On the infectivity of the blood and other fluids in some forms of septic disease, and the reputed occurrence therein of an increase of virulence in successive inoculations / by G.F. Dowdeswell ; communicated by M. Foster.

From the PROCEEDINGS OF THE ROYAL SOCIETY, No. 223, 1883.]

"On the Infectivity of the Blood and other Fluids in some Forms of Septic Disease, and the reputed occurrence therein of an Increase of Virulence in Successive Inoculations."

By G. F. DOWDESWELL, M.A. (Cantab.), F.L.S., F.C.S., &c.

Communicated by Dr. M. FOSTER, Sec. R.S. Received January 15, 1883.

The remarkable fact that in some cases the blood of an animal, intoxicated with putrid matter, becomes itself "infective," capable of

reproducing in other animals those symptoms which have occurred in it, though previously asserted by others, was first conclusively demonstrated in 1866 by MM. Coze and Feltz in France,* who further stated that in successive inoculations from one animal to another of the same species, such blood acquires a progressive increase of virulence, becoming more toxical than putrid matter itself, and that death follows inoculation with increasing rapidity. These observations were confirmed and greatly extended by Davaine,† in long investigations; hence the form of septichæmia here in question has since been known by the name of the latter observer. The first-mentioned writers founded their assertion of an increase of infective virulence upon the alleged fact that in successive inoculations‡ the incubation period was progressively shortened; whereas Davaine sought to show, by numerous experiments, that in the same manner successively smaller quantities of blood were required to create infection; that whereas to originate it some drops were necessary, ultimately, after several generations of transmitted infection, the billionth (in the French notation the trillionth) part of a drop, or less, was sufficient; but he regarded, indeed defines, septichæmia as a putrefaction of the blood in the living animal, a view naturally encouraged by the decomposition which in these cases occurs so rapidly after death.

Davaine's experiments were repeated by many observers, both in France and elsewhere.§ It is remarkable, however, that they one and all contented themselves with merely reproducing his earlier observations, inoculating in succession several animals with constantly diminishing quantities of blood, but without making any control experiments to ascertain whether in the first stages the blood was not already infective in the degree supposed to be attained only after several generations, and overlooked the fact that, in his later writings‖ Davaine had qualified his first statements by showing that a maximum of infectivity was attained in the earlier generations. These conclusions and the theory of an increase of infective virulence in successive inoculations have since been generally accepted; but the question here

* "Recherches Cliniques et Expérimentales sur les Maladies Infectieuses, &c.," Strasbourg, 1866. and Paris, 1872.

† "Comptes Rend. de l'Acad. des Sc.," Paris, Feb. 1, 1869, *et passim.* Also "Bull. Acad. Méd.," Paris, 1872, p. 907, &c.

‡ Or, as it was termed, "generations," with reference to the microphytes found to be present in the blood of these cases, which were regarded as constituting the active contagium, and which by propagation in the blood of living animals, it was said, acquired "renewed vigour" in successive generations. I have retained this term "generations" as convenient, but without here implying anything more than successive inoculations from one animal to another.

§ As by H. Dreyer, "Archiv. f. Expt. Path. u. Pharm." 1874, Bd. II, s. 149, &c., and Clementi and Thin.

‖ *Loc. cit.*, "Bull. d. l'Acad. de Méd.," 1873, p. 124, &c.

involved has recently acquired fresh importance in reference to the relations of the lower fungi to disease, and the occurrence in them, as lately asserted by Dr. Hans Büchner,* P. Grawitz,† and others, of a transmutation of physiological species under altered conditions.

In observations recently made upon septichæmia in the mouse,‡ and other experiments, I obtained results which seemed to me to negative the occurrence of any progressive increase of virulence in the blood in successive inoculations, or of any transformation, physiological or morphological, in the organisms present, an opinion which was confirmed and the question discussed by Dr. R. Koch,§ in a work recently published.

