(60)

Royal Institution of Great Britain.

WEEKLY EVENING MEETING.

Friday, June 8, 1900.

Sir James Crichton-Browne, M.D. LL.D. F.R.S., Treasurer and Vice-President, in the Chair.

[A]llan Macfadyen, M.D. B.Sc., Director of the Jenner Institute of Preventive Medicine.

The Effect of Physical Agents on Bacterial Life.

(Abstract.)

[T]he fact that life did not exist upon the earth at a remote period of [t]ime, the possibility of its present existence as well as the prospect [o]f its ultimate extinction, can be traced to the operation of certain [p]hysical conditions. These physical conditions upon which the main[t]enance of life as a whole depends are in their main issues beyond [t]he control of man. We can but study, predict and it may be [u]tilise their effects for our benefit. Life in its individual manifesta[t]ions is, therefore, conditioned by the physical environment in which [i]t is placed. Life rests on a physical basis, and the main springs of [i]ts energies are derived from a larger world outside itself. If these [c]onditions, physical or chemical, are favourable, the functions of life proceed; if unfavourable, they cease—and death ultimately ensues. These factors have been studied and their effects utilised to conserve health or to prevent disease. It is our purpose this evening to study some of the purely physical factors, not in their direct bearing on man, but in relation to much lower forms in the scale of life—forms which constitute in number a family far exceeding that of the human species, and of which we may produce at will in a test-tube, within a few hours, a population equal to that of London. These lowly forms of life—the bacteria—belong to the vegetable kingdom, and each individual is represented by a simple cell.

These forms of life are ubiquitous in the soil, air and water, and are likewise to be met with in intimate association with plants and animals, whose tissues they may likewise invade with injurious or deadly effects. Their study is commonly termed bacteriology—a term frequently regarded as synonymous with a branch of purely medical investigation. It would be a mistake, however, to suppose that bacteriology is solely concerned with the study of the germs of disease. The dangerous microbes are in a hopeless minority in comparison with the number of those which are continually performing varied and most useful functions in the economy of nature. Their wide importance is due to the fact that they ensure the resolution and redistribution of dead and effete organic matter, which if allowed accumulate would speedily render life impossible on the surface of the earth. If medicine ceased to regard the bacteria, their study wou still remain of primary importance in relation to many industr processes in which they play a vital part. It will be seen, therefo that their biology presents many points of interest to scientific work generally. Their study as factors that ultimately concern us rea began with Pasteur's researches upon fermentation. The subject this evening's discourse, the effect of physical agents on bacterial li is important not merely as a purely biological question, though th phase is of considerable interest, but also on account of the facts I ha already indicated, viz. that micro-organisms fulfil such an importa function in the processes of nature, in industrial operations, and connection with the health of man and animals. It depends largely the physical conditions to be met with in nature whether the micr organisms exercise their functions, and likewise whether they die remain inactive. Further, the conditions favouring one organism m be fatal to another, or an adaptability may be brought about to unusu conditions for their life. To the technologist the effect of physic agents in this respect is of importance as a knowledge of their mode action will guide him to the means to be employed for utilising th micro-organisms to the best advantage in processes of fermentatio The subject is of peculiar interest to those who are engaged in com bating disease, as a knowledge of the physical agents that favour retard bacterial life will furnish indications for the preventive measur to be adopted. With a suitable soil and an adequate temperatu the propagation of bacteria proceeds with great rapidity. If th primary conditions of soil and an adequate temperature are n present, the organisms will not multiply, they remain quiescent or th die. The surface layers of the soil harbour the vast majority of th bacteria, and constitute the great storehouse in nature for these form of life. They lessen in number in the deeper layers of the soil, an few or none are to be met with at a depth of 8–10 feet. As a matte of fact, the soil is a most efficient bacterial filter, and the majority the bacteria are retained in its surface layers and are to be met wit there. In the surface soil, most bacteria find the necessary physica conditions for their growth, and may be said to exist there under natur conditions. It is in the surface soil that their main scavengin functions are performed. In the deeper layers, the absence of air an the temperature conditions prove inimical to most forms.

