STUDIES ON BIOGENESIS BY SIR WILLIAM ROBERTS

by

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WILLIAM ROBERTS (1830–99) is one of that small, but important, band of workers whose labours are generally ignored by historians of the great nineteenth-century controversy over abiogenesis or 'spontaneous generation'. It is for the purpose of directing attention to Roberts's contributions, which were significant even though outshone by those of the great savants, Pasteur and Tyndall, that this essay is written.

Before entering into a detailed account and critical evaluation of Roberts's work, it should be noted that events of his personal life have been admirably documented in a long obituary notice by D. J. Leach[1] (see also those of the Royal Society,[2] and in the *British Medical Journal*[3] among others). It will be sufficient to note here that Roberts received his M.D. at the University of London in 1854, and was for twenty-eight years an active staff member of the Manchester Royal Infirmary (1855–83). A noted authority on renal diseases, Roberts was a Fellow of the Royal College of Physicians. In 1877 he was elected a Fellow of the Royal Society, and eight years later he was knighted. It appears that for approximately seven years (1870–7) Roberts devoted a portion of his busy life to a study of abiogenesis, and during this time his written works on this subject were published.

In a paper[4] read on Roberts's behalf before the Royal Society by Henry E. Roscoe, F.R.S., on 16 April 1874, certain remarkable results of studies on the biogenesis of micro-organisms were communicated. They were of interest in that some observations of H. C. Bastian,[5] the heterogenist, were confirmed, particularly those dealing with the extraordinary heat-resistance of bacteria in neutralized hay-infusions. In a sense, then, some of Pasteur's experimental procedures, as detailed in his monumental researches[6] on 'les corpuscules organisés qui existent dans l'atmosphère', were to require re-evaluation, since apparently not *all* organic infusions and decoctions could be sterilized by the simple Pasteurian technique of boiling for two or three minutes. It may be recalled that Pasteur almost always used flasks of 250 to 300 ml. capacity containing 100 to 150 ml. of a yeast extract—sugar solution (sugar, 10 g., albuminous matter from beer yeast, 0·2 to 0·7 g., water, 100 ml.) in this work. The results described in Roberts's communication also agreed with observations (but *not* conclusions) of F. A. Pouchet[7] and his collaborators (vanquished by Pasteur in their mighty clash of 1859–61), as well as with those of F. Cohn,[8] and the lone American worker, J. Wyman.[9] The point at issue was not the correctness of Pasteur's views concerning the impossibility of abiogenesis as observed under the laboratory conditions designed by its proponents, but rather the propriety of his summary dismissal of all results obtained with hay-infusions. The French master was not aware of the existence of heat-resistant bacterial