In the case of the mouse, from the small size of the animal and the blood in most cases being more or less coagulated when examined, it was not easy to obtain accurately measured minute fractional quantities, by successive dilutions, for the purposes of experiment; for which reason, and also as it was with reference chiefly to the blood of the rabbit that the statements had originally been made, I selected that animal for the investigation of this question by the following methods.

To originate infection in the same manner as in other experiments on the same subject, putrid blood was used, generally that of the ox; but I have also employed that of the pig, sheep, &c., or of the rabbit itself, all with similar results. Of such blood, diluted with an equal bulk of normal saline solution and strained, a few drops were injected into the subcutaneous tissue of the back or abdomen, by means of a Pravaz syringe. In the subsequent high dilutions five or ten drops were generally injected, and these comparatively large quantities were employed in order that there might be less chance of error in the quantity of blood actually received by the animal, than would be the case with smaller injections, *e.g.*, of one drop, as used by Davaine; though his method probably reduces the liability to formation of abscesses at the site of injection, with the accompanying failure of infection, which, however, only occurred to me in a few instances. In these experiments, where it was requisite to guard against the possibility of accidental infection or contamination, instead of a syringe I used a glass tube drawn out to a fine point; this is readily inserted under the skin of the back in young animals, still more so under that of the abdomen. When the quantity to be injected requires to be accurately measured, it is easy once for all to calibrate the tubing by the usual method, and extemporise a graduated pipette, containing a given quantity, between two marks of the file.

* "Sitzber. k. B. Akad. Wiss. zu München," 1880.

† Virchow, "Archiv.," Bd. 81, H. 2, s. 355.

‡ "Qrly. Journ. Micros. Sc.," N.S., No. 85, January, 1882, pp. 66–75.

§ "Untersuch. ü. d. Ætiol. d. Wundinfectionskrankheiten, &c.," Leipzig, 1878.

The symptoms which are observed after the injection of small quantities of putrid blood into a rabbit, when primary infection occurs, are very constant, and similar in most essential respects to those in the subsequent cases of transmitted infection, excepting that the inflammation at the seat of injection is more severe and extensive in the former than in the latter case, owing no doubt to the comparatively large quantity of septic* matter used. The period of incubation too is here very variable, in accordance with the uncertain toxicity of putrid blood; its duration is usually from twenty to forty, or sometimes sixty, hours, but if in specific infection an animal survives the latter period, to my experience, it invariably recovers. Putrid blood, however, in the quantities here used may be toxical, whether fatally or not, owing to the chemical poison it contains in solution, the sepsin of Panum, Bergmann, and other writers, whereas in subsequent cases of transmitted infection, when infinitely smaller quantities of the blood itself are used,—the thousandth or the millionth of a drop or less,—it is either fatally infective or, where not so, no symptoms of disturbance can be recognised. In these latter cases the incubation period is remarkably constant, being in the great majority of instances from twenty to twenty-four hours.

Beyond an extreme coagulability, which in this specific disease I have found invariable, in this differing from the observations of others, and frequently a great increase in the number of the white corpuscles of the blood, I have not recognised any constant change in the characters of that fluid nor in the form of the red corpuscles, as described and figured by Coze and Feltz, and as stated by others, in this case; and it seems to me that the appearances there described are often rather those which occur in normal blood from the methods of preparation, exposure to the air, &c., than constant pathological features. These authors, however, describe (*op. cit.*, p. 67) filamentous processes developed from the red corpuscles, of the nature of which they are uncertain, and conjecture that they may be parasitical micro-organisms. This statement of theirs has not been noticed, as far as I know, previously. I have observed the same thing, as already recorded,† in the blood of septichæmic mice. These bodies, which I have investigated and fully described elsewhere,‡ are mere processes developed from the stroma of the red corpuscles. They may be produced artificially and are indicative of a pathological condition of the blood.

The rapid coagulation of the blood upon death, often within a

* I here use the term "septic" in its proper signification of "causing putrefaction," or accompanying it.

† "Qrly. Journ. Micros. Sc.," *loc. cit.*, p. 69.

