Report on experimental investigations upon the intimate nature of the contagium in certain acute infective diseases / by G.F. Dowdeswell.

With the Author's Compliments

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REPORT

ON

EXPERIMENTAL INVESTIGATIONS

UPON THE

INTIMATE NATURE OF THE CONTAGIUM IN CERTAIN ACUTE INFECTIVE DISEASES.

BY

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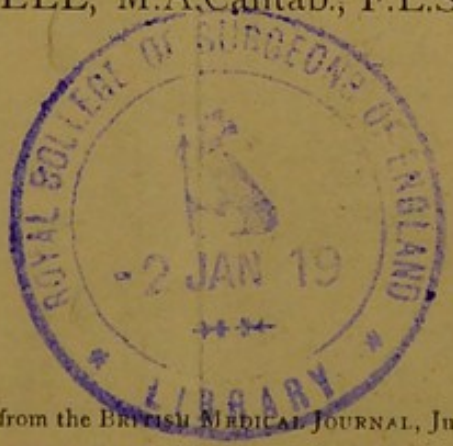

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REPORT ON EXPERIMENTAL INVESTIGATIONS UPON THE INTIMATE NATURE OF THE CONTAGIUM IN CERTAIN ACUTE INFECTIVE DISEASES.

By G. F. DOWDESWELL, M.A. Cantab., F.L.S., F.C.S., etc.

[The principal results of the following investigation have been published in detail, at different times, in the *Proceedings of the Royal Society*, hereinafter referred to.]

It is upon septichæmia in various forms that the earliest and most numerous experiments upon the nature of the contagium in infective diseases were made. The two opposite views upon this question, long held and warmly contested, may now be considered, in some measure, as finally settled; the investigations of Panum,[1] Bergmann,[2] Schmidt, and Schmiedeberg, with others, in Germany; in this country of Dr. Burdon Sanderson,[3] proved, as is now generally recognised, that in putrid matter there is a soluble chemical poison acting toxically on animals inoculated with it; whilst, on the other hand, it has been shown in a perfectly conclusive manner, first and foremost by the remarkable researches of MM. Rayer and Davaine[4] in France, more

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[1] A summary of these experiments, originally published in a Danish journal, is given by the author, Virchow's *Archiv*, Bd. 60, 1874, S. 301-351.

[2] *Das putride Gift und die putride Intoxication*, Dorpat, 1868.

[3] Report of Medical Officer of Privy Council, 1877-78.

[4] M. Rayer was clearly the first who described the *bactéridie*, as constituting the contagium in charbon (anthrax) (*Bull. de la Soc. de Biol.* (Paris), 1850, p. 142). The passage (*loc. cit.*, p. 142), which is of great historical interest, is as follows: "Les globules" (of the blood in charbon), "au lieu de rester bien distincts commes les globules du sang sain, s'agglutinaient généralement en masses irregulières, il y avait en outre dans le sang, de petits corps filiforms, ayant environ le double en longeur d'un globule sanguin. Ces petits corps n'offraient points de mouvements spontanes." Shortly afterwards, experiments at Chartres, in conjunction with M. Davaine, proved the infectivity of the blood in these cases. These observations were followed, as it appears independently, by those of Pollender (1855), Brauell, and others in Germany; but it was M. Davaine who afterwards completely and unanswerably, in an admirable series of experiments (*Comptes Rendus de l'Acad. des Sciences de Paris*, vol. 57, 1863, p. 222, etc.), demonstrated the causative agency of the micro-organisms, the characters of which he describes with great accuracy, and in some measure, too, anticipates the methods now in vogue for the preservation and microscopical examination of these organisms. Davaine's right in this respect is fairly admitted by Koch in his own first notable memoir on the subject (*Beit. zur Biologie der Pflanzen*, Band ii, S. 277); though he overlooks the claim of M. Rayer. Koch, however, has the merit of having completed this demonstration by the method of artificial cultivations.

The belief, or rather a vague conjecture, that epidemics and infective diseases were caused by a *contagium animatum*, is as old as history itself, though, till the last two centuries, destitute of any objective basis. The first definite observations upon and description of these microscopic organisms are generally accredited to Ehrenberg, who placed them amongst the Infusoria. In the previous century, however, Leeuwenhoek, *Phil. Trans.*, vol. xiv, 1864, p. 568, etc., and *Continuatio Arcanorum Naturæ Detectorum* (Delphis Batavorum, p. 10), also, *Select Works translated by Samuel Hoole*, London, 1798 (vol. i, p. 118, pl. iv, fig. 3), had described

recently extended in an admirable and well known manner by Dr. R. Koch, of Berlin, by Dr. Klein in this country, and others; that in some cases, a microphyte constitutes the actual contagium, the true *materies morbi.* Hence, in this relation, these diseases fall into two distinct classes, the one of microparasitical origin, designated by Dr. Burdon Sanderson[5] specific septichæmia, the other induced by a chemical poison, better distinguished as septic intoxication (Bergmann, etc.); a primary distinction between the two being that, in the latter case, the effect is distinctly proportionate to the quantity of septic or toxical matter used for inoculation, whilst the blood and tissues of the animal affected do not themselves, during life or immediately after death, become in any wise infective or toxical; but, in the former case—a specific microparasitical disease—the relation between the quantity of matter used for inoculation and the effect produced, is not so direct, the smallest conceivable particle frequently producing the same result in every respect, as an immeasurably larger quantity, showing that here the contagium can only be an organism capable of reproducing itself and multiplying in the animal tissues, which in this case become as actively infective as, or more virulent than, the original septic[6] matter.

Another primary distinction between the two cases is that, with an organised contagium, there is a latent, or so termed "incubation" period between the occurrence of inoculation or infection, and the appearance of any symptoms of disturbance; whereas, in the case of a chemical noxa, this is absent. This distinction, however, in the case of the lower animals, where the symptoms are sometimes obscure, is not always sharply defined; though the absence of any "incubation" period must be regarded as tending to disprove the agency of a microparasite, as in some cases hereinafter recorded, *e,g.*, in the so-termed Pasteur's septichæmia, in rodent's, when death occurred within four hours of inoculation; for a simple arithmetical calculation shows that, according to all reliable observations on this point, it is impossible that any forms of the lower fungi, introduced in small quantities, can develop in sufficient numbers within the period named, to pervade the organism, so as to be capable of effecting any serious disturbance. This consideration, in investigating the nature of the contagium, in some cases seems to have been lost sight of. Obviously, however, the converse does not hold good; and the occurrenceof a latent period between inoculation and any perceptible resulting disturbance, does not of itself preclude the casual agency of an unformed or soluble ferment.

The study of these diseases, and, more particularly, the characters and properties of the micro-organisms which occur in them, has lately received an additional stimulus, as is well known, from the asserted means of obtaining protective inoculation by certain methods of cultivating the microphytes which therein occur, inducing apparently, as it was termed, a transmutation of physiological species in them—a modification of their action on the animal organism, causing an abortive or mitigated form of disease.

Two distinct views are held by schizomycologists respecting the constancy or mutability of species in these organisms. The doctrine upheld by Professor F. Cohn[7] and his school, including Dr. R. Koch,[8] is that a difference either of form or of physiological function con-

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and figured with remarkable accuracy some forms which he had observed, and termed "animalcula." These, undoubtedly the first recorded observations on bacteria, possess a great interest now that the subject of these organisms has assumed such a wide importance.

[5] Brown Lectures, British Medical Journal, 1877 and 1878.

[6] The term septic is here used in its etymological meaning of putrescent, or more strictly, putrefactive.

[7] *Beiträge zur Biol. der Pflanzen*, Bd. i, H. 2, p. 127, and H. 3, p. 142, etc.

[8] *Ibid.*; also *Untersuch. über die Ætiol. der Wundinfectionskrankheiten*, Leipzig, 1878.

stitutes a distinct species, in the biological sense of the term ; that such differences are constant, and do not merge or become transformed into one another ; that, *e.g.*, the short cylindrical cells, known as bacteria in the classification of Cohn, are specifically distinct from the longer cells termed bacilli, from leptothrix filaments, or those forms termed spirilla ; and again, that those which occasion chromogenic, pathogenic or zymotic action, even when morphologically similar, or apparently identical, in like manner constitute distinct independent species. On the other hand, these different morphological and physiological forms are by others regarded as mere developmental phases, or varieties modified by external conditions, of one and the same, or but a very few distinct species. The chief advocate of this doctrine is Professor von Nägeli, of Munich.[9] It was previously, however, clearly stated by Billroth, in an elaborate work (*Die Vegetations forme der Coccobacteria Septica*, Berlin, 1874), followed by Cienkouski (Zur. morph. der Bact., *Mem. de l'Acad. Imp. de St. Petersb.*, 1877, ser. iii, T. 25, pp. 3-17), in this country by Professor E. Ray Lankester,[10] and more recently and explicitly by Dr. W. Zopf, of Halle, in various papers published in different journals, and summarised in his last work, *Zur Morphologie der Spaltpflanzen* (Leipzig, 1882).[11]

With respect to some at least, of these, it must be said that, minutely and carefully, in many cases, as the different forms, therein regarded as merely various stages of development of one and the same species, are described and figured, the conclusions cannot be regarded as established, inasmuch as they are not the results of continuous observation, of cultivation from spore to spore ; nor, in the case of Billroth's work, is it indeed attempted to show that the cultivations were in any way "pure," without which no circumstance of the life-history of these organisms can be regarded as satisfactorily established.

