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The limits of microbial existence

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Introduction

Many parts of the world, such as the ocean depths, polar and geothermal regions, where the environment severely restricts most forms of life, are nevertheless colonized to some extent by specially adapted micro-organisms. There are also man-made environments, some especially designed to inhibit microbial growth, such as the strong brines used to pickle fish and meat, and cold stores, in which some micro-organisms can survive and grow.

Although such organisms have been known since the early days of microbiology only recently have they been systematically studied. Modern interest in the ability of micro-organisms to tolerate such unfavourable conditions stems largely from the need of the food industries to prevent microbial growth in their products. A further stimulus to understand the limiting conditions for microbial life, and to collect and systematize the available information has been provided by the requirements of space research (see Vallentyne 1963, 1965).

Growth at extremes of temperature

Most species of micro-organisms grow between 20 and 45 °C and are said to be mesophilic. The thermophiles, comprising fewer species, grow from 45 to *ca.* 75 °C with optima between 50 and 55° C: some of these are obligate, requiring high temperature for growth; others only tolerate it and have comparatively low-temperature optima. The psychrophiles grow well at low temperatures, sometimes even below 0 °C, and are inhibited above *ca.* 30 °C.

Thermophiles

The first known thermophile, a bacterium that could grow at 73 °C but not at low temperatures, was isolated by Miquel (1888) from the River Seine. Since that time thermophiles, mostly bacteria, have been found in most samples of soil, mud or water from regions of widely differing climate. They also occur in composts, sewage, in deep ocean floor deposits (Bartholomew & Paik 1966) and in hydrocarbon-containing rocks (Bairiev & Mamedov 1963). Thus, though thermophiles are ubiquitous they mostly occur in habitats that offer little or no scope for growth at high temperature. Indeed, it is not uncommon for micro-organisms to be adapted to conditions they do not normally encounter.

Microbes are at the temperature of their environment (see Kempner 1963), but when growing they produce heat and so can raise the temperature of that environment if its thermal properties impede heat loss. Thus, rapid mesophilic growth can provide conditions in which thermophiles can flourish, and by so doing raise the temperature even further. An example of this is provided by the heating of hay stacks or bales when prepared from hay too wet for proper storage. Rapid growth of an initially very mixed microflora of fungi, actinomycetes and bacteria quickly raises the temperature to a level at which thermophilic representatives of these groups proliferate. A few species of thermophilic fungi such as *Mucor pusillus*, *Absidia ramosa*, *Aspergillus fumigatus* and *Humicola lanuginosa* develop vigorously in heating hay as also do large numbers of thermophilic actinomycetes (Gregory, Lacey, Festenstein & Skinner 1963; Festenstein *et al.* 1965).

Upper layers of the soil are usually too cool to permit extensive development of thermophiles. However, thermophilic fungi have been isolated from the zone of vegetation litter just above soil level where they seem to be well established. This zone, which is often rather warmer than the surrounding air, may be a natural habitat in which thermophiles can grow, though probably below their temperature optima (Apinis 1963*a*, *b*).

Few bacteria are really obligate thermophiles; most are facultative such as the aerobic sporeformers, *Bacillus coagulans* and *B. circulans*. Economically, the most important thermophile is *B. stearothermophilus*; this is an obligate thermophile though some merely thermotolerant strains exist. It is particularly troublesome in the food industries because its spores are so resistant to heat; some strains will grow at 80 to 85 °C. There are some anaerobic thermophilic bacteria such as the cellulose decomposers (McBee 1950) and sulphate reducers (Postgate & Campbell 1966).

The transient conditions that favour thermophiles in heating hay are in striking contrast to the hot springs that provide unusual but constant habitats for a few specialized thermophiles, especially algae and bacteria (Kempner 1963; Brock 1967).

The main problems concerning life at high temperatures are to explain how these organisms can grow at temperatures at which most proteins are denatured. It is possible that the proteins and other important macromolecules of thermophiles are intrinsically more stable at high temperature than the corresponding compounds or structures of mesophiles, or that they are protected against denaturation in the cell. Alternatively, proteins may be denatured just as easily in the thermophile as in the mesophile, but that the former can repair the damage more readily (Allen 1950, 1953; Recknitz & Janota-Bassalik 1967).