‡ *Ib.*, Vol. 25, January, 1881.

very few minutes, rendered it necessary to obtain it with the least possible delay. In the latter cases, *i.e.*, of transmitted infection, where the period of incubation is constant within a few hours, it is easy to watch the death of an animal and examine it immediately; but in the case where it is originated by putrid matter, this period varies within comparatively wide limits, death perhaps occurring during the night or when not expected. It is then more difficult to obtain it before coagulation occurs, and hence several failures were experienced in endeavouring to prepare successively diminishing quantities of the blood of this generation by fractional dilution.

Experiments.

Infection was originated in a young rabbit, No. 1, after some previous failures, by injection under the skin of the back of 3·0 gtt. of putrid bullock's blood, diluted, the animal was found dead forty hours after inoculation, with the appearances mentioned as usually occurring in these cases. Around the site of injection was observed diffuse hyperæmia, with extravasation of blood from the small vessels, a marked induration and discoloration of the subcutaneous tissue at the same spot, which appearance I have found invariably whenever infection occurred. There was no œdema, nor, beyond congestion, was any change in the spleen or other organs apparent. The blood on examination was much coagulated; in that of the heart were found, though irregularly distributed, numerous micro-organisms, a form of bacterium hereinafter more particularly referred to. These were not found in this or subsequent cases in any of the organs or other tissues, but as they are minute, and even in the blood when unstained, are difficult to recognise, in other tissues they may easily escape observation. The case of this rabbit so intoxicated by putrid blood, I have, in accordance with the phraseology of Davaine and other previous writers, termed the first generation of infection, though obviously for the reasons referred to, it would be more strictly accurate to designate such animal, as itself, not infected but poisoned, and originating infection.

Another rabbit, No. 2, thereupon received in like manner by injection, one drop diluted of the blood of No. 1. It died within twenty-four hours; blood from its heart was immediately diluted to different degrees, and ten drops of the various dilutions were injected into five other rabbits, all of similar size and condition, so that they received respectively the following quantities of blood, viz.:—No. 3, $\frac{1}{10}$th gtt.; No. 4, $\frac{1}{100}$th gtt.; No. 5, $\frac{1}{1000}$th gtt; No. 6, $\frac{1}{10000}$th gtt.; and No. 7, $\frac{1}{100000}$th gtt. No. 3 died in 25 hours, No. 4 in 24 hours, No. 5 in 25 hours, No. 6 within 40 hours, and No. 7 in 27 hours; all, with the exception of No. 6, in my presence, and all with very similar symptoms; thus showing (1) that in this form of septichæmia the

period of incubation is in nowise proportionate to the quantity used for inoculation, and (2) that in the so-termed second generation of infection, septichæmic blood is already infective in the $\frac{1}{100000}$th of a drop. Infection was then transmitted through the fourth, fifth, and sixth generations, by inoculation with similar small quantities and like results, the animals dying respectively within 23, 48, and 20 hours. The blood of this latter—the sixth generation—was diluted to different degrees, and ten drops of the solution injected into four other rabbits, which received—No. 11, $\frac{1}{100}$th gtt.; No. 12, $\frac{1}{10000}$th; No. 13, $\frac{1}{100000}$; No. 14, $\frac{1}{10000000}$th of a drop. These died all in my presence in, respectively 20, 26, 25½, and 20 hours, showing in addition to the first point above-mentioned, that there is no appreciable shortening of the incubation period between the second and the sixth generation. As the blood here proved to be infective in the ten-millionth part of a drop, it was now necessary to determine the limits of infectivity in the first generation.

Again five drops of putrid bullock's blood, diluted with an equal bulk of saline solution, were injected into a rabbit, No. 15, which died within 48 hours; its blood was diluted as before, and ten drops injected into each of five other rabbits, so that they received—No. 16, one-thousandth; No. 17, one hundred-thousandth; No. 18, one-millionth; No. 19, one ten-millionth; and No. 20, one hundred-millionth of a drop. No. 16 died in 24 hours, Nos. 17 and 18 died within (that is, were found dead in) 35 hours, and No. 19 within 48 hours; No. 20 was apparently unaffected; subsequently, however, it lost flesh, though continuing to feed, without any material rise in temperature or the occurrence of microphytes in the blood, and a small abscess was found at the spot of injection, which suppurated and healed spontaneously in about eighteen or twenty days, and the animal recovered.