This doctrine, *i.e.*, of the occurrence of a transformation of physiological species in the Schizomycetes, has recently been expressly advocated by Dr. Hans Büchner,[12] who, working under the auspices of von Nägeli, alleges that he has established this fact by direct experiment, in the case of the asserted transformation by certain methods of cultivation of a harmless mobile saprophyte, the hay-bacillus, into the virulent and immobile bacillus anthracis, and conversely that he has, by certain other suitably modified methods, transformed the latter deadly organism into the former bacillus subtilis. These statements have been fully examined both by Dr. R. Koch,[13] in Berlin, and by Dr. E. Klein in this country ;[14] so that it need merely here be said that, whatever the fact be as to the occurrence of any such transformation, nothing can be regarded as shown by these experiments, in which the occurrence of contamination in the cultiva-

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[9] *Die Niederen Pilze, etc.*, Munich, 1877 ; and more explicitly in his latest work, *Zur Umwandlung der Spaltpilsformen*, in *Untersuchungen über niedere Pilze*, Munich and Leipzig, 1882. The argument therein (p. 138), that different forms or varieties of pathogenic micro-fungi must have originated by transmutation or modification from others, not pathogenic, because obviously they could not have existed as such previously to the appearance of their hosts, and that therefore they cannot constitute constant or "good" species in the biological sense of the term, is no more conclusive, neat as it is, than is a similare argument in the case of higher organisms, and does not show that they are not *now* constant, whatever their origin may have been.

[10] *Quarterly Journ. of Microsc. Science*, vol. xiii, 1873, p. 408 ; *ib.*, vol. xvi, 1876, p. 27 ; and *ib.*, April, 1883, p. 142.

[11] Also in *Die Spaltpilze*, etc., Breslau, 1883.

[12] Uber die Erzeug. des Milzbrandcontagiums, etc., in *Sitzungsber. der Königl. Bayer. Akad. der Wissensch. zu München*, Band x, 1880, S. 368, and *ib.*, Band xii, 1882, S. 147 ; and the latest "Erzeugung des Milbrandes Contag.," in *Untersuch. über niedere Pilze*, von Prof. C. V. Nägeli, Munchen und Leipzig, 1882.

[13] Zur Ætiologie des Milzbrandes, in *Mittheil. aus dem Kaiserl. Gesundheitsamte* (Berlin, 1881), p. 75, etc.

[14] Report of Medical officer of Local Government Board for 1881.

tion is admitted, and the paradoxical conclusion arrived at (*loc. cit.*, Erzugung, etc., p. 152 *et seq.* and p. 170 *ib.*), that the oxygen of the atmosphere is the agent both by which the fatal contagium of anthrax is deprived of its virulence, and also by which the innocuous saprophyte, fancifully, in opposition to the generally accepted nomenclature of Cohn, termed by the author "bacterium" subtile, in an inverse process of cultivation, loses its mobility, and becomes the virulently infective bacillus anthracis.[15]

It must, however, be admitted that, while, as stated, the observations here in question can establish nothing, whatever may be the fact as to the mutability of species, morphological or physiological, in these organisms, the occurrence of protective inoculation by certain, though not as yet clearly defined, methods of cultivation, which appears to be established by the extensive series of experiments recently recorded, involves a modification of virulence, an alteration of physiological function, in the organism ; and that, consequently, the views of Cohn and his school as to the constancy and immutability of species in the lower fungi cannot be maintained in their entirety.[16]

The first recorded experiments upon putrid poisoning or septic infection are generally credited to Gaspard, who, in conjunction with Majendie,[17] tried the effects of injecting a great variety of substances, septic and other, into the blood-vessels of living animals, but without obtaining any very definite results ; though, previously, Haller,[18] had shown, in 1765, that putrid water injected into the blood of living animals was toxical. Leuret,[19] however, was the first who proved by experiment that the blood of an infected animal was itself infective when injected into another. His experiments were made chiefly upon cases of charbon (anthrax), which he attributed to a putrid—chemical —poison ; he has the merit, too, of having conclusively shown that the blood in such cases decomposes more readily than that of healthy animals.

MM. Coze and Feltz[20] greatly extended these experiments, about the year 1866, in France, showing more particularly, by numerous experiments, that when certain animals are intoxicated by the injection of septic matter, their blood becomes infective to others. Further, they asserted that, in successive inoculations from one animal to another of the same species, such blood acquires an increase of infective virulence,

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[15] The method, too, here described as employed for obtaining the infective matter with which to originate the cultivations, viz., by triturating a portion of the spleen of an infected animal in an open mortar, without any precautions against atmospheric contamination, could have but one result, as is well known to every one who has made any such experiments.

[16] I may here add briefly, with respect to the question of the morphological transformations of these organisms, that while, obviously, certain species pass through a wide cycle of different phases in their life-history, as, *e.g.*, in the case of Beggiatoa roseo persicina, described by Professor E. Ray Lankester (*Quarterly Journal of Microscopical Science*, 1873, etc.) and some others ; yet that there is no reason to doubt the constancy of such species, or to suppose that all the different forms that occur are merely phases of one and the same species, any more than to assert this in the case of all insects, which in their life-cycle pass through various transformations ; others, too, are "Protean and adaptive" in their transformations, the forms they assume being controlled by alterations in external conditions ; the Bacillus anthracis was shown by Toussaint (*Recherches sur la Maladie Charbonneuse.* Lyons, 1879) to assume various abnormal forms in certain methods of cultivation, this has quite recently been confirmed and extended by Dr. Klein in this country. (*Report of Medical Officer to the Local Government Board*, 1882-83, and *Quarterly Journal of Microscopical Science*, 1883).

[17] *Journal de Physiologie de Magendie*, t. 2, s. 4, 1822 and 1823. The work is somewhat scarce, though in the University Library at Cambridge ; but its interest is chiefly historical.

[18] *Elementa Physiologiæ*, Göttingen and Leyden, 1765, vol. iii, p. 154, etc.

[19] Recherches et Expériences sur l'Alteration du Sang, par M. Leuret, M.D., *Arch. Gén. de Médecine*, Paris, 1826, p. 98.

[20] *Recherches Cliniques et Expérimentales sur les Maladies Infectieuses*, Strasburg and Paris, 1866 and 1872. The work is interesting in many respects. There is a copy in the Library of the Royal Medical and Chirurgical Society.

becoming more toxical than putrid matter itself, so that death succeeds inoculation with increasing rapidity. In such blood they found numerous microphytes invariably present; these they have particularly described and figured, regarding them as constituting the true cause of the disease, the actual contagium. To a change in their properties, an adaptation to external conditions, acquiring "renewed vigour" in successive generations in the blood of animals of the same species which they infested, they attributed the asserted increase of virulence in successive inoculations from one animal to another, which they consequently termed successive *generations*.

These observations are highly interesting, both as an early instance of the adoption of the then recent views of Pasteur on the "vital" nature of fermentation, for the explanation of pathological subjects, and as one of the first explicit statements of the occurrence of a modification of physiological function (*transmutation of physiological species*), an adaptive increase of virulence, from external conditions, in the lower fungi in a particular case, in accordance with the doctrine of evolution and the origin of species then just promulgated.

These experiments, made upon a variety of septic and infective diseases, attracted considerable attention at the time, and, indeed, many of the observations of these authors not here referred to, and but little noticed, as, *e.g.*, those on the alteration in the chemical constitution of the blood in pathological conditions, would form a very useful basis for further exact investigation.

This subject was shortly afterwards taken up by Dr. Davaine in Paris, who, in his first published experiments,[21] showed that, in rabbits, when specific septichæmia was excited by the injection of a small quantity of putrid blood, usually that of the ox, some few drops of which were requisite, yet that, after several generations, ultimately in the twenty-fifth, the trillionth of a drop or less was sufficient to produce fatal infection.