Amongst pathogenic bacteria the organisms of lockjaw and c malignant œdema appear to be eminently inhabitants of the soi As an indication of the richness of the surface soil in bacteria, I ma mention that 1 gramme of surface soil may contain from severa hundred thousand to as many as several millions of bacteria. Th air is poorest in bacteria. The favouring physical conditions to be me with in the soil are not present in the air. Though bacteria are to b met with in the air, they are not multiplying forms as is the case i ıe soil. The majority to be met with in air are derived from the oil. Their number lessens when the surface soil is moist, and it inreases as the surface soil dries. In a dry season the number of air rganisms will tend to increase.

Town air contains more bacteria than country air, whilst they ecome few and tend to disappear at high levels and on the sea. A hower of rain purifies the air greatly of bacteria. The organisms eing, as I stated, mainly derived from the surface of the ground, heir number mainly depends on the physical condition of the soil, nd this depends on the weather. Bacteria cannot pass independntly to the air, they are forcibly transferred to it with dust from arious surfaces. The relative bacterial purity of the atmosphere s mainly, therefore, a question of dust. Even when found floating bout in the air the bacteria are to be met with in much greater umber in the dust that settles on exposed surfaces, e.g. floors, arpets, clothes and furniture. Through a process of sedimentaion the lower layers of the air become richer in dust and bacteria, nd any disturbance of dust will increase the number of bacteria in he air.

The simple act of breathing does not disseminate disease germs from a patient, it requires an act of coughing to carry them into the air with minute particles of moisture. From the earliest times great weight has been laid upon the danger of infection through air-borne contagia, and with the introduction of antiseptic surgery the endeavour was made to lessen this danger as much as possible by means of the carbolic spray, etc. In the same connection numerous bacteriological examinations of air have been made with the view of arriving at results of hygienic value. The average number of micro-organisms present in the air is 500–1000 per 1000 litres; of this number only 100–200 are bacteria, and they are almost entirely harmless forms. The organisms of suppuration have been detected in the air, and the tubercle bacillus in the dust adhering to the walls of rooms. Investigation has not, however, proved air to be one of the important channels of infection. The bactericidal action of sunlight, desiccation and the diluting action of the atmosphere on noxious substances will always greatly lessen the risk of direct aerial infection.

The physical agents that promote the passage of bacteria into the air are inimical to their vitality. Thus, the majority pass into the air not from moist but from dry surfaces, and the preliminary drying is injurious to a large number of bacteria. It follows that if the air is rendered dust-free, it is practically deprived of all the organisms it may contain. As regards enclosed spaces, the stilling of dust and more especially the disinfection of surfaces liable to breed dust or to harbour bacteria are more important points than air disinfection, and this fact has been recognised in modern surgery. In an investigation, in conjunction with Mr. Lunt, an estimation was arrived at of the ratio existing between the number of dust particles and bacteria in the air. We used Dr. Aitken's Dust-counter, which not only renders the dust particles visible, but gives a means of counting them in a sample of air. In an open suburb of London we found 20,000 dust particles in 1 cubic centimetre of air; in a yard in the centre of London about 500,000. The dust contamination we found to be about 900 per cent. greater in the centre of London than in a quiet suburb. In the open air of London there was on an average just one organism to every 38,300,000 dust particles present in the air, and in the air of a room, amongst 184,000,000 dust particles, only one organism could be detected.

These figures illustrate forcibly the poverty of the air in micro-organisms even when very dusty, and likewise the enormous dilution they undergo in the atmosphere. Their continued existence is rendered difficult through the influence of desiccation and sunlight. Desiccation is one of nature's favourite methods for getting rid of bacteria. Moisture is necessary for their development and their vital processes, and constitutes about 80 per cent. of their cell-substance. When moisture is withdrawn most bacterial cells, unless they produce resistant forms of the nature of spores, quickly succumb. The organism of cholera air-dried in a thin film dies in three hours. The organisms of diphtheria, typhoid fever and tuberculosis show more resistance, but die in a few weeks or months.