There is some evidence for the first hypothesis. Oprescu (1898) demonstrated the existence of thermostable hydrolytic enzymes that could be isolated from culture filtrates of thermophiles, and since then, thermostability of enzymes has been frequently reported (e.g. Militzer, Sonderegger, Tuttle & Gorgi 1949; Marsh & Militzer 1956*a*, *b*; O'Brien & Campbell 1957; Amelunxen 1967; Daron 1967). Heat stability of enzymes is often related to the growth temperature of the organism; thus, Campbell (1955) found that α-amylases produced by a strain of facultatively thermophilic bacteria grown at 35 and 55 °C respectively differed in that the latter was more stable at 90 °C (see also Militzer *et al.* 1949; Marsh & Militzer 1956*b*; Brown, Militzer & Georgi 1957). Differences in the stability of bacterial flagellar proteins to heat have also been demonstrated. Whereas the flagella of mesophiles usually disintegrated easily above 50 °C, those of thermophiles remained intact even at 70 °C (Kofler 1957; Kofler, Mallet & Adye 1957).

Ribosomes from *Bacillus stearothermophilus* (the most frequently used organism in thermophilic studies) are much more resistant to high temperatures than those from the mesophile *Escherichia coli*, as judged by experiments on the incorporation of amino acids (Algranati & Lengyel 1966; Friedman & Weinstein 1966; Friedman, Axel & Weinstein 1967). The thermal denaturation point is also higher for ribosomes from thermophilic than for those from mesophilic bacteria, but this greater resistance to heat seems not to arise from intrinsic differences in the *RNA* (Mangiantini, Tecce, Toschi & Trentalance 1965; Saunders & Campbell 1966*a*; Friedman *et al.* 1967; Stenesh & Yang 1967) or the ribosomal proteins (Saunders & Campbell 1966*a*). The greater resistance of ribosomes to heat is correlated positively with maximal growth temperatures (Pace & Campbell 1967), but the reason for this stability is not known.

Magnesium ions may play a part in enzyme stability, for increasing the concentration of magnesium increases the resistance of some enzyme preparations to inactivation by heat (Brown, Militzer & Georgi 1957). Friedman & Weinstein (1966), working with enzyme systems from *Bacillus stearothermophilus* and the mesophile *Escherichia coli*, also emphasized the importance of magnesium ions in stabilizing the reaction between ribosomes, *s-RNA* and *m-RNA*, and suggested that thermophiles might make their protein synthetic apparatus stable to heat by maintaining a high concentration of magnesium or other polyvalent cations.

Intrinsic resistance of macromolecules to heat is also demonstrated by the existence of phages not easily inactivated at high temperature (Lark & Adams 1953). Saunders & Campbell (1966*b*) described a bacteriophage able to lyse strains of *Bacillus stearothermophilus* over the range of 43 to 76 °C; it was inactivated rapidly at 65 °C in citrate buffer, but not in broth during a 12 h period. White, Georgi & Militzer (1954) also described a bacteriophage for thermophilic bacteria; this strain remained infective after 2 h at 95 °C in broth.

The thermophilic actinomycete *Thermoactinomyces* (*Micromonospora*) *vulgaris* also has its phages (Agre 1961), and current work with these at Rothamsted shows that they are fully active after 30 min at 70 °C but many particles are not infective at 75 °C and inactivation is almost complete at 80 °C (J. J. Patel, personal communication).

Brock (1967) measured the photosynthetic efficiency at different temperatures of algae taken from growths in hot springs at several temperatures in the Yellowstone National Park. Photosynthetic efficiency of each sample was greatest at the temperature of the water from which that particular sample of algae was taken, even though the biomass at the limiting temperature of 73 to 75 °C was smaller than at the optimal range of 50 to 55 °C. Possibly, some part of the photo-synthetic mechanism does not operate above *ca.* 75° C, because non-photosynthetic bacteria grow well in these springs at 85 to 88 °C and make slight growth at 91 °C, which is only two degrees below the boiling point of water at that altitude. Growth has not been reported at a higher temperature than 91 °C at atmospheric pressure. Hot spring bacteria also have different temperature optima according to the position along the stream (Brock & Brock 1966).

Psychrophiles

Bacteria that can grow well at or near 0 °C are widely distributed and can be readily isolated from many sources, especially the sea, most of which is always colder than 5 °C. Mostly they are Gram-negative non-sporeforming rods (e.g. *Pseudomonas* spp.). Although these bacteria tolerate cold, most of them have optima between 20 and 40 °C so it has been suggested that they should be called 'psychrotrophic' and the term 'psychrophilic' reserved for those organisms that grow optimally below *ca.* 35 °C (Eddy 1960). Few species have optima below 20 °C. There has been much disagreement among microbiologists about this terminology (see Ingraham & Stokes 1959).