From this series of experiments it appeared that the blood of an animal poisoned by inoculation with putrid matter, in the so-termed first generation, is infective in less than the millionth part of a drop, and the considerations mentioned below showed that it was useless attempting to investigate the limits of infectivity beyond this point; but in order to ascertain whether there occurred subsequently any appreciable shortening of the incubation period, as was considered to be the case by MM. Coze and Feltz, infection was transmitted in succession up to the tenth generation, in which the blood of a rabbit that had died septichæmic, was again diluted as before, and ten drops, each of the same dilutions as in the last-recorded experiments, injected into four other rabbits, one-thousandth, one-millionth, a ten-millionth, and a hundred-millionth of a drop respectively into rabbits Nos. 21, 22, 23, and 24, which died, No. 21 in twenty-one hours, while the other three survived upwards of twenty-seven, but died during the night within

forty hours; here obviously there is no appreciable shortening of the incubation* period; but the instances recorded show how constant in the large majority of cases this period is, the quantity used for inoculation is absolutely without any influence upon it, whether some drops, or the hundred-millionth of that quantity is used; it is even seen frequently that inoculation with materially smaller quantities of the same blood produces death in a shorter period than larger quantities do, in such cases it would appear that the result can only be owing to a difference in constitutional vigour, the power of resisting infection, in the animals experimented upon. It must be remarked, too, that the apparent variation in the length of the incubation period as recorded herein, appears sometimes greater than was actually the case, for when an animal died, *e.g.*, during the night, it is recorded as having occurred "within" a certain time, whereas it may have occurred, and in some instances certainly did so, some hours previously; if the death of all the animals had been actually witnessed, this period would appear even more uniform than it does here.

In all these cases the appearances on death above mentioned were observed without material variation, and in every case there were found in the blood large numbers of a microphyte which is very characteristic and distinctive; yet although some previous writers have clearly recognised its presence, regarding it as constituting the active contagium and *materies morbi*, no accurate account of its microscopical characters has been given, nor its direct relation to the infective virulence of the blood which it infests considered.†

This microphyte is somewhat minute and in fresh preparations

* I have here used the term "incubation period" in the same manner as done by Davaine and others, not in its proper sense of denoting the period between infection and the first appearance of any symptoms of disturbance, but in reference to the duration of the malady between inoculation and the death of the animal; this, though not strictly correct in the proper signification of the term, may, perhaps, be excused, inasmuch both as the first occurrence of any constitutional disturbance is not well marked nor easy to recognise, in the animals the subjects of these experiments, and also as the period between the appearance of such symptoms and subsequent death is, in ordinary cases, very short, seldom exceeding a few hours.

† MM. Coze and Feltz, indeed, described and figured a microbe which they found in the cases they examined. If their figures are drawn to scale, this cannot be the same bacterium here in question, or otherwise the figures are erroneous, for they represent a form fully double in breadth that which I have obtained, and more nearly resembling the common septic ferment, *B. termo* (Cohn), than any other species with which I am acquainted. If, however, these cases were examined immediately after death, as stated, this form could not have developed, being a septic and not a pathogenic species, nor capable of multiplying or subsisting in the tissues of the living animal. It must, however, be remembered that at the time of their observations, nearly twenty years since, the available microscopical appliances were greatly inferior to what we now possess.