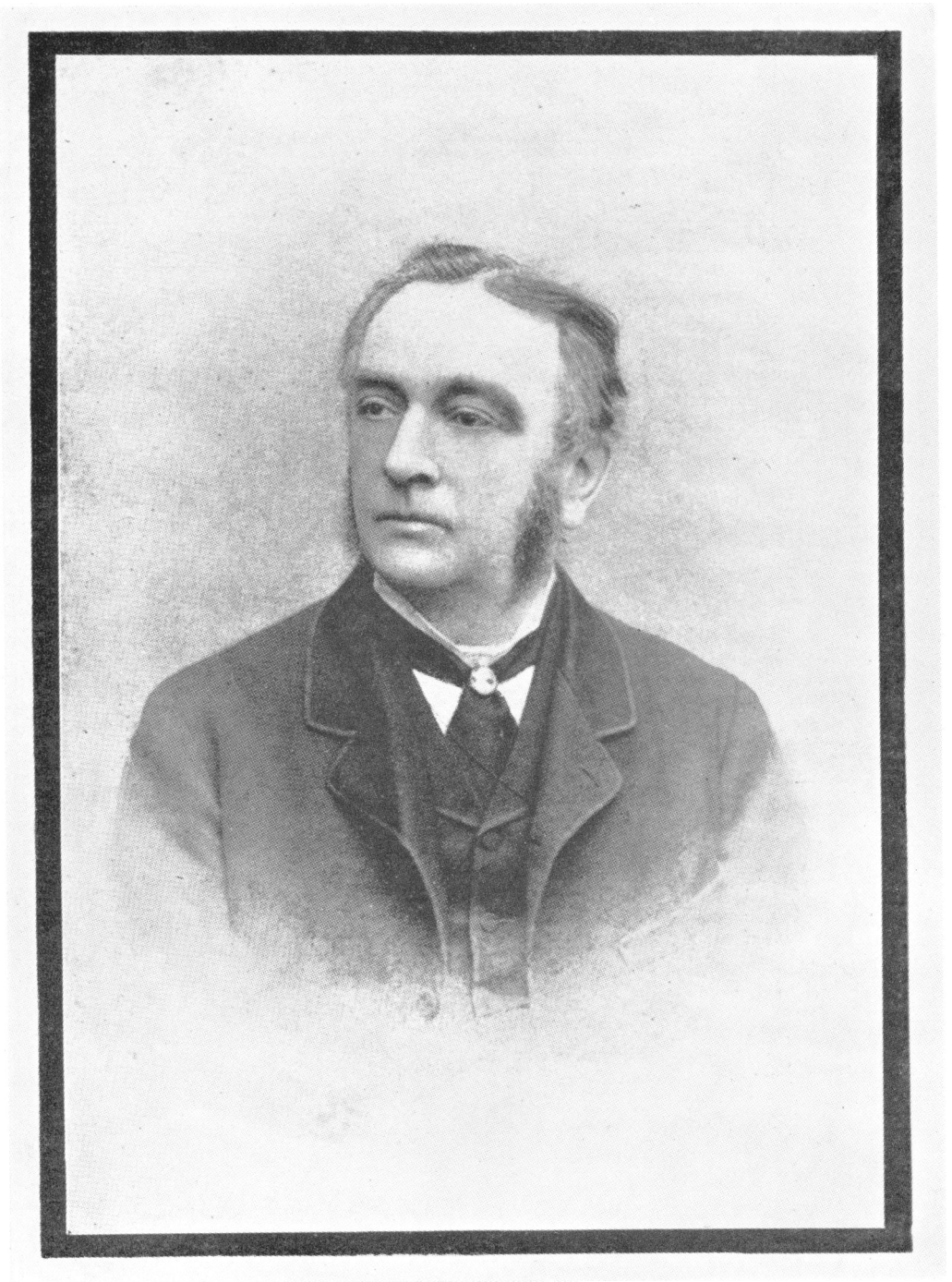

Fig. 1. Sir William Roberts, 1830–99

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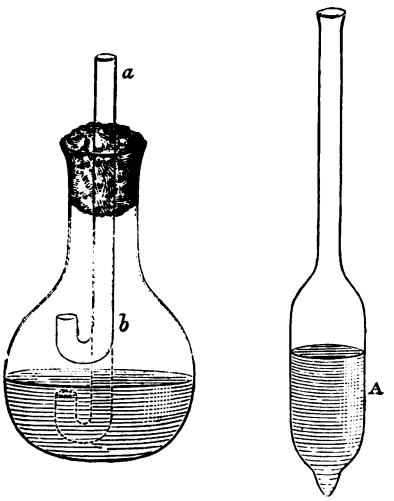

Fig. 2

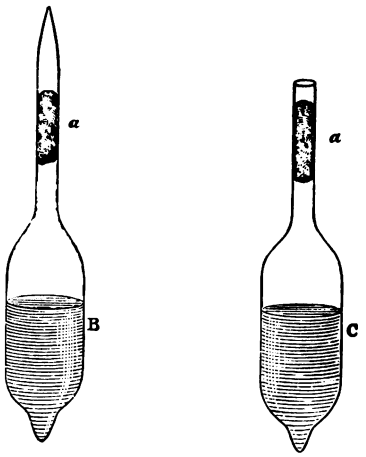

Fig. 3

Fig. 2 Roberts's modification of Pasteur's 'swan-neck' flask. (*From Roberts, 1874*)

Fig. 3 Roberts's 'plugged bulbs'. (*From Roberts, 1874*)

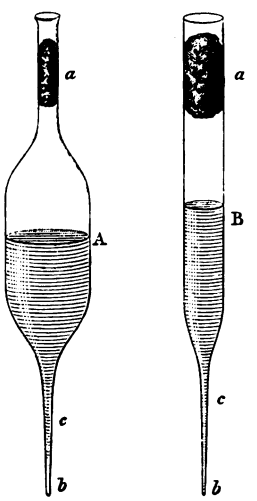

Fig. 4

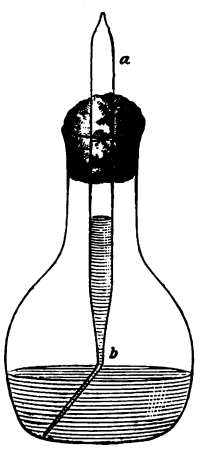

Fig. 5

Fig. 4 Device used by Roberts for sampling natural substances. (*From Roberts, 1874*)

Fig. 5 Flask used by Roberts for neutralizing infusions after boiling. (*From Roberts, 1874*)

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spores at the time he carried out his work, nor of their ability to remain dormant and germinate only *after* the re-entry of air (oxygen) into sealed flasks of such previously boiled infusions.