Psychrophilic, facultatively anaerobic, bacteria can also be easily isolated from many sources; their fermentation activities are not necessarily the same at temperatures near 0 °C as they are at *ca.* 20 °C, presumably because enzymes are either not formed or are inhibited at low temperatures (Upadhyay & Stokes 1962). Strictly anaerobic psychrophiles seem to be rare in most habitats, though sulphate reducers occur in ocean deposits (ZoBell & Morita 1957) and some sporeforming anaerobes, such as *Clostridium carnofoetidum* var. *amylolyticum* and *Cl. perfringens* will grow well at 6 °C though their growth optima are at *ca.* 30 °C (Beerens, Sugama & Tahon-Castell 1965). Bacteriophage with a growth temperature maximum only slightly above that of the psychrophilic host has also been reported (Olsen 1967).

Some bacteria, including *Bacillus megaterium*, micrococci and coryneform bacteria, and the fungus *Sporobolomyces* have been found growing in strongly saline pools in Antarctica at *ca.* −23 °C, the lowest temperature at which active life has been observed: these organisms grew in laboratory media at room temperature, thereby displaying remarkable adaptability to changing conditions (Meyer *et al.* 1962). The flagellate alga, *Dunaliella salina*, has also been seen to swim in brine pools at −15 °C (Zernow 1944).

Some small algae, such as *Chlamydomonas nivalis*, live on snow surfaces (Lewin 1962). The resistance to freezing of these algae is probably partly explained by the high osmotic pressure of the cell contents and perhaps by the ability of the cytoplasm to survive damage caused by freezing and thawing (Levitt 1956; Kanwisher 1957). Typically, these algae are red with carotenoid pigments.

Other examples of microbial growth at very low temperatures have been given in the review by Geiger, Jaffe & Mamikunian (1965).

Barophiles

Many bacteria occur in the sediments of the ocean floor, even at the greatest known depths of water. A characteristic feature of this environment is the enormous hydrostatic pressure, which increases at the rate of about one atmosphere for each 10 m depth. Most bacteria can tolerate considerable pressure, but some grow only at very high pressures and not at atmospheric pressure. ZoBell & Johnson (1949) found that whereas terrestrial mesophilic bacteria did not grow at 30 °C under 600 atm pressure, and that some strains were killed, marine bacteria isolated from depths where this order of pressure obtains grew readily; they introduced the term 'barophilic' for such bacteria whose growth or metabolism was favoured by high pressure.

Many barophiles were recovered from samples of the uppermost layers of sediments taken from the Philippine Trench at depths greater than 10000 m. In culture at 2·5 °C (ocean floor temperature) 10 to 1000 times more bacteria developed at 1000 atm pressure than at atmospheric pressure. Mostly, the barophiles are rod-shaped cells 2 to 4 μm long, many encapsulated and many forming endospores. The genera *Pseudomonas*, *Vibrio*, *Spirillum*, *Bacillus* and *Clostridium* were all represented. Bacteria also occurred in the deeper sediments, down to about 1 m below the ocean floor, an environment likely to favour longevity of cells and activity of anaerobes (ZoBell 1952).

Attempts to grow these deep sea bacteria at various pressures and temperatures revealed that the two factors were not acting independently. For example, some cultures grew under 400 to 600 atm at 40 °C, a temperature too high for their growth at atmospheric pressure. The same high pressures retarded growth at lower temperature. Cold greatly accentuated the growth-inhibiting and killing effects of pressure. Conversely, the adverse effects of pressure were alleviated by heat.

Pressure does not cause damage by crushing the cells, but is thought to act by changing molecular volumes. It so happens that high pressure acts on bacterial growth in the same direction as cooling. These temperature–pressure relationships are affected by the specific temperature characteristics of each organism. For example, high pressure might prevent a thermophile growing at its usually high optimal temperature but permit growth at temperatures higher than this. ZoBell (1958) found that one strain of thermophilic sulphate-reducing bacteria that did not grow above 85 °C at atmospheric pressure, grew at 104 °C under a pressure of 1000 atm, the highest growth temperature recorded.

It is considered that barophiles possibly adapt to high pressure by developing metabolic mechanisms that operate with little or no changes in molecular volumes: thus, pressure would have little effect. This could explain why some barophiles can be cultivated at atmospheric pressure, even for several years, and still retain their barophilic properties (ZoBell & Johnson 1949).