Dust containing tubercle bacilli may be carried about by air currents, and the bacilli in this way transferred from an affected to a healthy individual. It may, however, be said that drying attenuates and kills most of these forms of life in a comparatively short time. The spores of certain bacteria may, on the other hand, live for many years in a dried condition, e.g. the spores of anthrax bacilli which are so infective for cattle and also for man (wool-sorters' disease). Fortunately few pathogenic bacteria possess spores, and, therefore drying by checking and destroying their life is a physical agent that plays an important rôle in the elimination of infectious diseases. This process is aided by the marked bactericidal action of *sunlight*. Sunlight, which has a remarkable fostering influence on higher plant life, does not exercise the same influence on the bacteria. With few exceptions we must grow them in the dark in order to obtain successful cultures; and a sure way of losing our cultures is to leave them exposed to the light of day. Direct sunlight is the most deadly agent, and kills a large number of organisms in the short space of one to two hours; direct sunlight proves fatal to the typhoid bacillus in half an hour to two hours, to the diphtheria bacillus in half an hour to one hour, and to the tubercle bacillus in a few minutes to several hours. Even anthrax spores are killed by direct light in three and a half hours. Diffuse light is also injurious, though its action is slower. By exposing pigment-producing bacteria to sunlight colourless varieties can be obtained, and virulent bacteria so weakened that they will no longer produce infection. The germicidal action of the sun's rays is most marked at the blue end of the specrum, at the red end there is little or no germicidal action. It is vident that the continuous daily action of the *sun* along with *desication* are important physical agents in arresting the further developnent of the disease germs that are expelled from the body.

It has been shown that sunlight has an important effect in the pontaneous purification of rivers. It is a well-known fact that a iver, despite contamination at a given point, may show little or no vidence of this contamination at a point further down in its course. Buchner added to water 100,000 colon bacilli per cubic centimetre, nd found that all were dead after one hour's exposure to sunlight. He also found, that in a clear lake the bactericidal action of sunlight xtended to a depth of about six feet. Sunlight must therefore be aken into account as an agent in the purification of waters, in adlition to sedimentation, oxidation and the action of algæ.

Air or the oxygen it contains has important and opposite effects on he life of bacteria. In 1861, Pasteur described an organism in conection with the butyric acid fermentation which would only grow in he absence of free oxygen. And since then a number of bacteria, howing a like property, have been isolated and described. They re termed anaerobic bacteria as their growth is hindered or stopped n the presence of air. The majority of the bacteria, however, are erobic organisms inasmuch as their growth is dependent upon a free upply of oxygen. There is likewise an intermediate group of rganisms, which show an adaptability to either of these conditions, being able to develop with or without free access to oxygen. Preminent types of this group are to be met with in the digestive tract of animals, and the majority of disease-producing bacteria belong to this adaptive class. When a pigment-producing organism is grown without free oxygen its pigment production is almost always stopped. For anaerobic forms N and H_2 give the best atmosphere for their growth, whilst CO_2 is not favourable and may be positively injurious, as e.g. in the case of the cholera organism.

The physical conditions favouring the presence and multiplication of bacteria in water under natural conditions are a low altitude, warmth, abundance of organic matter and a sluggish or stagnant condition of the water. As regards water-borne infectious diseases such as typhoid or cholera, their transmission to man by water may be excluded by simple boiling or by an adequate filtration. The freezing of water, whilst stopping the further multiplication of organisms, may conserve the life of disease germs by eliminating the destructive action of commoner competitive forms. Thus the typhoid bacillus may remain frozen in ice for some months without injury. Employment of ordinary cold is not therefore a protection against dangerous disease germs.

As regards *electricity*, there is little or no evidence of its direct action on bacterial life, the effects produced appear to be of an indirect character due to the development of heat or to the products of electrolysis.