These experiments, of Davaine, though at first regarded with incredulity, were repeated by several[22], both in France and Germany, who confirmed his statements, though without further elucidating the subject; and henceforth his conclusions wers generally accepted, and the doctrine of an "adaptive increase of virulence" of the blood in infective diseases, in successive inoculations, was by most writers regarded as demonstrated.[23] In subsequent experiments, however, Davaine himself showed that a high degree of infective virulence in the cases in question is attained in the earlier stages; and he consequently modified his former conclusions. These latter results, being published in the *Bulletin of the Academy of Medicine* (Paris), were apparently overlooked by those who repeated his earlier observations, which they mostly did without any control experiments to ascertain the minimal degree in which the blood in question was infective in the earlier generations. It is equally remarkable, too, and a circumstance that has reference especially to the subject of the present paper, how generally microscopical observation was neglected in these cases. Coze and Feltz, indeed, as mentioned, described and figured the microorganisms which they found in the blood, and apparently under what should have been ample magnification; but, to my observation, their description and drawings are inaccurate in the points which will be more particularly mentioned below.

Davaine regarded this disease as a putrefaction of the blood in the living animal, and, adopting the then recent views of Pasteur on the

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[21] *Comptes Rendus de l'Acad. des Sciences*, 1873, *passim*, and *Bull. de l'Acad. de Méd.*, 1872, etc.

[22] Dreyer, *Arch. für Experim. Path.*, etc., 1874, Band ii, s. 149, etc.; and Clementi and Thin, *Wiener Med. Jahrb.*, H. 3, 1873.

[23] As by Birch-Hirschfeld, in an exhaustive summary and review of the subject up to that date, Schmidt's *Medicinische Jahrbücher*, 1875, c. 16, p. 169.

nature of fermentation, expressly considered the micro-organism which he found present in the blood as the cause of the disease, but regarded it as identical with the putrid ferment (B. termo)[24]—a mistake which, it must be remarked, may easily be made, without the most careful examination and the best microscopical appliances, in the absence of the present methods of preparing and staining these organisms. In the investigation of the relations of microparasitic organisms to disease, the very definition would seem to imply the paramount necessity of intimate microscopical examination. The fact, however, is, that this has been, in these cases, very generally overlooked. Of those who repeated Davaine's experiments, none have attempted to give any exact account of the microscopic characters of the microphyte. M. Vulpian alone remarked that the number of micro-organisms in the blood appeared to exceed in number the red corpuscles,[25] but deduced no further consequences from it; whilst some of those who repeated Davaine's experiments do not even allude to the presence of these organisms, apparently overlooking the necessity for microscopical observation.

Another form of so-called septichæmia—though the term is here a misnomer—is induced typically by the injection of putrid matter into the abdominal cavity, exciting acute peritonitis, with a copious inflammatory exudation containing large numbers of micro-organisms—a form of bacillus. Upon injection of a small quantity of this fluid into another animal subcutaneously, acute inflammation and local serous effusion are produced, the exudation of which likewise contains the same form of microphyte, and similarly is found infective in small quantities. In these cases, I have found that the blood, immediately upon death, is not in any wise infective or toxical; nor does it usually contain any micro-parasites, though very shortly after death these develop rapidly and pervade the whole organism. Pasteur himself states, in one passage, [26] that in these cases the blood is free from micro-organisms, and not infective; but others have asserted their occurrence in it. This may probably be accounted for by the observations having been made some hours after death, when these organisms had developed and pervaded the tissues of the animal.

This affection is generally known as the septichæmia of Pasteur, by whom it was lately described in France; but it has hitherto been over-looked that, in 1869, a similar form of disease was previously described by one who has done so much to elucidate the subject, and who never occupies himself with "reclamations of priority"—viz., M. Davaine—who[27] excited the affection by injecting a few drops of putrid blood into the abdominal subcutaneous tissue in guinea-pigs, and describes the characters of the disease thus produced as follows. 1. The bacteria which occurred in the blood and tissues were immobile. 2. The blood preserved its normal characters, and was not altered as in anthrax, the changes in which he clearly describes. 3. The spleen was unaltered, not enlarged, as is invariably the case in anthrax. 4. The blood, though found infective in minimal quantities, did not preserve its virulence on being dried, as in anthrax. M. Davaine has thus the credit of having first clearly distinguished this form of septichæmia from anthrax, with which it was often confounded; and M. Pasteur's subsequent observations on the same subject are merely a repetition of those of M. Davaine, though he extended them in another direction with his usual ability. It is bare justice to M. Davaine to note this.

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[24] *Bull. de l'Acad. de Méd.*, 1872, p. 1,005.

[25] *Bull. de l'Acad de Méd.*, 2 ser., tome ii, part 13, p. 393 (1873). In blood diluted 1,000 times, he found rods and globules "moderately numerous," but few "granulations and bacteries" in dilutions of one-millionth.

[26] *Bulletin de l'Acad. de. Med. de Paris*, 2 ser., t. vii., 1878, p. 4334, etc., La Theorie es Germes, and *ib.*, February 1st, 1881.

[27] *Comptes Rendus de l'Académie des Sciences (Paris)*, t. lxviii., 1869, p. 19, etc.

In 1873,[28] Drs. Sanderson and Klein, in this country, published some remarkable observations upon the effect on healthy animals of inoculation with different pathological products; and ultimately they showed that, by injecting into the peritoneal cavity a small quantity of a chemical irritant itself germ-free, peritonitis was induced, the exudation in which abounded in micro-organisms, and was itself infective when injected in small quantities into the peritoneum of another animal, the specific organisms in all cases being reproduced. Here was an instance of presumably a microparasitical disease induced by the injection of an antiseptic germ-free fluid, which obviously offered a good case for investigating the actual relations of these organisms to the diseases in which they occurred; but, though this subject was fully considered at the time referred to by Dr. Sanderson, it seems to have been lost sight of since, in this view; nor have the observations, as far as I know, ever been repeated; constantly as these relations, in different cases, have been the subject of experiment and discussion. Quite recently, however, in two instances, it has been alleged that infective microparasitical diseases have been excited by the injection of sterilised or germ-free fluids, though this is found, on examination, to be an error, as is described below; and the experiments recorded by Drs. Sanderson and Klein still stand alone in respect to this result. It has been thought that, in the case of this disease also, similarly to what was asserted in that of Davaine's septichæmia, an increase of virulence in the infective matter occurred in successive inoculations, which point is more particularly the subject of investigation here. In several series of experiments, however, I have found that this last mentioned affection, originated by the injection of a germ-free chemical irritant in its most interesting phase, or otherwise by various pathological products, is essentially the same as the more recently described and so-termed Pasteur's septichæmia. Originated by septic or putrid matter, they are interchangeable at will, according to the site of inoculation or injection. The pathological appearances are exactly similar, and the micro-organisms which occur in both are identically the same.

Experiments Upon Davaine's Septichæmia.

Primary Infection and Appearances.—Infection was here excited, as in the original experiments of Davaine himself, by inoculation with a few drops of putrid bullock's blood; though that of other animals may be used with, as far as I have observed, similar results. In several series of experiments during some years past, I have found the action of such blood, as regards its specific infectivity, always uncertain. When from five to ten days old, in summer, it is usually the most infective; after some time further, it loses this property. In summer, it is much more readily infective than in winter, which clearly is not owing to the temperature or to the stage of putrefaction, as, to test this, I have, during the winter, at different times, placed fresh blood in the incubator, keeping it at a temperature of about 100° Fahr. for a considerable time, and making inoculations with it at frequent intervals, but always without success; and I failed to get specific infection through the whole of the last winter, during which I worked at this subject.

The inoculations were made by injecting into the subcutaneous tissue of the back, a few drops, not exceeding five, of the blood, generally diluted with an equal bulk of normal salt-solution, and filtered or strained. In order to obtain specific infection, a much larger quantity must not be used, or the liability to the formation of abscesses, and the occurrence of putrid intoxication by the chemical products of decomposition—"sepsin"—is increased. In putrid blood there are observed, under the microscope, numerous microphytes of

[28] *Med.-Chir. Trans.*, vol. lvi., p. 345, etc.

very various forms ; in some few instances, when the blood proved infective, I have been able to recognise the specific organism here in question, though in but very small numbers, and only in stained preparations ; in unstained, it is most difficult to do so certainly, or to distinguish it from b. termo, which always abounds in the earlier stages of putrefaction in both animal and vegetable substances.