Pressure changes can have profound effects on the morphology of marine bacteria. For example, in sea-water broth at ordinary pressures, *Serratia marinorubra* grew as a small rod, 0·3 to 0·5 μm × 0·6 to 1·5 μm, but at 600 atm the cells grew to form filaments up to 200 μm long with no sign of division. When the long forms were transferred to atmospheric pressure they began to fragment after a few minutes (ZoBell & Oppenheimer 1950).

Halophiles

Micro-organisms tolerate greater or lesser concentrations of sodium chloride in their environment; some, the halophiles, require this salt. Flannery (1956) defines facultative halophiles as those that will grow with less than 2 % of salt but grow better with more, and obligate halophiles as those that grow only with more than 2 % of salt, 15 to 25 % being usually required for satisfactory growth.

Halophiles, especially bacteria, occur in natural briny waters such as the Dead Sea (Wilkansky 1936) and in man-made habitats wherever high concentrations of salt are employed. Typically, the extreme halophilic species are red-pigmented (carotenoids) and their presence is often indicated by the reddening of suitable substrates such as crude salt, salted fish and salted hides. The brine flagellate, *Dunaliella salina*, is also coloured bright red by carotenoids.

No Gram-positive extreme halophiles are known (Brown 1964) though some strains of *Staphylococcus* and *Micrococcus* tolerate very high salt concentrations (Christian & Waltho 1962): such tolerance is a reflexion, at least for some species, of a very small requirement for water (Scott 1953). Most extreme halophiles are Gram-negative rods, and some have growth temperature optima of 40 to 50 °C which is high for Gram-negative bacteria (Gibbons & Payne 1961). Some bacteria can adjust to higher salt concentrations; this adjustment can be reversible (Kluyver & Baars 1932) or irreversible (Hof 1935).

The fact that the freezing-points of halophiles are often lower than those of non-halophiles (Brown 1964) suggests that their cells contain more concentrated solutes. Certainly, the intracellular enzyme systems of halophilic bacteria are active in high concentrations of salts, although the concentration favouring optimal activity of cell-free preparations is often lower than that necessary for growth of the organism. For example, the nitritase and lactic dehydrogenase of *Micrococcus halodenitrificans* were not resistant to the sodium chloride content optimal for growth. Thus, the salt concentration within a halophilic cell, though possibly large compared with that of a non-halophile, may be less than in the surrounding medium (see Robinson 1952; Robinson & Gibbons 1952; Robinson, Gibbons & Thatcher 1952; Baxter & Gibbons 1954).

Though the cell envelopes of halophiles and non-halophiles have properties in common, for instance, they all permit cells to accumulate K^+ ions and exclude Na^+ ions against concentration gradients, there are chemical and structural differences between them. The cell membranes of extreme halophiles (e.g. *Halobacterium* spp.) contain little or no mucopeptide, but proportionately more protein and less carbohydrate than non-halophilic Gram-negative bacteria. Moreover, such cell membranes (e.g. of *H. halobium*, one of the few species examined) break up in solutions of low ionic strength; the membranes require a high concentration of cations for mechanical stability. This cation-dependent stability of the membranes is correlated with a larger proportion of aspartic and glutamic acid in the protein than of lysine and arginine. Isolated membranes of a non-halophilic pseudomonad acquired halophilic properties when acidic groups (—COO^-) were substituted for lysine-ϵ-NH_2^+ groups. It is suggested that all bacteria needing much salt have membranes of this type, which are stabilized by a high concentration of cations (Brown 1964).

Extreme halophiles are strict aerobes; perhaps very saline environments with restricted aeration and adequate supplies of organic nutrients are rare so that there is little evolutionary opportunity for anaerobes to develop. However, some sulphate-reducers tolerate concentrated salt solutions (ZoBell 1958), as do some facultative anaerobes such as staphylococci and a *Bacillus* sp. (Eddy & Ingram 1956). One species of obligate anaerobe, the sulphate-reducer *Desulfovibrio salixigens*, requires 2·5 to 5 % of salt (Postgate & Campbell 1966). However, here the requirement is for chloride ions and not, as is more usual, for cations.