In his introductory statement Roberts points out that the main purpose of his investigations is to determine whether micro-organisms originate *de novo*. He states, however, that the doctrines of panspermism and abiogenesis are not necessarily mutually exclusive, and that whereas the usual mode of production of micro-organisms is from predecessors like themselves, there may exist *rare* conditions under which they arise spontaneously. This is an extraordinarily modern point of view, and doubtless a statement acceptable to most present-day biologists with but little emendation. In order '. . . to establish the fundamental propositions of the panspermic theory . . . without prejudice to the question of abiogenesis',[10] Roberts adopts '. . . . the attitude assumed by pathologists in regard to contagious diseases. No pathologist doubts, for example, the contagiousness of smallpox, nor that the ordinary production and spread of the disease is due to infection. And this belief is not inconsistent with the notion, very commonly held, that in some previous age smallpox did arise *de novo*; nor would it now be shaken, nor the practical deductions therefrom be set aside, if it were proved that under certain rare etiological combinations smallpox might still arise *de novo*.'[11] This position is similar to but not as strong as that advanced by Roberts's contemporary, Doctor William Budd of Bristol. In his 1873 classic, *Typhoid Fever*, Budd stated that, 'cases of this fever, like cases of smallpox, are constantly arising which cannot be traced to contagion. There is the strongest conceivable evidence, not withstanding that whatsoever its first origin, smallpox does not spring up *de novo* now.'[12]

Roberts's paper of 1874 is divided into three sections. Section I deals with the heat-sterilization of organic mixtures. Section II considers 'the capacity of the juices and tissues of animals and plants to generate *Bacteria* and *Torulae* without extraneous infection',[13] and Section III is a summation of Roberts's position '. . . on the origin of *Bacteria* and *Torulae*, and on the real explanation of some of the alleged causes of abiogenesis'.[14]

In Section I Roberts describes how beef-tea or turnip-infusion is sterilized by boiling several minutes in a cotton-wool-plugged flask. These 'plugged flasks' could be 'kept for months and even years exposed to the most favourable conditions of warmth and light, with a constantly renewed supply of air; but so long as the cotton-wool plug remains undisturbed, neither *Bacteria* nor *Torulae*, nor any other organisms make their appearance in it'.[15] An ingenious modification of Pasteur's 'swan-neck' flask was devised. A glass tube (*a*, *b* in Fig. 2) 4 inches long was bent at one end into a U-shape. The longer portion was wrapped round with cotton-wool and used to plug a flask half filled with beef-tea or turnip-infusion. The bent glass tube also was plugged at *a* with cotton-wool and positioned above the surface of the liquid. The infusions were boiled for five minutes, cooled, and then the cotton-wool plug at *a* was removed. The flask contents remained sterile until the U-tube was submerged, soon after which 'mold appeared on the surface, or the liquid becomes turbid from *Bacteria*'.[16] Roberts states that,

these experiments admit of an easy explanation on the panspermic theory. The living germs and organisms contained within the flasks are killed by the heat during ebullition; and the fresh supplies of air which enter the flasks on cooling are deprived of their germs—in the first case by filtration through the cotton-wool plug; in the second case, they are arrested in the bend of the tube *a b*, the shorter limb of which they are unable to ascend against the force of gravity; and thus the necrosis at first effected by the heat is succeeded by a state of permanent sterility through want of living germs to start the process of germination.[17] [Then follows the significant statement] The degree of heat required to induce this state of permanent sterility varies greatly according to the nature of the materials operated upon.[18]

Roberts then altered his experimental procedure by substituting 'plugged bulbs' for the flask and U-tube (see Fig. 3). The bulbs were designed to obviate evaporation and allow better temperature control. In these experiments the bulb (of 30 to 50 ml. capacity) consisted of an ordinary delivery pipette sealed at one end. The infusion was added to the bulb until two-thirds full. A plug of cotton-wool was inserted to the middle of the neck (Fig. 3A), and the latter was drawn out above the plug and sealed in a flame (Fig. 3B). The bulbs were placed in boiling water for various periods and then cooled. The glass neck above the cotton plug was filed off (Fig. 3C) and the bulbs were incubated at suitable temperatures (15 to 32°C.) and periodically observed.