Ozone is a powerful disinfectant, and its introduction into pollute water has a most marked purifying effect. The positive effects c the electric current may therefore be traced to the action of th chemical products and of heat. I am not aware that any direct actio of the X-rays on bacteria has up to the present been definitel proved.

Mechanical agitation, if slight may favour, and if excessive ma hinder bacterial development. Violent shaking or concussion ma not necessarily prove fatal so long as no mechanical lesion of th bacteria is brought about. If, however, substances likely to produc triturating effects are introduced, a disintegration and death of th cells follows. Thus Rowland, by a very rapid shaking of tubercl bacilli in a steel tube with quartz sand and hard steel balls, produce their complete disintegration in ten minutes.

Bacteria appear to be very resistant to the action of *pressure* At 300–450 atmospheres putrefaction still takes place, and at 60 atmospheres the virulence of the anthrax bacillus remained unim paired. Of the physical agents that affect bacterial life, tempera ture is the most important. Temperature profoundly influence the activity of bacteria. It may favour or hinder their growth, o it may put an end to their life. If we regard temperature i the first instance as a favouring agent, very striking difference are to be noted. The bacteria show a most remarkable range o temperature under which their growth is possible, extending fron zero to 70° C. If we begin at the bottom of the scale we fin organisms in water and in soil that are capable of growth an development at zero. Amongst these are certain species of phosphor escent bacteria which continue to emit light even at this low tempera ture. At the Jenner Institute we have met with organisms growin and developing at 34–40° F. The vast majority of interest to us fin however the best conditions for their growth from 15° up to 37° C Each species has a minimum, an optimum and a maximum tempera ture at which it will develop. It is important in studying any give species that the optimum temperature for their development b ascertained, and that this temperature be maintained. In thi respect we can distinguish three broad groups. The first grou includes those for which the optimum temperature is from 15–20° C The second group includes the parasitic forms, viz. those which grov in the living body and for which the optimum temperature is a blood heat, viz. 37° C. We have a third group for which th optimum temperature lies as high as 50–55° C. On this accoun this latter group has been termed thermophilic on account of it growth at such abnormally high temperatures—temperatures whicl are fatal to other forms of life. They have been the subject o personal investigation in conjunction with Dr. Blaxall. We foun that there existed in nature an extensive group of such organisms t which the term thermophilic bacteria was applicable. Their growtl and development occurred best at temperatures at which ordinar protoplasm becomes inert or dies. The best growths were always obtained at 55–65° C. Their wide distribution was of a striking nature. They were found by us in river water and mud, in sewage, and also in a sample of sea water. They were present in the digestive tract of man and animals and in the surface and deep layers of the soil as well as in straw and in all samples of ensilage examined. Their rapid growth at high temperatures was remarkable, the whole surface of the culture medium being frequently overrun in from fifteen to seventeen hours. The organisms examined by us (fourteen forms in all) belonged to the group of the Bacilli. Some were motile, some curdled milk, and some liquefied gelatin in virtue of a proteolytic enzyme. The majority possessed reducing powers upon nitrates and decomposed proteid matter. In some instances cane sugar was inverted and starch was diastased. These facts well illustrate the full vitality of the organisms at these high temperatures, whilst all the organisms isolated grew best at 55°–65° C. A good growth in a few cases occurred at 72° C. Evidence of growth was obtained even at 74° C. They exhibited a remarkable and unique range of temperature, extending as far as 30° of the Centigrade scale.

As a concluding instance of the activity of these organisms we may cite their action upon cellulose. Cellulose is a substance that is exceedingly difficult to decompose, and is therefore used in the laboratory for filtering purposes in the form of Swedish filter paper, on account of its resistance to the action of solvents. We allowed these organisms to act on cellulose at 60° C. The result was that in ten to fourteen days a complete disintegration of the cellulose had taken place, probably into CO_2 and marsh gas. The exact conditions that may favour their growth, even if it be slow at subthermophilic temperatures, are not yet known—they may possibly be of a chemical nature.