Osmophiles

Only a very restricted microflora can develop in media of very high osmotic pressure such as that provided by 20 to 50 % sugar solutions. Some microbes have adapted to this environment, the outstanding examples being the osmophilic yeasts able to grow in honey and molasses. These yeasts have been referred to the genus *Saccharomyces*, mostly to *S. rouxii*. Some of these yeasts are obligate osmophiles; others can adapt themselves to grow in more dilute sugar solutions. Osmophilic yeasts form only a small proportion of the naturally occurring microflora on fruits but they predominate on the surfaces of dried grapes and dates (Scarr 1953).

Some other fungi have osmophilic properties: examples are *Xeromyces bisporus* (Bunker 1967) and *Aspergillus glaucus*, both of which will grow in 60 to 65 % sugar solutions (see Scarr 1953; Borgstrom 1961).

Acidity and alkalinity

Most micro-organisms grow in the pH range of 3·5 to 9·0, but a few tolerate extreme acidity: the thiobacilli being notable examples. *Thiobacillus thiooxidans* grows at pH values down to 0·5, although there is evidence that the internal pH of the cell is about 6 to 7. Certainly, enzymes isolated from such cells operate at neutrality rather than in acid solution. *T. thiooxidans* brings about its own extremely acid conditions by forming sulphuric acid. It can survive in 7 % sulphuric acid and will stand subculture directly into a medium of not less than pH 4 (H. J. Bunker, personal communication). The mould *Acontium velatum* also grows in media containing up to 2·5 N sulphuric acid, and in media saturated with copper sulphate (Starkey & Waksman 1943).

The remarkable alga *Cyanidium caldarium* lives in hot acid springs and has excited as much interest by virtue of its thermophilic as by its acidophilic properties. It has an optimal growth temperature range of 45 to 50 °C but will grow at up to 75 °C at a pH of *ca.* 1·2 (corresponding to about 0·1 N sulphuric acid) (see Allen 1959).

There are also examples of adaptation to high alkalinity. Bacteria such as *Streptococcus faecalis* and *Bacillus circulans* can grow at pH 10 to 11 and there are reports of algae occurring in alkaline lakes at pH 9 to 11. The blue-green alga *Plectonema nostocorum* grows at pH 13 which seems to be the highest at which active life has been recorded (Geiger *et al.* 1965).

This is a convenient point to refer to work with atmospheres enriched with ammonia gas. Some higher plants and animals tolerate gaseous ammonia and it has also been found that many micro-organisms grow from soil samples incubated in ammonia-rich atmospheres (Siegel & Giumarro 1966). When soil samples from Harlech, Wales, were inoculated on to a meat extract peptone agar, and the cultures incubated in an 'ammonia-air' mixture (containing 25 % ammonia and 25 % methane) an unusual micro-organism appeared (Siegel & Giumarro 1966). This was an umbrella-like structure of *ca.* 5 μm diam. with a stalk *ca.* 10 μm long, and although it had no obvious affinity with any known species of soil micro-organism, it was remarkably similar to the Pre-Cambrian fossil organism *Kakabekia umbellata* occurring in the Gunflint chert formation of Southern Ontario (Barghoorn & Tyler 1965*a*, *b*). It may be that the modern form flourishes only in atmospheres similar to those in which *Kakabekia* may have lived (Symposium 1965) and which are now to be found only in micro-environments such as soils where ammonia is produced and aeration is restricted. Other organisms of more familiar type also grew in these ammonia-rich mixtures; bacteria, fungi, actinomycetes, blue-green algae and protozoa.

Anaerobiosis

Although probably all multicellular and most unicellular organisms require oxygen, many species of bacteria are obligate anaerobes and cannot grow in its presence. Moreover, many other bacteria and yeasts are facultative anaerobes which usually grow better with oxygen but can grow without it provided suitable nutrients are available.

The obligate anaerobes are of great practical importance in clinical pathology and food preservation, and accounts of their activities form a substantial part of the literature of bacteriology. From less specialized viewpoints, these organisms pose some interesting and difficult problems, such as their significance in nitrogen fixation and the breakdown of organic matter in their natural environments, soils and muds.

The survival and growth of anaerobes is of particular interest to workers concerned with problems of contamination of extra-terrestrial environments, most of which are likely to be deficient in oxygen.