After four years' work, during which several hundred experiments were performed using the 'plugged flask' and 'plugged bulb' techniques, Roberts concluded that natural materials could be divided into categories depending on the time required to sterilize them. In group I (substances in plugged flasks sterilized by exposure to boiling water for five to ten minutes) were infusions of beef, mutton, pork, codfish, mussel, carrot, turnip, hay, malt, pear, apple, cucumber, cabbage, lettuce, tomatoes, vegetable marrow and parsnip; solutions of organic salts—citrate, acetate and tartrate; and healthy and diabetic urine.

In group II (substances in plugged bulbs requiring twenty to forty minutes' exposure to boiling water) were mixtures of chopped green vegetables, pieces of flesh meat, or fish, or boiled egg, blood, dropsical fluids, milk, albuminious urine and turnip-infusion with cheese. Finally, group III contained but one substance, namely, 'superneutralized' hay-infusion. This was found to require at least one to two hours' boiling in a plugged bulb to achieve sterilization. Roberts reported that 'the maximum resistance to sterilization resided in infusions alkalized with about 5 drops of *liquor potassae* (potassium hydroxide) per ounce (about 1 per cent); 2 or 3 drops less than this, or 4 or 5 drops more than this, considerably diminished this resistance'.[19]

In Section II Roberts reports results of investigations made on fresh eggs, blood, urine, blister-serum, milk, grape-juice, orange-juice, tomato-juice, turnip and potato. In each instance the materials were sampled with the bulbs and tubes depicted in Fig. 4. These were drawn out into sealed capillary points at one end (Fig. 4, *b*, *b*), and the other ends were plugged at *a*, *a* with cotton-wool. The bulbs were sterilized

by introducing water into them before the plugging, and boiling over the flame for 10 minutes. The tubes were sterilized by passing and repassing them through the flame of the spirit-lamp until they were quite hot, as shown by commencing charring of the cotton-wool plug. If the conditions of the experiment required that there should be some water in the tubes, their

capillary ends were snipped off (after sterilization) and boiling water was sucked into them, and the ends again resealed.[20]

In examining the above-mentioned natural substances the capillary end of a sterilized bulb or tube (Fig. 4, *b*, *c*) was immersed for a few seconds in boiling water, the sealed end snipped off, and the capillary portion plunged into the centre portion of the material. The bulbs or tubes were charged by sucking or squeezing the liquids into them, and when this was accomplished, the bulb or tube was quickly withdrawn and its capillary end sealed in a flame. Roberts concluded from the results of ninety experiments (in which sixty-seven tubes or bulbs remained sterile) that,

the ideal conditions of the experiments could not in any case be carried out with absolute stringency. Some risk of extraneous infection was always encountered in conveying the materials of the experiments into the sterilized bulbs and tubes. The results obtained are therefore not altogether uniform; but as this was in accordance with the expectation of the experiments, it adds to, rather than detracts from, their validity. Where the conditions of the experiment could be carried out in almost ideal perfection, as with egg-albumin, urine, blister-serum, grape- and orange-juice, the results were nearly uniform; but when, on the contrary, the risk of extraneous infection was obviously considerable, as with blood, milk, turnip, and potato, the results were less uniform, though even in these cases with the single exception of milk, the sterile tubes were in a majority. . . . It was scarcely possible to obtain a more clear demonstration of the general conclusion that the normal tissues and juices have no inherent power to originate organisms, and that when organisms appear therein their development is due to germs imported from without.[21]

J. H. van den Broek had published a series of papers between 1858 and 1860 on this question.[22] His experimental approach was somewhat more complex than Roberts's, but the conclusion of his work was that grape-juice, urine, eggs, and the like, did not possess the power to generate *de novo* organisms capable of effecting their decomposition. In this regard it may be recalled that Pasteur reconfirmed his earlier results in similar studies after the death of Claude Bernard in 1878, when the latter's notes were revealed to contain statements that seemed to indicate he believed in the possibility of alcoholic fermentation apart from living organisms.