When infection occurs, the symptoms observed are tolerably constant, and similar both in primary infection with putrid matter, and in subsequent transmitted infection. There is for some hours, marked pyrexia, as indicated by the rectal temperature,[29] and perceptible to the hands placed on the ears or other part of the body ; this generally rises from 99° or 100° Fahr. to 103° or 104° Fahr., in some cases to 106 Fahr. ; this is followed by dulness, loss of appetite, prostration, some dyspnœa, frequently a short convulsive struggle, and death, after a period which is here inconstant, averaging about 40 hours, but in subsequent transmitted infection by septichæmic blood, varies but slightly, averaging about 24 hours. The *post mortem* appearances are not well marked, and the statements of the earlier writers on this point are somewhat discordant ; to my observation, they are the following. Around the site of the subcutaneous injection is a circumscribed hyperæmia, with slight extravasations, a discoloration, and invariably a considerable thickening of the subcutaneous tissue, chiefly of the corium ; these appearances are always more marked and extensive in the case of primary infection by septic blood, than subsequently in transmitted infection ; there is seldom any material serous effusion. The lungs, liver, and spleen are generally congested ; the latter sometimes, but by no means invariably, enlarged and softened, with the occurrence of extravasations, which are sometimes very considerable. The most noticeable symptom is that, within a very few minutes, and sometimes apparently almost at the instant of death, the blood in the cavities of the heart and the great vessels is found coagulated, and immediately, or very shortly afterwards, throughout the other vessels. Contrary to the observations of others, I have found this invariable, without exception. The red corpuscles are not constantly nor materially altered,[30] but the white are killed, have become round, granular, and swollen, and are generally augmented in number. The blood is distinctly venous in appearance. Another noticeable feature, in every form of septichæmia alike, as far as I have hitherto observed, is the rapid decomposition of the blood, whether drawn from the vessels or not, and consequently, likewise, of all the organs and tissues. In most cases, even in winter, this occurs within a very few hours of death, as was first remarked and shown experimentally by Leuret *(loc. sit.)*, in the case of charbon (anthrax).

Several series of experiments on the subject here in question were made during the last few years ; the results in all were similar—on the main point, without a single exception—though some were not so clearly conclusive as those here recorded, through having been interrupted by the occurrence of coagulation, and the consequent difficulty of accurately measuring the blood of an animal that had died unobserved ; with which it was desired to experiment. The series here recorded, therefore, are the same as those already communicated to the Royal Society.[31]

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[29] In rabbits, however, as also in guinea-pigs, this, being normally variable from very slight causes within wide limits, does not afford a very reliable indication. In healthy animals I have sometimes observed it over 103° Fahr.

[30] In this, affording the most marked contrast to the case of anthrax, where the increased fluidity and loss of individuality in the coloured elements of the blood is strikingly conspicuous, and, to my observation, the most obvious and unmistakable characteristic of this affection, clearly distinguishing it from all other forms of septichæmia.

[31] *Proceedings of the Royal Society*, vol. xxxiv. (1883, p. 449, etc.), where detailed tabular statements of the experiments are given.

Infection was originated in a young rabbit by the subcutaneous injection of a few drops of putrid bullock's blood ; it died within forty hours of inoculation, with the appearances already described as characteristic of these cases. In the blood, which was much coagulated, were found many of the specific micro-organisms. One drop of this blood diluted, was then injected into another rabbit, No. 2, which died within 24 hours ; blood from its heart was immediately diluted with from 10 up to 100,000 times the quantity of salt-solution, and ten drops of each solution were injected respectively into five other rabbits of similar size and condition ; No. 3 thus receiving of blood 1-10th of a drop (minim), No. 4 1-100th, No. 5 1-1,000th, No. 6 1-10,000th, and No. 7 1-100,000th drop. Nos. 3, 4, 5, and 7 died in my presence in respectively 25, 24, 25, and 27 hours, and No. 6 in 27 hours, all with exactly similar symptoms. Hence it was shown that the incubation-period, so termed, is in nowise proportionate to, or influenced by, the quantity used for injection, and also that the blood of the so-termed second generation is already infective in the 100,000th part of a drop. Infection was then continued up to the sixth generation, in which the blood of an animal was diluted in increasing degrees, and inoculated into four other rabbits, so that they received respectively the 100th, the 10,000th, the 100,000th, and the 10,000,000th of a drop ; these all died in similar matter as before in from 20 to 26 hours, thus further showing that there is absolutely no shortening of the incubation-period from the second to the sixth generation ; and as the blood of this generation proved to be infective in the 10,000,000th of a drop, it was requisite to prove the limits of infectivity in the first generation.

Infection having been again originated in another animal by the injection of putrid matter, its blood was diluted, and ten drops of the different dilutions injected into each of five other rabbits, so that they received respectively 1,000th, 100,000th, 1,000,000th, 1-10,000,000th, and a 100,000,000th of a drop ; the first died in 24 hours, the next three were found dead within 48 hours, while the last survived ; it was apparently unaffected at first, but an abscess was found to have formed at the place of injection, and it lost flesh somewhat ; there was no appreciable rise of temperature or other symptoms of infection ; and, after some days, the abscess healed, and the animal recovered.

It was thus shown that the blood of an animal infected with specific septichæmia, in the first generation, is fatal in less than the millionth part of a drop. Numerous other experiments confirmed this ; and showed that, whether in the first or in any subsequent generation, the blood was infective, excepting in the very few cases when putrid intoxication or purulent abscesses occurred, in the hundred-millionth of a drop ; but, in much smaller quantity, its infectivity was uncertain.

These experiments proved that there is no increase of virulence in septichæmic blood in successive generations, from the first to the sixth, as had been supposed from the statement of Davaine and others, with respect to the minimal quantity requisite to produce infection, nor with respect to the incubation-period, as supposed by MM. Coze and Feltz. To ascertain whether any such increase might occur in later generations, infection was now transmitted successively up to the tenth, in which the blood of an animal was diluted as before, and injected into four other rabbits, so that they received respectively the 1,000th, the 1,000,000th, the 10,000,000th, and the 100,000,000th of a drop. The first of these died in 21 hours, the others survived upwards of 27 hours, but were found dead within 48 hours—thus showing, in the three latter cases, a somewhat longer incubation-period than had occurred in some of the earlier generations.

In the experiments here detailed, the appearances before mentioned as characterising this disease were found, in all cases, without material

alteration ; there was also found, in great though variable numbers, a microphyte of constant and distinctive character ; preparations of this organism have been shown under the microscope, and fully described already.[32] It is somewhat minute, being but about half a micro-millimètre (nearly 1-50,000 inch) in breadth. Its length varies in different stages of development, from a little more than equal, to about three times the breadth. These cells do not, in the blood of a living animal, form torula-chains, leptothrix-filaments, nor zoogloea-masses. They frequently appear, to superficial observation, in the dumb-bell or figure-of-eight form ; this is due to the plasma of the cell being aggregated at the two ends. In unstained preparations, this is the most highly refractive portion ; and, when stained, it takes the colouring matter deeply, the central portion remaining uncoloured, and the cell-wall consequently being very indistinct or imperceptible. This character, *i.e.*, the retention of the cylindrical form of the cell, with aggregation of its contents at the two ends, serves to distinguish this species from *Bacterium termo*, which it superficially resembles, but which is nearly double the size in breadth, with most of the cells distinctly constricted in the centre. The mode of multiplication of this species—which may appropriately be named *Bacterium Davainii*—is very obscure ; and, as I have hitherto failed, as all others appear to have done, in cultivating it satisfactorily, I can say nothing on the subject. It is as well marked and determinate a species as any known form of schizophyte, and, from every point of view, well deserving further study. In the blood of an animal, it has certainly sometimes appeared to me to be mobile, though never very actively so ; but, unstained, it is rather difficult to distinguish clearly, and I cannot speak positively upon this point. The methods of microscopical examination and preparation are mentioned below.

Experiments upon the so-termed Septichæmia of Pasteur in Guinea-Pigs.

In the first series of my experiments upon this form of disease, it was originated, as by Pasteur, himself, by injecting into the peritoneal cavity of a guinea-pig a few drops, usually five, of putrid blood ; subsequently, however, it was found that exactly the same results followed the method of Drs. Sanderson and Klein, before cited, by employing instead, a chemical irritant, *e.g.*, a few drops of a dilute solution of liquor ammoniæ, which, being germ-free, not introducing a variety of septic organisms, appeared preferable. In these cases, I found the infectivity of the exudation-serum, whether peritoneal or subcutaneous, infinitely less virulent than that of the blood in the case of Davaine's septichæmia ; and consequently, from using too small a quantity of the fluid for inoculation, infection was lost in some of the first series of experiments.

Infective inflammation having been excited in a guinea-pig, by injection into the abdominal cavity of a few drops of liquor ammoniæ diluted, it was found dead the following morning, with a copious exudation of serum, abounding in bacilli, with some micrococci or spores, which also occurred in the lower layers of the abdominal wall. Of this fluid, 0.05 and 0.022 cubic centimètre respectively, diluted with equal quantities of normal salt-solution, were injected into the peritoneal cavities of two other animals, which both died within 18 hours under similar appearances, with recurrence of the same organism as in the first case. At the same time, 0.0044 and 0.0022 cubic centimètre of the same fluid were injected into two other animals, which were not perceptibly affected, and survived.

