022 |
| Merthiolate | 0.014 | 0.014–0.02 | 0.0056–0.0067 | 0.0033 |
| same + aerosol | 0.0028–0.0033 | 0.0033 | 0.0028 | 0.0020–0.0022 |
| Hexyl resorcinol | 0.033 | 0.025 –0.033 | 0.013–0.02 | 0.0067–0.0084 |
| same + aerosol | 0.004–0.005 | 0.004 | 0.0033–0.004 | 0.0025–0.00275 |
| Zonite | 0.05 | 0.125 –0.167 | 0.167 | 0.25 –0.33 |
| same + aerosol | 0.033 | 0.125 | 0.125 –0.167 | 0.20 |
| Zephiran | 0.00125–0.00142 | 0.005 | >0.2 | >0.2 |
| same + aerosol | >0.2 | >0.2 | >0.2 | >0.2 |

Data of Gershenfeld and Perlstein, 1941.

between aqueous and alcoholic solutions is slight, and in favor of the aqueous solution. Frobisher's generalization is erroneous. The surface of wood or skin which contains airspaces, is not comparable with the spaces between molecules of a cell membrane which are already completely surrounded by water molecules.

However, while not a general phenomenon, the efficiency of *some* disinfectants at *specified* concentrations may be increased by surface tension depressants. The increase may be quite noticeable. Gershenfeld and Witlin (1941) could find no change in the efficiency of disinfectants by the addition of detergents, but in the same volume, a little later, Gershenfeld and Perlstein did report such an effect (table 15). It is worth noting that at pH 7, Aerosol in concentration

of 0.02% increased the efficiency of phenol 2.2 times, of hexyl resorcinol 7 times, of merthiolate 5 times, of zonite a little, of mercuric chloride not significantly. It seems as if only surface-active substances are affected by the presence of wetting agents.

This assumption is in agreement with the experiments by Frobisher (1927) who found that a small quantity of sodium oleate (0.25 ml of a 1% solution) increased the efficiency of phenol slightly, while 0.5 ml decreased it greatly. Ethyl acetate (0.1 ml) increased the efficiency of phenol and of hexyl resorcinol

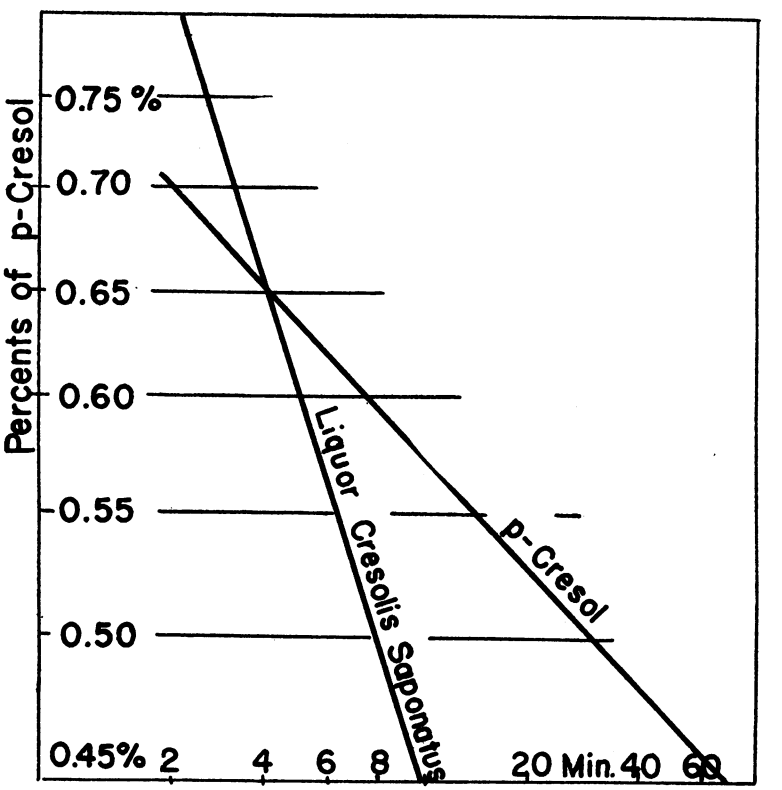


FIG. 6. THE EFFECT OF SOAP ON THE GERMICIDAL POWER OF CRESOL. LOGARITHMS OF DEATH TIMES PLOTTED AGAINST LOGARITHMS OF CRESOL CONCENTRATION. (DATA OF TILLEY, 1939)

considerably. All compounds tested decreased surface tension. The assumption also agrees with the extensive experiments of Schaffer and Tilley (1930) with mixtures of soap and phenolic compounds. The addition of 1 part of soap to 2 of phenol increased the germicidal efficiency considerably. Figure 6 from Tilley's later experiments (1939) shows that this increase is limited to a narrow range of concentration. In this connection, the following statement of Gershenfeld and Witlin (1941) is of interest: "The addition of twelve wetting agents to aqueous solutions of mercuric chloride and arsenic trioxide did not increase the penetration of the inorganic compounds when applied to the unbroken skin of rabbits." Mercury and arsenic compounds do not affect surface tension greatly.

*4. Bile solubility.* Another phenomenon usually ascribed to surface tension depression is the bile solubility of certain bacteria, notably pneumococci. Höber and Höber (1942) treat this solubility in a general way, although they are discussing primarily blood cells. They describe the action of bile salts and detergents as follows:

> "Their molecules are composed of two portions, one polar hydrophilic, which has a tendency to anchor the molecule to water, and one nonpolar hydrophobic which is attracted towards the nonaqueous phase. The result is a molecular orientation at the interface. . . . An increase of hydrophilic affinity will lead to a stronger pull on the nonaqueous phase toward the water and may have a disintegrating or a dispersing effect. Stronger organophilic properties on the other hand will favor a wetting action, *viz.*, the fixation of a film of water on the nonaqueous phase or its separate components. Regarding especially the interface between a cell and its surroundings, hydration, swelling and dissolution can be due to the wetting action on the colloidal structures or the micellae. Protein molecules are unfolded or disrupted by the pull, and thus denatured. In conjugated proteins, the bonds between the protein part and the prosthetic group are severed, viruses and enzymes are inactivated, and the final result of such effects on cells is cytolysis."

This phenomenon of complete dissolution by such compounds as bile and bile salts is not caused by the decreased surface tension as such, for other depressants like the alcohols, phenols and saponin do not dissolve bacteria. Of special interest in this differentiation between dissolving and non-dissolving agents is the report by Falk and Yang (1926) that sodium oleate dissolves washed pneumococci when re-suspended in distilled water, but not when re-suspended in 0.85% NaCl solution. Leonard and Feirer (1927) reported that *Endamoeba coli*, *Iodamoeba williamsi*, *Leptospira icterohaemorrhagiae*, *Trypanosoma lewisi*, and *Trichomonas hominis* are instantly destroyed and disappear completely on contact with hexyl resorcinol solutions while bacteria do not. The reviewer has observed that cationic detergents did not dissolve pneumococci at pH 9.8 where they were most efficient as germicides. While germicidal power depends primarily on the concentration of the undissociated base, dissolving power depends upon other properties.

Our knowledge of the interaction between surface tension depressants and bacteria is not at all perfect or complete. The sterilizing properties of the compounds of this group are certainly not due to the decreased interfacial tension as such. However, the intensity of the chemical reaction of these compounds is influenced by their effect upon interfacial tension, and they may, by this effect, increase or decrease the germicidal power of other disinfectants.

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