Attention is directed in Section III to those puzzling cases in which bacterial development occurs (without admitting contamination) *after* exposure to boiling water. Roberts's attack on this problem is most instructive. He carried out two series of experiments designated 'A' and 'B'. In 'A' he used sixteen bulbs of alkalized hay-infusion that had been heated for more than two hours and consequently were sterilized. These sixteen bulbs were divided into three sets. The first set of six bulbs had the plugs withdrawn and their contents exposed for six hours to unfiltered air, then they were replugged, and boiled for five minutes. Three of these tubes showed bacterial growth within four days. The second set of six bulbs was also unplugged, and from one to sixty drops of tap-water added to them. They were then replugged and boiled for five minutes. Four of these tubes showed bacterial growth. The third set of four bulbs was unplugged, and several drops of an alkalized contaminated hay-infusion were added to each; they also were replugged and boiled for five minutes. All of these bulbs showed bacterial growth. Finally, the three negative tubes from the first set, and the two

from the second set, were unplugged after showing no growth after one month and inoculated with alkalized contaminated hay-infusion. After replugging they were boiled for five minutes, and all were found to germinate in four days. Roberts concludes that,

> these experiments prove directly and positively that there exist in ordinary air and water, particles which, in certain liquids, are capable of preserving their germinal activity after exposure to a boiling heat for 5 minutes. They also prove that certain types of *Bacteria*, or their germs, are capable of surviving a similar heat . . . it would seem that the vital resistance to heat of *Bacteria* and their germs varies greatly according to the nature of the liquid in which they subsist, and probably also according to the species or type of *Bacteria*. It does not seem unreasonable to suppose that different races of *Bacteria*, or different phases of their development, are capable of offering very different degrees of resistance to the destructive influences of heat.[23]

Prophetic words, indeed!

In his 'B' series of experiments Roberts again repeats his statement that whereas unneutralized hay-infusion is sterilized by five minutes' boiling, neutralized hay-infusion requires more than one hour. Two possible explanations may be offered: either neutralization increases the resistance to heat of the germs contained in the infusion, or it increases the 'abiogenic aptitude' of the infusion itself. Roberts tested these alternative explanations using the flasks of the sort depicted in Fig. 5. He charged ten such flasks with unneutralized hay-infusion.[24] Five were simply plugged with cotton-wool and boiled for five minutes, and the remaining five were plugged with cotton-wool through the centre of which there was passed a sealed glass tube, shown as *a b* in Fig. 5, containing previously sterilized *liquor potassae*. These infusions also were boiled for five minutes. None of the contents showed bacterial growth after two weeks. At the end of this period the first set of five flasks were placed under a bell-jar close by a beaker of *liquor ammoniae* (ammonium hydroxide). After two hours a copious sediment in the flasks indicated that the infusions had been neutralized. These flasks were then set aside for observation. The second set of five flasks were neutralized by breaking the glass capillary and forcing out the *liquor potassae* with gentle heating of the upper end at *a*. These also were re-incubated. At the end of two months the infusions were sterile and Roberts summarizes his case by saying,

> . . . this result was strictly conformable to the view that the effect of the alkali was to increase the power of survivance of the germs, but it was wholly unconformable to the alternative view. According to the former view, the germs contained in the infusions were destroyed by the preliminary boiling, and no subsequent addition of alkali could, of course, restore their vitality. But on the opposing theory there was no reason why the alkali should not have been equally effective in promoting germination, whether added before or after the short preliminary boiling. By carrying the experiments a step further it was shown that, although the contents of the flasks had not acquired the power to germinate, they had acquired the property of enabling freshly introduced germs to survive a boiling heat; for when the flasks were unplugged and infected with ordinary air or water and then replugged and boiled for 5 minutes, their contents in every instance germinated in a few days. These experiments appear to warrant the following conclusions: 1. That the germinal particles of air and water (or some of them) are capable of surviving the heat of boiling water in certain media. 2. That when we speak of different liquids and mixtures as possessing different degrees of resistance to sterilization by heat, it would be more exact to say that the germinal particles of air and water possess

varying degrees of vital resistance to heat according to the nature of the media in which they subsist.[25]