Organisms may be gradually *acclimatised* to temperatures that prove unsuited to them under ordinary conditions. Thus the anthrax bacillus with an optimum temperature for its development of 37° C., may be made to grow at 12° C., and at 42° C. Such anthrax bacilli proved pathogenic for the frog with a temperature of 12° C., and for the pigeon with a temperature of 42° C.

Let us in a very few words consider the inimical action of temperature on bacterial life. An organism placed below its minimum temperature ceases to develop, and if grown above its optimum temperature becomes attenuated as regards its virulence, etc. and may eventually die. The boiling point is fatal for non-sporing organisms in a few minutes. The exact thermal death-point varies according to the optimum and maximum temperature for the growth of the organism in question. Thus for water bacteria with a low optimum temperature, blood heat may be fatal; for pathogenic bacteria developing best at blood heat, a thermophilic temperature may be fatal (60° C.); and for thermophilic bacilli any temperature above

75° C. These remarks apply to the bacteria during their multipl ing and vegetating phase of life. In their resting or spore sta the organisms are much more resistant to heat. Thus the anthr organism in its bacillary phase is killed in one minute at 70° C.; its spore stage it resists this temperature for hours, and is only kill after some minutes by boiling. In the soil there are spores bacteria which require boiling for sixteen hours to ensure their deat These are important points to be remembered in sterilisation disinfection experiments, viz. whether an organism does or n produce these resistant spores. Most non-sporing forms are killed 60° C. in a few minutes, but in an air-dry condition a longer time necessary. Dry heat requires a longer time to act than moist hea it requires 140° C. for three hours to kill anthrax spores. Dry he cannot therefore be used for ordinary disinfection on account of i destructive action. Moist heat in the form of steam is the mo effectual disinfectant, killing anthrax spores at boiling point in a fe minutes, whilst a still quicker action is obtained if saturated stea under pressure be used. No spore, however resistant, remains ali after one minute's exposure to steam at 140° C. The varying therm death-point of organisms and the problems of sterilisation cannot b better illustrated than in the case of milk, which is an admirable so for the growth of a large number of bacteria. The most obvio example of this is the souring and curdling of milk that occurs aft it has been standing for some time. This change is mainly due the lactic acid bacteria, which ferment the milk sugar with the pr duction of acidity.

Another class of bacteria may curdle the milk without sourin it in virtue of a rennet-like ferment, whilst a third class precipita and dissolve the casein of the milk, along with the development butyric acid. The process whereby milk is submitted to a heat 65° to 70° C. for twenty minutes is known as pasteurisation, and th milk so treated is familiar to us all as pasteurised milk. Whil the pasteurising process weeds out the lactic acid bacteria from th milk, a temperature of 100° C. for one hour is necessary to destro the butyric acid organisms: and even when this has been accom plished there still remain in the milk the spores of organisms whic are only killed after a temperature of 100° C. for three to six hour It will therefore be seen that pasteurisation produces a partial, no a complete sterilisation of the milk as regards its usual bacteri inhabitants. The sterilisation to be absolute would require si hours at boiling point. But for all ordinary practical purpose pasteurisation is an adequate procedure. All practical hygienic re quirements are likewise adequately met by pasteurisation, if it i properly carried out, and the milk is subsequently cooled. Mil may carry the infection of diphtheria, cholera, typhoid and scarle fevers as well as the tubercle bacillus from a diseased animal to th human subject. For the purpose of rendering the milk innocuou freezing and the addition of preservatives are inadequate method procedure. The one efficient and trustworthy agent we possess is heat. Heat and cold are the agents to be jointly employed in the process, viz. a temperature sufficiently high to be fatal to organisms producing a rapid decomposition of milk, as well as to those which produce disease in man; this to be followed by a rapid cooling to preserve the fresh flavour and to prevent an increase of the bacteria that still remain alive. The pasteurising process fulfils these requirements.