difficult to examine; it is a form of Bacterium, but when stained is readily recognised by the size of the cells, which is but little variable, and by its form, which is distinctive.* The numbers in which it appears in the blood in different cases, in various portions of the same blood, or even in different parts of the same preparation, are very variable; this appears to arise in some measure from a disposition to agglomerate together in places, and may be due to the tendency of the blood to coagulate. To enumerate these organisms in unstained preparations is impossible with any of our present microscopical appliances, as they cannot be sufficiently clearly distinguished, and in dried and stained preparations, not knowing the depth of the layer of blood, it can only be done by comparison with the number of the red corpuscles, obviously a rough and uncertain method. In some cases, however, they are fully ten times as numerous as the red corpuscles, and taking the number of the latter at about 5,000,000 to the cubic millimetre, we have of the former, 50,000,000, or in a drop upwards of 3,000,000,000, which corresponds as nearly as could be expected from their variable numbers and irregular distribution to the minimal quantities in which I have found the blood to be infective, viz., almost invariably in the 100 millionth of a drop;† in much smaller quantities its action is uncertain, in correspondence with the view that the microphyte does constitute the active contagium; for in that case, or if the contagium be particulate, whatever its intimate nature, to whatever degree the fluid in which it is contained may be divided by successive dilution, it is evident that any given portion may, and some one or more portions must, contain the infective particle; and hence that to determine the least quantity in which it is constantly infective is impracticable. From the dimensions of the organisms and the numbers that can be comprised in a given space,‡ it is evident that blood containing them cannot be constantly infective in the quantities stated by some observers, viz., in the trillionth of a drop or less, yet it might be so exceptionally, and consequently the original statement of Davaine may have been strictly correct, in the instances he has recorded, though the inference usually drawn from them is erroneous.

In regard to the origination of infection by putrid blood, in several series of experiments, I have found that during the summer and

* Its characters I have already described ("Journ. R. Micros. Soc.," 1882, vol. ii, p. 310): it is easily distinguished from *B. termo*, which it superficially resembles, by its size; being but half the breadth (0·5 μ), and by its form; the cell-wall not being constricted in the centre as in the latter species.

† In one instance I found 10 gtt. of the blood diluted 10,000,000,000 times fatally infective within about the usual period.

‡ Viz., as already shown ("Journ. Roy. Micros. Soc.," 1882, p. 311), 250,000,000,000 in a drop taken as the sixteenth part of a cub. centim.

autumn months, it is generally obtained in two or three trials; in the winter I have also obtained it; but during the present winter (1882) I have failed in numerous attempts. In putrid blood, not too old, a large number of septic microphytes of many different forms may be observed; in cases when such blood proved infective, I have sometimes, though not always, been able to recognise the specific organism herein described as invariably occurring in the blood of infected animals, and apparently constituting the actual contagium of the disease, though they were never numerous; but in these cases when the blood did not prove specifically infective, I never in one instance could recognise them, often as they were sought for. Davaine states that blood taken fresh and kept at a temperature of about 38° C. for forty-eight hours,* becoming putrid, is as virulently infective as blood in the later generations of transmitted infection, in the rabbit, viz., in the hundred-millionth of a drop or less. In several experiments, made at different times and places, I found that such blood, though rapidly developing a variety of septic bacteria, was not specifically infective in any case, even when injected in quantities of some drops; neither in such blood did I ever find the specific organism, although sometimes, and in one instance most conspicuously, there were present a large number of bacteria proper, very similar in appearance to the specific organism here in question, from which in fresh or unstained preparations it is somewhat difficult to distinguish, but when stained after the usual methods they were found on measurement, to be fully double the size in breadth of the others, and with a perceptible difference in form. But few other species were observed in these cases; that which was present is the *B. termo* of Cohn, usually the first to develope in putrefying animal or vegetable matter, they very shortly disappear and are replaced by other forms. Not having succeeded during these latter experiments in getting infection, I was unable to try the effect of inoculating fresh blood from the infection, and then incubating or artificially putrefying it.