Although Roberts considered that these results fully confirmed the panspermic theory, he nevertheless could not refrain from attempting to explain rare cases of delayed germination occurring in certain infusions as possible illustrations of the abiogenic mode of microbial reproduction. In one case the interval between initial sterilization and appearance of micro-organisms was eight months. In these instances the organisms which ultimately grew were found to be *Penicillium glaucum* and *Saccharomyces* sp., Roberts says,

The facts just enumerated are far too few, and of too exceptional a character, to permit a deduction in favour of abiogenesis; but they certainly impose a reserve which is highly significant. If future investigations should establish the occurrence of abiogenesis, this would not overturn the panspermic theory, it would only limit its universality; and it may be predicted with some confidence that if abiogenesis exists the conditions of its occurrence can only be determined by an inquirer who is fully alive to the truth and penetrating consequences of the panspermic theory.[26]

In 1876 a short note by Roberts entitled 'A Word on the Origin of Bacteria, and on Abiogenesis' appeared in the *British Medical Journal*.[27] In this paper he indicates that H. C. Bastian has erroneously cited his experimental results as lending support to the latter's views on heterogenesis. This Roberts vigorously denies, and he says that,

the results obtained by me were in substance identical with those obtained by Pasteur and Professor Tyndall. The circumstance that Professor Tyndall did not encounter those examples of great resistance to sterilization by heat that I encountered involves no contradiction in our results. His procedure was different from mine, but our results were the same. We both succeeded by boiling in sterilizing our infusions without impairing their aptitude for the growth of bacteria. As well might we say that two chemists contradict each other when they obtain the same metal from the same ore by different processes.[28] [His paper concludes with the remark that] . . . it is absolutely certain that up to the present time no case of abiogenesis has been presented which has stood the test of accurate investigation; nor can it be doubted that, in so far as the antiseptic treatment of disease rests on the origin of bacteria, the advocates of that treatment stand on unassailable ground.[29]

Roberts thus presents a vigorous defence of the panspermic theory, and he now does not doubt that any apparent exceptions to it are due to faulty experimental technique.

At the 1877 meeting of the British Medical Association held in Manchester, Roberts delivered an address entitled 'The Doctrine of *Contagium Vivum* and its Applications to Medicine'. This lecture seems to have been well received and subsequently it was reprinted in three different journals, finally appearing as a small forty-two-page book.[30] In this essay, which was to be Roberts's last formal consideration of biogenesis, a departure was made from the purely biological aspects of the problem to its consequences for medical practice.

Roberts begins by comparing a contagious fever (smallpox) with the action of yeast during fermentation. This well-known example involves the analogy of the incubation period, commencement of the disturbance (vigorous fermentation), rise in temperature, cessation of reaction and immunity from further attack. It is interesting to note that Roberts touches upon the subject of

immunity by saying that, 'the comparison fails in at least one important point— . . . sugar is replaced by alcohol and carbonic acid; but we are not aware that any pronounced chemical changes occur in the blood or tissues during an attack of smallpox'.[31] Further on he develops the proposition 'that organic matter has no inherent power of generating bacteria and no inherent power of passing into decomposition'.[32] Roberts considers bacteria to be the actual agents of decomposition, and in all instances 'the organisms which appear as if spontaneously in decomposing fluids owe their origin exclusively to parent germs derived from the surrounding media'.[33] He also returns to the conclusions of his 1874 paper in which survival to boiling heat, but not *de novo* generation, seemed the most reasonable explanation of his results, by citing the demonstration of bacterial spores by F. Cohn, and noting how the discovery of these heat-resistant bodies fully substantiates his position.

Turning next to the problem of *contagium vivum* Roberts begins with the remark that,

> if . . . the doctrine of a *contagium vivum* be true, we almost forced to the conclusion that a *contagium* consists (at least in the immense majority of cases) of an independent organism or parasite, and it is in this sense alone that I shall consider the doctrine. . . . My object is to establish the doctrine as a true doctrine; to produce evidence that it is undoubtedly true in regard to some infective inflammations and some contagious fevers. In an argument of this kind, it is of capital importance to get hold of an authentic instance; because it is more than probable—looking to the general analogy between them—that all infective diseases conform in some fashion to one fundamental type.[34]

Roberts then considers, in order, the evidence for the connection between *contagium vivum* and septicaemia, relapsing fever and splenic fever (anthrax).