In conjunction with Dr. Hewlett, I had occasion to investigate in how far the best pasteurising results might be obtained. We found that 60° to 68° C. applied for twenty minutes weeded out about 90 per cent. of the organisms present in the milk, leaving a 10 per cent. residue of resistant forms. It was found advisable to fix the pasteurising temperature at 68° C., in order to make certain of killing any pathogenic organisms that may happen to be present. We passed milk in a thin stream through a coil of metal piping, which was heated on its outer surface by water. By regulating the length of the coil, or the size of the tubing, or the rate of flow of the milk, almost any desired temperature could be obtained. The temperature we ultimately fixed at 70° C. The cooling was carried out in similar coils placed in iced water. The thin stream of milk was quickly heated and quickly cooled as it passed through the heated and cooled tubing, and whilst it retained its natural flavour, the apparatus accomplished at 70° C. in thirty seconds a complete pasteurisation, instead of in twenty minutes, i.e. about 90 per cent. of the bacteria were killed, whilst the diphtheria, typhoid, tubercle and pus organisms were destroyed in the same remarkably short period of time, viz. thirty seconds. This will serve to illustrate how the physical agent of heat may be employed, as well as the sensitiveness of bacteria to heat when it is adequately employed.

Bacteria are much more sensitive to high than to low temperatures, and it is possible to proceed much further downwards than upwards in the scale of temperature, without impairing their vitality. Some will even multiply at zero, whilst others will remain alive when frozen under ordinary conditions.

I will conclude this discourse by briefly referring to experiments recently made with most remarkable results upon the influence of low temperatures on bacterial life. The experiments were conducted at the suggestion of Sir James Crichton-Browne and Professor Dewar. The necessary facilities were most kindly given at the Royal Institution, and the experiments were conducted under the personal supervision of Professor Dewar. The action of liquid air on bacteria was first tested. A typical series of bacteria was employed for this purpose, possessing varying degrees of resistance to external agents. The bacteria were first simultaneously exposed to the temperature of liquid air for twenty hours (about − 190° C.). In no instance could any impairment of the vitality of the organisms be detected as regards their growth or functional activities. This was strikingly illustrated in the case of the phosphorescent organisms tested. The cells emit light which is apparently produced by a chemical process of intra-cellular oxidation, and the phenomenon ceases with the cessation of their activity. These organisms therefore furnished a very happy test of the influence of low temperatures on vital phenomena. These organisms when cooled down in liquid air became non-luminous, but on re-thawing the luminosity returned with unimpaired vigour as the cells renewed their activity. The sudden cessation and rapid renewal of the luminous properties of the cells despite the extreme changes of temperature was remarkable and striking. In further experiments the organisms were subjected to the temperature of liquid air for seven days. The results were again *nil.* On re-thawing the organisms renewed their life processes with unimpaired vigour. We had not yet succeeded in reaching the limits of vitality. Prof. Dewar kindly afforded the opportunity of submitting the organisms to the temperature of liquid hydrogen—about $-250°$ C. The same series of organisms was employed, and again the result was *nil.* This temperature is only 21° above that of the absolute zero, a temperature at which, on our present theoretical conceptions, molecular movement ceases and the entire range of chemical and physical activities with which we are acquainted either cease, or it may be, assume an entirely new rôle. This temperature again is far below that at which any chemical reaction is known to take place. The fact then that life can continue to exist under such conditions affords new ground for reflection as to whether after all life is dependent for its continuance on chemical reactions. We, as biologists, therefore follow with the keenest interest Prof. Dewar's heroic attempts to reach the absolute zero of temperature; meanwhile his success has already led us to reconsider many of the main issues of the problem. And by having afforded us a new realm in which to experiment, Prof. Dewar has placed in our hands an agent of investigation from the effective use of which, we who are working at the subject at least hope to gain a little further insight into the great mystery of life itself.

[A. M.]

LONDON: PRINTED BY WILLIAM CLOWES AND SONS, LIMITED,
STAMFORD STREET AND CHARING CROSS.