The exudation-fluid of the first generation thus appearing to be infective in quantities of 0.022 cubic centimètre, but not in 0.0044,

[32] *Jour. Royal Micros. Soc.*, 2nd ser., 1882, vol. ii. part 2, page 310.

infection was continued by inoculation of another animal, of the third generation, with 0.05 cubic centimètre of the serum of the last ; this also died in 30 hours with similar appearances ; but the exudation from it, being injected into another animal of the fourth generation, this survived without being materially affected. It was thus evident that the exudation had gained nothing in virulence from the first to the fourth generation of transmitted infection, and was not invariably infective or fatal in quantities of 0.05 cubic centimètre. Further experiments confirmed this ; and ultimately, infection having been again originated by the injection of ammonia, it was transmitted by the successive injection of 0.22 cubic centimètre of the serum-fluid to the fifth generation ; the exudation of an animal, in this last generation, was injected immediately upon its death into four other animals, in quantities of 0.22, 0.022, 0.0022, and 0.00022 cubic centimètre ; the first, receiving 0.22 cubic centimètre, died in four hours ; the second, receiving 0.022 cubic centimètre, a quantity which in the experiments of a former series had proved fatally infective within 24 hours, was unaffected, as were the other two which received smaller quantities.

It thus appeared that the infective virulence of these inflammatory fluids, infinitely less than that of the blood in Davaine's septichæmia, from the first to the fifth generation, is in nowise increased. In the cases recorded, a quantity which in a former series was fatal in the second generation, here in the sixth was innocuous. The toxicity or infectivity of the exudation, however, is very variable ; both as evinced by the quantity requisite for fatal infection, and by the period between inoculation and death ; to me it appears that this is in a great measure due to constitutional idiosyncrasy in the animal inoculated—its power to resist infection ; in some cases at least, this appeared to be evidently associated with constitutional activity and vigour.

Neither was there any constant shortening in the incubation-period in successive generations, though, as compared to the case of Davaine's septichæmia, this period is here variable throughout my experiments, being from four to forty hours. Yet the absence of any progressive shortening in it is evident ; as an instance may be noted case No. 14, of the third generation dying in four hours, and No. 15 of the following (fourth) generation, receiving the same quantity, and surviving for upwards of twelve hours.

In all these cases, similar symptoms were observed without material variation. In the experiment here recorded, infection was transmitted by intraperitoneal injection, in other series by subcutaneous, as in the method of M. Pasteur. In both cases, micro-organisms of identically the same form were found in the peritoneal and in the subcutaneous exudation. In the former case they occurred also in the connective tissue of the lower layers of the abdominal wall, in the latter in that of the subcutaneous tissue, together with, in some cases, a certain number of micrococci, or, more probably, spores.

In the case of originating infection by the intraperitoneal injection of a chemical irritant, their appearances in these situations indicated that they originated from within the animal organism. They were not introduced either by the needle, previously purified by heating, nor by the fluid injected, boiled, and prepared, as in the original experiments of Drs. Sanderson and Klein, with antiseptic precautions ; nor is it conceivable that they penetrated from without through the perforation made by the needle, which was very minute, and, closing again immediately, frequently left no trace of any lesion, even to microscopical examination. It is disproved further by the circumstance, found in numerous experiments, that injections of an indifferent fluid, *e.g.*, "of normal salt-solution," made in similar manner, did not cause their development ; the only conclusion being that they were present previously, in parts of the animal organism, the pathological condition

—inflammation—caused by injection of the chemical irritant, enabling them to develop.

The microphyte which occurs in these cases is a form of bacillus somewhat similar to the hay-bacillus (bacillus subtilis) or the bacillus anthracis, in width about 1 micromillimètre (0.001 mm.), the length of the single cells being three or four times the diameter. Sometimes it forms chains of a few of the single cells united endwise, longer than those usually formed by bacillus anthracis in the tissues of a living animal; and also, unlike that organism again, the separate segments have distinctly rounded ends, instead of rectangular, as in the latter; from which, too, they are distinguished by their being generally distinctly active, the bacillus anthracis being invariably perfectly immobile. A further obvious distinction between the two organisms is that the bacillus here described forms numerous spores in the tissues of the animal which it infests, which the bacillus of anthrax never does. It is possible that, under different circumstances, as in different nutrient media, the characters of the organisms here described may vary, as do those of bacillus anthracis; but that does not affect the fact that, in the case here referred to, namely, in the tissues of the living animal, the characters mentioned I have as yet found to be constant, and to afford a perfectly reliable means of discrimination.

The organism here mentioned as typical of Pasteur's septichæmia, has recently been described by Dr. Koch in similar cases, which he terms more suitably "malignant œdema," and the microphyte, bacillus œdematis. He has shown that it frequently occurs on the surface of the soil, and appears to be the common saprophyte, which develops even more readily and universally than Bacterium termo in all decomposing animal or vegetable matter. In preparations, dried and made in the usual manner, chains of separate cells are less numerous than in the serum examined fresh, nor are the individual cells so long, obviously they break up into their constituent segments in preparing. This affords an exemplification of the necessity for examining all such organisms in their natural state in fresh preparations, and not merely in those dried and stained. In no case were any microparasites found in the blood or other tissues, excepting as described, when the case was examined immediately upon death.

The Occurrence of Microparasites in the Organs and Tissues of Healthy Animals.

The question of the occurrence of these organisms or their germs in the tissues of healthy animals, is a fundamental one in the determination of their relation to disease. Several exact experiments upon this subject have been made, some of which, more particularly those of Billroth,[33] and more recently of Nencki and Giakosa,[34] have shown that the germs of microphytes are present in these cases, chiefly as it appears, in the liver and pancreas. The specific characters, however, of the microparasites found, and the distinction between septic and pathogenic bacteria, do not appear to have been kept in view. Other authors, from experiments apparently carefully performed, have asserted confidently that the organs and tissues of healthy animals are free from such germs, and that, when excised under sufficient precautions, and placed in suitable conditions for the development of germs, they remain sterile. It is, however, generally known and readily demonstrated that, in certain situations at least, these micro-organisms do occur, especially in the lower intestine, consisting there of various ordinary septic forms. The presence of these, their multiplication and invasion of other tissues after death, has no pathological significance nor relation to the question of the contagium in any particular case; but if it

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[33] *Untersuch über Coccobact. sept.* (Berlin, 1874.)
[34] *Jour. für prakt. Chem.*, 1879.

be shown that the specific form (pathogenic) characteristic of any particular disease, and which was consequently regarded as constituting the contagium, occurs normally and harmlessly in the animal organism, the view of its specific infectivity would have to be profoundly modified in such case. On this subject two papers have recently appeared, which directly involve the question at issue here.

The first is that of Professor Rosenberger, of Würzburg,[35] who states that he sterilised by heat the blood and exudation-fluids of the rabbit in the two forms of septichæmia known as those of Davaine and Pasteur, and injected these sterilised fluids into other animals, that they were found to be infective ; and that in these cases the specific organisms which characterise the disease were reproduced in each case ; he therefore considers these micro-organisms as merely secondary or accidental phenomena of the disease in which they occur, having no causal relation to it. He also found, as he asserts, that in both affections there was an increase of virulence in successive inoculations. The results are merely thus stated in general terms ; no detailed account is given, either of the methods employed or of the experiments. To determine this question, I proceeded in the following manner.[36]

1. *Pasteur's Septichæmia in the Guinea-Pig.*—Infection was excited in the manner adopted by Pasteur by injection into the peritoneum of a small quantity of putrid blood. A few drops of the serous exudation resulting from this, on the death of the animal, were injected into the subcutaneous tissue of the abdomen of another guinea-pig, from which infection was transmitted in similar manner to another animal, very shortly after the death of which a portion of the subcutaneous infiltration, which was copious, and contained numerous active bacilli and spores, was mixed with an equal quantity of normal salt-solution, with the addition of 1 per cent. potassic carbonate, to prevent coagulation upon heating. Some of this fluid was then put in vacuum-tubes by breaking their points under its surface ; these were boiled in salt-solution, and while boiling their points were resealed, placed in the hot air oven and kept at a temperature of 140° Cent. (284° Fahr.) for one hour. Subsequently 0.3 cubic centimètre of fluid, prepared as above stated, but not heated, was injected into the subcutaneous tissue of another guinea-pig, which the next day died with the same symptoms as in other cases. At the same time, a like quantity, 0.3 cubic centimètre of the dilute alkaline fluid, which had been superheated, was injected into another animal, which remained free from any symptoms of affection for several days, but subsequently died from an accidental cause. The experiment was repeated by diluting, rendering alkaline, and superheating as before, the exudation-serum of another case, 1.0 cubic centimètre of which was injected into a fresh animal, which remained unaffected.