Growth and survival in simulated extra-terrestrial environments

Rather little attention has hitherto been given to the effect of two or more extreme environmental conditions acting together, though some examples of complex extreme habitats (very cold and saline; cold and high pressure; hot and acid) have been mentioned above. The study of such complex extreme sets of conditions is, however, an important part of space research programmes. The basis of simulation work is to study how terrestrial organisms react to environments likely to be found on other planets. Such experiments can indicate whether any particular environment, such as that of Mars, prohibits life as we know it, and they can also demonstrate whether terrestrial micro-organisms contaminating space vehicles are likely to survive or grow in their new environment. Emphasis is very much on micro-organisms in this work because they are the only living things likely to be carried in a space vehicle without being noticed, and they are also the most likely to find ecological niches suited to them. Many of these studies have also been aimed at establishing the effects of environmental factors acting in a cyclical manner. Such changing factors have been little investigated: the usual concern of the microbiologist is to keep experimental conditions as constant as possible (see Siegel *et al.* 1965).

Much recent information derives from numerous studies on the simulated 'Martian' environment. Experimental details vary considerably but the following conditions are typical: soil samples inoculated with the organisms under test are subjected to low-pressure atmospheres and a daily freeze–thaw cycle with about 4 h spent above 0 °C. Very small amounts of water are usually provided, and organic matter is sometimes included. Many such experiments have been recorded and reviewed (Jenkins 1966).

Bacteria survive these treatments extremely well, especially the sporeformers as would be expected. For example, in some experiments bacteria survived for at least 6 months in all soil samples tested in a low pressure, almost dry anoxic atmosphere and a 12 h freeze–thaw cycle ranging from −60 to +20 °C (Packer, Scher & Sagan 1963). Dryness and cold are not inconsistent with prolonged survival, and even very small and transient amounts of water permit some growth. Hawrylewicz *et al.* (1967) showed in environmental studies of this kind that bacterial species have different requirements for water, but with all the organisms studied, *Staphylococcus albus*, *S. aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum* and PA 3679, the extreme diurnal temperature cycle aided conservation of water, and a limited amount of growth occurred.

It seems clear that terrestrial microbes might survive when introduced to the Martian environment, but would be unlikely to find favourable growth conditions. A large gap in our knowledge is the nature and amount of organic matter that might be available to terrestrial contaminants, and on which their growth must depend.

Conclusions

Micro-organisms grow in what seem to be remarkably forbidding environments, and to meet these conditions the cell becomes modified in many different ways. Cell enzymes and enzyme systems are formed with optimal activities at very different temperatures or at different ionic strengths, or are stabilized against heat denaturation by cations. Cell envelopes are modified to prevent their dissolution by extreme salinity, and there are cell repair mechanisms, possibly operating to counteract the damaging effect of heat, and certainly to offset radiation damage (Lett *et al.* 1967).

Considered above are some extreme physical and chemical factors characteristic of whole environments, and which manifest themselves by restricting the numbers of species that flourish. The more extreme the conditions, the fewer the species able to tolerate them, and the slower they grow. For instance, there are very few records of growth above 80 °C, and only one above 90 °C, yet in such situations as hot springs a suitably adapted organism may flourish untroubled by competitors.

It must be remembered, however, that these extreme factors are superimposed on others such as nutritional factors, that are limiting the growth of individual cells or species within that environment. Moreover, conditions for growth can be 'unfavourable' and thereby growth-limiting, without any one factor being obviously 'extreme'. The vigorous development of any one species of micro-organism is not so common in nature, except, paradoxically, in certain extreme conditions such as the hot springs mentioned above, and in some specialized saprophytic and parasitic associations. In soils, for example, any one species of micro-organism usually is not abundant even though the total numbers present are very large: many species exist together but growth of any one is limited.

No doubt, new man-made habitats, seemingly inimical to microbial life, will be developed and it will probably be found that some micro-organisms will be able to adapt to the new conditions. Moreover, not all natural environments have yet been exhaustively examined for the presence of unusual organisms: some have only recently been discovered. Thus, Miller (1964) and Charnock (1964) drew attention to the existence of pools of warm brine in depressions at the bottom of the Red Sea. The hottest of these pools, Atlantis II, is at 56 °C, is under 2000 m of normal sea water (hydrostatic pressure of *ca.* 200 atm), has 20 to 30 % total solids mostly sodium chloride, is rich in iron and manganese, and has no free oxygen (Munns, Stanley & Densmore 1967). This water is similar to that of oil field and deep well brines in which bacteria have been found (ZoBell 1958). So far, micro-organisms have not been reported from the Red Sea brines, though it is probably too soon for investigations to have been made, yet it would seem rash to regard these pools as sterile in view of what has been noted above about the ability of organisms to survive a hostile environment.

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