An analysis of the course of septicaemia leads Roberts to the conclusion that,

> . . . an unprotected wound receives infection from the septic organisms of the surrounding media. If the discharges are retained in the sinuosities of the wound, decomposition of them sets in with the production of the septic poison. This is absorbed into the blood, a toxic effect follows, and septicaemia is established. As this effect increases with the continuous absorption of the poison, the vitality of the system is progressively lowered, and especially the vitality of the tissues bordering the wound, which may be topically affected by the poison which percolates through them. These tissues at length become moribund or die outright; the septic organisms then invade and breed in them, more septic poison is produced and absorbed; the toxaemia becomes intense, embolic centres of inflammation and suppuration are formed, and the end comes. In all this history there is no necessity to assume, nor even a probability, that septic organisms invade, or at least multiply in the blood. They may do so at the near approach of death, but scarcely before that period.[35]

In regard to relapsing fever and splenic fever the same careful analysis of symptoms is made. Roberts shows that he is conversant with all the important literature published on these diseases, and he cites the work of Obermeier, Cohn, Heydenreich, and Motschutoffsky on relapsing fever, and that of Pollender, Brauell, Davaine and Koch on anthrax. His careful analysis of the relationships of specific bacteria and these diseases leads him to this final eloquent conclusion:

> There is nothing in all nature more wonderful than the intimate and subtle nexus which unites a parasite to its host . . . even different varieties or races of the same species have

different and exclusive parasites. . . . The same minute correlation is seen in specific contagion —all are confined to one or a few species . . . I believe that the doctrine of a *contagium vivum* is established on a solid foundation; and that the principle it involves, if firmly grasped in capable hands, will prove a powerful instrument of future discoveries. And let no man doubt that such discoveries will lead to incalculable benefits to the human race: our business in life is to do battle with disease, and we may rest assured that the more we know of our enemy the more successfully we shall be able to combat him.[36]

Except for some minor work on bacteriaemia done in 1881–2, Roberts never returned to the subject in which he had made so auspicious a contribution.[37]

Shortly after Roberts's main paper was published, Tyndall's brilliant work began to appear,[38] fully substantiating the former's observations. The best commentary that probably has been made on Roberts appeared in the Royal Society obituary notice[39]

. . . Roberts' record of work shows well what a busy physician can do in the way of accurate work in the scanty leisure afforded him in the intervals of practice, and all his work, whether chemical or histological, was characterized by the same scrupulous accuracy and neatness. It is probable that few, if any, of his statements of fact will require emendation, although doubtless some of the views founded on them may alter. . . .

After the existence and heat-resistance of bacterial spores had been established beyond doubt, the puzzling cases that seemed understandable only by evocation of some abiogenic manifestation, now were subject to more precise explanation, and, of course, much of the remaining structure of abiogenic theory collapsed. In addition, a realization of the importance of the *environment* on the expression of physiological characters of bacteria emerged. It is in these areas, then, that William Roberts made his small, but none the less sound, contributions, and it is for these that we ought to recognize him as a neglected predecessor of Tyndall and Cohn, and an able worker on the side of Pasteur and his colleagues.

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24. The infusion was always made in the same way. Hay was soaked in a minimum of water for 4 hours at blood heat. The infusion was then filtered and diluted with ordinary water until it had a specific gravity of 1·006. Prepared in this way, hay-infusion had the colour of sherry; it was slightly acid in reaction, and it boiled without forming any sediment. When neutralized with ammonia or *liquor potassae*, it threw down a copious sediment which increased on boiling; this sediment subsided completely on standing, and left a perfectly transparent supernatant liquor. This perfect transparency permitted the detection of the slightest turbidity from the development of Bacteria. *Ibid.*, pp. 462–3.
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31. *Ibid.*, p. 168. 32. *Ibid.*, p. 169. 33. *Ibid.*, p. 169. 34. *Ibid.*, p. 170. 35. *Ibid.*, p. 171. 36. *Ibid.*, p. 173.
37. There is a final statement in the *Proc. roy. Soc. Lond.*, 1876, **25**, 454–6 entitled, 'Note on the influence of *liquor potassae* and an elevated temperature on the origin and growth of microphytes.'
38. TYNDALL, J., Further researches on the deportment and vital persistence of putrifactive and infective organisms from a physical point of view, *Phil. Trans.*, 1877, **167**, 149–206. Roberts's work is also quoted at length by F. Cohn, in his 'Untersuchungen über Bacterien', IV. *Beitr. z. Biol. d. Pflanzen*, 1876, **2**, 249–76.
39. J.R.B., *op. cit.*, p. 71.