2. *Davaine's Septichæmia in Rabbits.*—Infection was here originated, as in previous experiments, by the injection of a small quantity of putrid blood into the subcutaneous tissue of a rabbit. This having died with the symptoms of specific infection, and the occurrence of the characteristic microphytes in its blood, another rabbit was infected ; immediately upon the death of which, blood was taken from its heart, and mixed with two parts of salt-solution, containing 3 per cent. of carbonate of potash. Of this solution, 0.3 cubic centimètre (containing 0.1 cubic centimètre of the blood) was injected into a fresh rabbit, which died with characteristic symptoms in about 24 hours. Other portions of the dilute alkaline blood were then placed in tubes, similarly to the former experiments, and heated to 140° C. for one hour, as before, and the next morning a Pravaz' syringe full of the superheated blood was injected into another animal, which showed no

[35] *Centralblatt für die Med. Wiss.*, 1882, No. 4, p. 65 ; *see* also *Proc. Roy. Soc.*, vol. xxxiv. (1882), p. 150, etc.

[36] See *Proc. Roy. Soc.*, vol. xxxiv, 1882, p. 150, etc.

symptoms of being affected, except by a slight rise of temperature, not exceeding 100.0° Fahr. This experiment was subsequently repeated with the blood of another rabbit, similarly treated, and heated to 120° C. for one hour. Of this diluted blood, unheated, a minute quantity, less than the 100 millionth of a drop of the blood itself, was injected into a rabbit, to test its infectivity, the animal dying with the usual symptoms within 24 hours. Of the dilute superheated blood, 0 6 cubic centimètre (= 0.2 cubic centimètre of the blood) was injected into another rabbit, which remained for 20 hours apparently unaffected, but died in about 39 hours infected, and in its blood were found the specific bacteria ; it was found to be infective when inoculated into another rabbit in a small quantity. Another portion (0.6 cubic centimètre) of the same dilute alkaline superheated blood was therefore injected into another rabbit, which remained perfectly unaffected, and healthy as long as observed. As it appeared, from these results, that the methods of filling the tubes with the blood to be sterilised might be defective, another method was adopted. The blood of an animal just dead of transmitted infection, was diluted with three times the quantity of salt-solution, and rendered alkaline by 3 per cent. potassic carbonate ; portions of this were then put, by a capillary pipette, into tubes previously prepared, avoiding contamination of the sides, these were then drawn out at the end, boiled, and sealed whilst boiling. They were placed in the hot-air oven, heated to 100° C. for six hours, raised to 130° C. for one hour, and then gradually cooled. Of this alkaline dilute blood unheated, further diluted to a high degree, .00006 cubic centimètre was injected into another rabbit, which died infected in 27 hours ; in its blood were found the specific bacteria, and it proved infective on inoculation. Of the dilute superheated blood, 0.6 cubic centimètre (=0.15 cubic centimètre of blood) and 1.1 cubic centimètres (=0.275 cubic centimètre) was injected respectively into two other rabbits, which both remained unaffected in any way ; notwithstanding that they each received by injection several thousand times the quantity that, of the unheated blood, had proved fatal by infection to another animal. Hence it appears that both in the case of Davaine's septichæmia in rabbits, and of Pasteur's septichæmia in guinea-pigs, the contagium is destroyed by a sufficient degree of heat, and the fluids so treated are innocuous when injected into healthy animals in immeasurably larger quantities than those of the unheated which are sufficient for fatal infection, nor do they reproduce the micro-organisms which appear when infection occurs ; and that consequently the relations of these organisms to the contagium are not disproved by the experiments in question, as asserted by Professor Rosenberger *(loc. cit.)*. It must, however, be stated that it cannot be asserted that it is proved, by these experiments alone, that the organisms which are destroyed by heat necessarily constitute the active contagium; it is only asserted that this view is not hereby disproved.

In the next case, with respect to the statement of Professor Rossbach,[37] that small quantities of a germ-free chemical poison, injected into the blood of a living animal, enabled the microphytes therein present to develop more rapidly than in the case of actual infection, the following experiments were made.[38] I was previously acquainted with the remarkable properties of papaiin, an extract from the juice of the papaw-tree ; and I had found, as had others,[39] that if a solution of it be made, and kept in a warm place for some hours, it always develops a characteristic form of septic bacillus. On this occasion, solutions of the substance were made and placed in test-tubes in the incubator at a temperature of 98° Fahr. ; other tubes, previously pre-

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[37] *Centralblatt für die Med. Wiss.*, 1882, No. 5, p. 82.
[38] See the *Practitioner*, vol. xxx, May 1883, p. 254, etc.
[39] M. Wurtz, *Comptes Rendus de l'Acad des Sciences (Paris)*, t. 90, p. 1379.

pared, of sterilised blood-serum, and of bouillon, tested by incubation, were also inoculated with small portions of the solution, and placed with the others. After 48 hours, it was found that all the tubes, both those of the plain solution, and of the serum and bouillon inoculated with it, teemed with numerous micro-organisms—a form of bacillus somewhat characteristic, and spores or micrococci. Thus it was shown that a solution of papaiin is not germ-free, as supposed by Professor Rosenberger; but, on the contrary, that it readily develops saprophytes, and that, consequently, the conclusions sought to be established by the Professor in the paper referred to cannot be maintained. The following experiments, however, were made, further to examine the action of this substance on the animal organism. A 10 per cent. aqueous solution of the drug was made with all antiseptic precautions, and one cubic centimètre of it was immediately injected into the jugular vein of a young rabbit, previously anæsthetised. In about 50 minutes, respiration had ceased, but the heart continued to contract for about 40 minutes longer. Blood now taken from the heart was found to be more fluid than usual, and in great measure to have lost the property of coagulation; but neither when examined fresh by the microscope, nor in preparations dried and stained by the usual methods, were any micro-organisms whatever apparent; the red corpuscles were not materially altered, though the white were killed; but the small bodies generally known as Zimmermann's or Max Schultze's corpuscles were much more numerous than usual; these are probably merely disintegration-products of the red corpuscles; though, as shown by Riess,[40] their unusual abundance is indicative of a pathological condition. Further to test the sterility of the blood, a capillary tube was inserted into the cavity of the ventricle, the point broken therein, and a droplet of blood inoculated through plugs of cotton-wool, after Dr. Klein's method, into tubes of sterilised serum and bouillon; these, placed in the incubator, remained perfectly clear, and without organisms.

This experiment was repeated upon another rabbit with essentially similar results.

Thus it was shown on every ground that the conclusions sought to be established in the paper above referred to cannot be maintained; in no case has it yet been shown that microparasites are developed in the blood of a living animal by the injection of a germ-free substance; the experiments of Drs. Sanderson and Klein, above recorded, alone have shown that by such injections into the peritoneal cavity these organisms are developed.

Further Experiments upon the Occurrence of Micro-organisms in Healthy Animals.

Further to elucidate this question, more particularly with reference to the assertion of Professor Rossbach, that the pathogenic microphyte characteristic of Davaine's septichæmia exists normally in the blood of rabbits, the following experiments were made. A young healthy rabbit, killed by asphyxiation, was placed in the incubator for 24 hours, when it was found largely distended with gas, and decomposition proceeding rapidly. In blood from the heart and other organs, there was found a bacillus, though not in large numbers; it was active, some of the cells containing spores at one end; it was in all respects similar to the bacillus which occurs in Pasteur's septichæmia above described. No other form of microphyte was found, nor anything in any way resembling the specific bacterium of Davaine's septichæmia. The blood was examined fresh, and numerous preparations were also made, dried and stained, with the same result.

A similar experiment was performed with a guinea-pig, likewise killed and placed in the incubator for 24 hours, when a bacillus similar

[40] *Reichart and Du Bois Raymond's Archiv.*, 1872.

to that in the case of the rabbit was found in the blood, but no other organisms. Here it is to be remarked that, while, upon infection with Davaine's septichæmia, the specific micro-organism pervades the whole blood, far outnumbering the red corpuscles—and that sometimes within 12 hours—in these experiments this microphyte failed to develop in 24 hours in a dead animal kept at the temperature of the body; from this it appears clear that it does not exist normally in the blood of rabbits, and is a pathogenic organism. The absence of bacterium termo in the blood of these cases is to be remarked; in decomposing matter, either animal or vegetable, exposed to the atmosphere, it is usually the first form to appear; and in blood placed in the incubator, in any vessel, it occurs in vast numbers within a few hours.

Cultivation-Experiments.

The most conclusive proof of the pathogenic action of any micro-organism is, as generally recognised, by reproducing the infection after several generations of its propagation in an artificial cultivating medium. In the case of the bacterium of Davaine's septichæmia, I have inoculated it into many various nutrient fluids, serum of different animals, and several sorts of bouillon, as well as vegetable infusions. In blood-serum and in beef-bouillon kept at a temperature of 98° Fahr. for 24 hours, it generally germinated, rendering the fluid opalescent, but forming principally at the bottom of the vessel; with the first cultivation, however, it appeared to die out, and other fluids inoculated from these growths invariably remained sterile, the second generation or cultivation never germinating. The last series of experiments made on this subject was during the winter, throughout which I failed to get specific infection with this disease, and consequently was unable to try further methods of cultivation. Much the same appears to have been the experience of others in attempting to cultivate this organism.

With the bacillus of Pasteur's septichæmia, the result at first was similar, its germination, in all the nutrient fluids employed, being sparse and uncertain. As, however, it occurred in situations where there was no free oxygen in the fluids or tissues, it seemed probable that it might thrive in suitable nutrient fluids *in vacuo*, which method was said to have succeeded in France. For this purpose, tubes were prepared, some containing a little beef-bouillon, others serum of bullock's blood; these were plugged with disinfected cotton-wool, after Dr. Klein's method, and the bouillon was sterilised by prolonged boiling, the serum by heating in a water-bath to 65° Fahr. for some hours on several successive days, after the manner introduced by Professor Tyndall, and since practised and described by Dr. Koch. After this, they were inoculated by introducing a droplet of the infective œdema contained in a capillary pipette passed through the cotton-wool into the nutrient fluid; the tubes were then drawn out, above the plug of cotton-wool, exhausted by the Sprengel pump, sealed with the blow-pipe, and placed in the incubator at 98° Fahr. The next day, after 24 hours' incubation, the tubes, both of bouillon and of serum, showed, by their opalescence, that micro-organisms were developing freely; on opening some of them, the fœtid odour evolved from the tubes of both classes was remarkable; on the second day of the cultivation, other tubes were inoculated again from them, in similar manner, exhausted, sealed, and placed in the incubator. In this manner, four successive cultivations were made, the results in each being similar. In all, bacilli of the same form occurred, of similar character to those contained in the original infective œdema; like them, too, they were moderately active, and many of the cells contained true distinct spores. This spore-formation occurring *in vacuo* is remarkable; and, in the instance at least, of this particular species of bacillus, shows that the views which regard spore-formation in these organisms as universally

dependent on the presence of oxygen are erroneous. From the fifth cultivation, containing numerous bacilli, five drops were injected into the subcutaneous tissue of the abdomen of a guinea-pig, which was found dead with the usual symptoms of infection within 40 hours, but one-hundredth of a drop of the same cultivation inoculated into another animal failed to produce infection, though the quantity employed must have contained several million bacilli.

Methods of Microscopical Examination.

For the microscopical examination of the objects here in question, preparations were made by the well known methods of Weigert and Koch, often described. Some points, however, which I have found useful may be here mentioned.

In preparing the specimens of blood or other infected fluid, spread and dried on the cover-glass in the usual manner, I have found it desirable not to heat them, if at all, nearly so strongly as is usually done. Exposure for two or three minutes to a temperature of 80° C. is quite sufficient to "fix" the film on the glass. For this purpose, it is easy to have a hot-air or water-oven fitted with a Page's regulator, and adjusted to the given temperature. No trouble or loss of time is involved in this. If the object be merely to demonstrate the presence of bacteria, or where their chemical reaction is sufficiently distinctive, as in the case of Koch's bacilli of tubercle, the rough method of passing though a flame is sufficient; but when it is desired to examine the specific characters and form of a microphyte, a higher temperature than necessary is to be avoided. In some cases it certainly alters and distorts the bacteria; for the same reason, treatment with any strong reagent is to be avoided, even absolute alcohol, which sometimes contracts the cells. Nitric acid, in Ehrlich's method of preparing the bacilli of tubercle, as might be expected, distorts them very materially; although, in the particular object for which that method was devised, this is immaterial.

Preparations of blood or serum, however, may be made without heating at all, by merely spreading on the cover-glass, drying in the air, and staining either with an alcoholic, a glycerine, or a concentrated aqueous solution of one of the aniline dyes, and, when sufficiently stained, washing lightly by floating in water for an instant. But, as even drying in the air is liable to alter the character of the micro-organisms—bacilli, for instance, sometimes splitting up into the primitive segments—preparations, both stained and unstained, should always be examined fresh. They are easily stained, without drying, by adding to the fluid on the slide a little of an aniline dye dissolved in some neutral fluid—normal salt-solution, or dilute glycerine—placing the cover-glass upon it, and, when sufficiently stained, running in slowly the plain preservative fluid. Different species of the schizomycetes require different methods of staining, as is exemplified strikingly in Koch's remarkable discovery of the tubercle-bacillus. I have, too, found other forms which do not stain by any of the usual methods. The species, however, which occur in the two forms of disease here in question, both stain readily by several of the aniline dyes. I have used, in general, aqueous solutions of methyl aniline violet, or preferably, in some cases, when it is desired to avoid colouring the other elements of the preparation, magenta. These preparations I have always mounted in Canada balsam; those stained with the aniline salt named, I have not found to fade, if not exposed to the light, when preserved in this medium, as is often said to be the case; neither will they run if sufficiently washed in alcohol, and passed through oil of cloves. The risk of this, however, may be avoided altogether by diluting the balsam with benzine instead of with chloroform or turpentine, or by using it undiluted and liquefied by heat. I

have not found acetate of potash, which is often recommended, a good preservative medium for these permanent preparations; in it they are liable to fade, and the bacteria to become materially altered in size and form by the osmotic action of the salt.

The importance of discriminating the microscopical characters of these pathogenic micro-organisms is now universally recognised, and has given an increased value to the use of the highest powers and means of measurement. In many cases, their dimensions afford the readiest means of distinguishing between different species,[41] as, *e.g.*, between the micro-organisms here described, and b. termo. For the measurement of objects of this size—less than 0.001 mm. ($\frac{1}{25000}$ inch)—instruments of the most perfect construction are requisite. I have here found great advantage in the use of a cobweb eye-piece micrometer, admirably made for me by Messrs. Ross, which has the second web movable, so that it can be readily brought into accurate contact with any object to be measured, while, in the usual form, it is most difficult to bring the object itself, by means of the mechanical stage, or still more of the hand, into contact with the fixed web. With any eye-piece micrometer, it is of course necessary to determine the value of the scale by means of a stage-micrometer; in using high powers, this must be done afresh for each series of observations. Of the stage-micrometers of English and continental construction, I have found that some differed amongst themselves to a considerable and serious extent. A standard micrometer, however, of the most beautiful and accurate construction, has lately been made by Professor W. E. Rogers, of Cambridge, U.S.A.,[42] one of which I have used; and as another has just been presented to the Royal Microscopical Society, it will no doubt be easy in future for microscopists to compare their own instruments, and ensure accuracy.

Both for the measurements and for the examination of ultra-minute objects, a great advantage will be found in the object-glasses (homogeneous immersion) of wide aperture, lately constructed by Messrs. Powell and Lealand. In these investigations, I have myself used their recently constructed $\frac{1}{25}$th homogeneous immersion-objective of the very wide N.A., 1.38 (balsam-angle 130°), which gives with a $\frac{3}{4}$-inch eye-piece an amplification of fully 3,400 diameters; with suitable illumination, of the most perfect definition, showing details of structure certainly not to be distinguished by the powers generally in use. The value of wide aperture in object-glasses, for resolving certain classes of objects, whether or not desirable for all purposes, is now pretty well known and understood through the clear explanations of the subject recently published by Professor Abbè.[43]

Those who have used the latest production of the makers above-named, viz., the $\frac{1}{12}$th oil-immersion of the highest N.A. yet constructed, or perhaps obtainable (1.47) will have fully recognised the value of this quality.

I may here remark, with regard to the value of the morphological characters of these organisms as a means of diagnosis, that, whatever may be the fact as to the occurrence in them of transformations of form, modifications in which, under different circumstances, as for instance in different cultivating media, certainly do occur; yet, under similar conditions, as in the blood and like tissues of animals of the same species, they are, to my observation, absolutely invariable, and afford the most valuable means of diagnosis. The *bacillus anthracis*,

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41 An instance of this is afforded in Dr. Klein's investigation of swine fever, *Qr. Jour. of Micros. Science*, vol. xviii, 1878, p. 170, etc.

42 Described in the *English Mechanic*, also in the *Journal of the Royal Microscopical Society*, 1882.

43 *Journal of the Royal Microscopical Society.*

44 *Rep. Med. Off. Loc. Gov. Board*, 1881-82, p. 166, etc., and *Quarterly Journal Micros. Science.*

the most carefully studied of pathogenic micro-organisms, in artificial cultivation is of variable size and form, as lately described, more particularly by Dr. E. Klein[44] and M. Toussaint[44*] ; but no reliable observations have ever asserted that its characters are not perfectly constant in the blood and tissues of animals of the same species.

Conclusions.

From this investigation I conclude that in Davaine's septichæmia in the rabbit, the bacterium therein always found in the blood does constitute the active contagium, in the strictest sense of the term ; it answers the three conditions laid down by Koch as the test for a true microparasitical disease,[45] viz., it is invariably present in all cases of infection ; it occurs in such numbers as will account for the symptoms ; and further, it alone is present, without the occurrence of any other organism. Davaine's statement of the minimal quantity in which the blood, in these cases, is infective, was in the main correct ; as I have shown, it is so almost invariably in quantities of the hundred-millionth of a drop ; in much smaller quanties its action is uncertain, though in one case I found the four thousand-millionth of a drop fatal. This degree of infectivity corresponds to, and depends upon, the numbers in which the micro-organisms occur in the blood ; for though, as already stated, it is impossible to enumerate them accurately, they are generally nearly equal to the red corpuscles, and in some cases outnumber these latter at least ten times ; but their distribution is so irregular, that scarcely two preparations of the same portion of blood can be made, in which their numbers are similar, nor can we conclude that each and every individual cell is capable of reproduction in the tissues of the living animal, the antagonisms of t he cells of which it has to overcome ; while, even in artificial cultivation, Professor von Nägeli[46] has shown the influence that the quantit y of the infected fluid, or the number of micro-organisms used for inoculation, has on the result, the same thing may obtain here in some degree, so that the quantities in which this blood is found to be actually infective appear to correspond well to, and to be dependent upon, the numbers in which the microparasite is therein present. With regard to the statement of Davaine, that the blood in these cases was infective in the trillionth or quadrillionth of a drop, or less, it is to be remarked that, though it can scarcely be constantly infective in such quantities, as was generally understood to be the conclusion from Davaine's statement, it may well be so in certain cases, or, indeed, in any conceivable quantities obtained by successive dilutions.

In infection originated by putrid blood, the action of which is uncertain and variable, as has been shown, the circumstances observed are to be accounted for by the infectivity here, too, being dependent upon the presence of the specific micro-organism, due to atmospheric contamination. It is clearly shown not to depend upon the stage of decomposition ; for, when this is accelerated by keeping it at an artificial temperature, it does not become more quickly or more certainly infective, and, indeed, in many cases, it never does so, whatever the temperature. This circumstance of its uncertain infectivity is quite conformable to the view that it is dependent upon the presence of the specific micro-organism, which sometimes does, and sometimes does not, occur in it. Further, the vast difference between the virulence of such septic—putrid—blood, and that of septichæmia, in subsequent transmitted infection, is explained by microscopical examination, which shows that the specific micro-organism, when present in the former, is only so in very small numbers, and amongst numerous other forms with which it has to

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[44*] *Recherches sur la maladie charbonneuse* (Lyons, 1879).
[45] *Untersuch. über die Ætiol*, etc., p. 27.
[46] *Die Niederen Pilze*, etc.

contend for existence ; whereas, in the latter case, as already shown, it is present in countless numbers, and without any such competitors.

In the case of "Pasteur's septichæmia" in rodents, it appears to me that it cannot be considered a true microparasitical affection, though the bacillus which characterises it is usually present in large numbers, and the toxicity of the fluids is destroyed by heat, they are not infective or toxical in minimal quantities at all comparable to the former case, or to that of anthrax. In all cases without exception, when I have employed much smaller quantities than about .01 cubic centimètre for inoculation, and frequently with larger quantities, infection has failed, though millions of the microphyte must often have been present ; cultivation-experiments, too, as far as they went, showed the same thing. I can only conclude that here the products of inflammation—the œdematous fluids or exudation—possess in themselves a toxical and pathogenic action, though this effect is, no doubt (Dr. Burdon Sanderson), modified by the action, perhaps merely mechanical, as some circumstances seem to show, of the micro-organism present in them. It must be remarked, too, that the microbe here in question, under some conditions at least, as in cultivations *in vacuo*, is distinctly a septic organism, exciting putrid fermentation of the most marked character in the fluids which contain it, in strong contrast to the organism in Davaine's septichæmia, which is merely pathogenic, and not, as far as observed, zymotic or septic (*i.e.*, causing fermentation or putrefaction). In the case of this bacillus, I have been able to discover no constant microscopical difference of character whatever, under similar conditions, between it and the most common and ubiquitous septic organism, the bacillus, which appears so readily and universally in nearly all and every decomposing substance, even more frequently and universally than b. termo, and is of far greater persistence.

Further, it has been rightly stated by Koch that the bacillus present in this disease—which he appropriately terms "malignant œdema," and the micro-organism itself, *bacillus œdematis*—occurs universally in the soil, from the droppings of animals, etc. ; so that, in the common acceptance of the term, it can only be regarded as an ordinary saprophyte.

With respect to the question more immediately proposed in this investigation, I conclude that, neither in the case of Davaine's septichæmia, nor in that, so termed, of Pasteur, in rodents, is there any increase of virulence in the infective fluids in successive inoculations. In the former disease, in the first case of transmitted infection, the blood is virulent in incomparably smaller quantities than in the original inoculation with septic—putrid—blood ; but, subsequently, there is not, nor indeed, for the reasons already stated, can there be constantly, in any succeeding generation up to the tenth, to which I carried it in the experiments here recorded, any further increase of virulence, either as shown by the duration of the so-termed inoculation-period, or by the minimal quantity requisite for infection. The misconception on the first point has apparently arisen from septichæmic blood, *i.e.*, blood in the cases of transmitted infection, being virulent in infinitely smaller quantities than in the case of originating infection by putrid blood ; and next from all former writers on the subject having followed Davaine's first recorded experiments, and worked with constantly diminishing quantities of blood, without any control-experiments, to ascertain the minimal quantities in which such blood was already infective. In regard to the conclusions of MM. Coze and Feltz, that an increase of virulence was shown by a successively diminishing incubation-period, the experiments here recorded show that not only is there absolutely no such diminution (after the first case), but that, in Davaine's septichæmia, this period in transmitted infection is constant in a very

remarkable manner; in the large majority of cases, including very numerous experiments not here recorded, varying only between about twenty-three and twenty-five hours. In those exceptional cases where the duration of this period materially exceeded this time, it was probably—in some instances, it appeared to me, obviously—due to the constitutional vigour of the animal, its individual power of resisting infection; to some degree it may be due to the greater age of the animal, though it was endeavoured to avoid this cause of disturbance as much as possible, by always using animals only of about the same age, viz., nearly full grown; the younger the animal, as is well known, the greater being the susceptibility to infection and the more rapid its course. Another cause which in some cases in my record of experiments contributed to make this period appear to be longer than it actually was, is that, where the animal was not actually observed to die before evening, it was not seen again till the next morning, when it may have been dead several hours.

Neither in the case of Pasteur's septichæmia, or *infective peritonitis—the malignant œdema* of Koch—is there any increase of virulence in successive inoculations after the first. The quantities requisite for fatal infection or intoxication are here infinitely greater than in the case of Davaine's septichæmia, and the result is far less certain, in one case 0.02 cubic centimètre being fatally toxical, in another, and that one of a subsequent generation, 0.05 being innocuous. In no case, however, in any successive generation up to the sixth of transmitted infection, was a materially smaller quantity than in the first found to be toxical. The period between inoculation and death, likewise, is here more variable than in the case of Davaine's septichæmia, dependent, in some measure at least, upon the different constitutional vigour of the animals; but there is obviously no constant diminution of this period.

This investigation, as I am fully aware, is very incomplete, leaving many essential points undetermined, and that more particularly in the case of Pasteur's so-termed septichæmia, or infective peritonitis, which, from the circumstance of its being the only instance as yet recorded of a microparasitical infective disease which can be excited by the action of a germ-free chemical irritant—deserves the most careful further investigation, with the view of elucidating the exact relations existing between these micro-organisms and the virulence of the fluids in which they occur. In this affection, I can only claim to have shown that, as in the former case, there is here no increase of virulence in successive inoculations, and that, consequently, with reference to the particular object with which this investigation was undertaken, it lends no support whatever to the doctrine of a transformation of physiological species, either through an "adaptive increase of virulence," or in any other way. The question of the occurrence of any such change is still, as it has been lately pronounced to be by Professor Virchow,[47] a fundamental one in the subject of the relations of these organisms to disease: to its elucidation and the avoidance of some errors previously made, the vastly improved and improving appliances of the microscope, more especially its highest powers now so greatly perfected, should be brought to bear more assiduously than hitherto in the requisite preliminary discrimination of the specific morphological characters of these micro-organisms.

In conclusion, I must express my sincere thanks to the British Medical Association for the assistance liberally afforded, through the Scientific Grants Committee, from time to time during this investigation.

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[47] *Address to the International Medical Congress*, 1881, Pathological Section.