The fact above referred to as established by MM. Coze and Feltz, and Davaine, was in the year 1872 further extended by the experiments of Drs. Sanderson and Klein,† in this country, who showed that by injection into the peritoneal cavity of an animal, either of various pathological products or, ultimately of a chemical irritant, itself free from living organisms, or even parasiticidal, and with antiseptic precautions, an inflammatory affection was induced, the exudation products of which, always abounding in micro-organisms, produced on inoculation into other animals similar symptoms, with the recurrence of the same microphytes. Further, it was thought that in

* "Bull. Acad. Méd.," Oct. 8, 1872.

† "Med. Chir. Trans.," vol. lvi, p. 345, &c.

this case, too, there occurred an increase in the infective virulence of the pathogenic fluids, in successive generations of transmitted infection, similarly to the case considered hereinbefore. The remarkable circumstance here shown of the origination of an infective bacterium-containing product by means of a chemical irritant, seems to have been since lost sight of in reference to its bearings on the question of the relations of micro-organisms to infective disease. The affection was first described under the title of the "Infective Products of Inflammation;" but recently a similar disease has attracted attention under the designation of Pasteur's septichæmia, a term which is decidedly a misnomer,* the blood neither in the living animal nor shortly after death, to my experience, being in anywise infective or septic. I have found in numerous experiments made since those herein recorded, some of which have lately been communicated to the Royal Society, that these two forms of disease, which may be originated by various methods, are essentially the same, inasmuch as they are interchangeable at will, merely by altering the place of injection, the pathological symptoms in both are similar, only differing in extent and severity; the serous exudation in each has a very similar character, though the subcutaneous œdema is more constantly highly coagulable than the peritoneal exudation, the period of incubation after inoculation is similar, and the micro-organisms which occur in each are identically the same, vaguely as they are characterised by recent French writers.†

Guinea-pigs were used in this case, healthy animals being selected; for comparative experiments young and as much as possible of the same size. To originate infection, a small quantity of a dilute solution of ammonia was injected by means of a Pravatz syringe into the peritoneal cavity of one of these animals; the water used was previously boiled, the syringe was new, rinsed out with boiling water, and the vessels employed were disinfected by heat. In subsequent experiments with infective fluids the syringes after use were treated first with boiling water, then with a strong solution of potassic permanganate, and again washed in boiling water; that these measures were sufficient to destroy infection was proved by several experimental injections with these syringes of normal saline solution, which in every instance were innocuous. I have found guinea-pigs as well suited as rabbits for experiments on this affection, and although here, as is shown, the period of incubation is somewhat less constant

* It has since been styled by Dr. R. Koch more appropriately "malignant œdema."

† M. Pasteur, however, shows that he is familiar with the microscopical characters and measurements of these organisms, "Bull. Acad. Med.," 1881, p. 97, and from this passage and other circumstances it seems to me shown that the "new disease" therein referred to as a form of rabies, is identical with Davaine's septichæmia.

than in the case of Davaine's septichæmia in rabbits, above recorded, yet it was found that when infection occurred death always followed within about thirty or forty hours; some few instances occurred in which animals that had been inoculated died subsequently to this period while under observation, but in such cases it was found that this was the result of accidental causes and not of infection. In some cases where infection failed and the animal was obviously in good health, as evinced by there being no loss of weight or of appetite, it was, after the lapse of two or three weeks, used for other experiments; it is quite contrary to my experience in this case or in that of Davaine's septichæmia, that an animal inoculated should die of septic infection after the lapse of several days or some weeks, as has been recorded by other observers. In the experiments on transmitted infection the septic fluids employed were always diluted with normal saline solution recently made and freshly boiled: in cases where moderate quantities—two or three drops or upwards—were used, equal parts of each were taken; when much smaller quantities were required, they were obtained by the method of "fractional dilution." In all cases the quantities given as having been used for injection apply to the actual quantities of the septic fluid and not to its dilution.

Experiments.

0·3 cub. centim. of a dilute solution of ammonia was injected into the peritoneal cavity of a guinea-pig which, when examined the next morning, had apparently died some hours previously, decomposition of the viscera and abdominal wall being far advanced, destructive inflammation around the site of injection had occurred, and there was a considerable quantity of peritoneal exudation, containing red blood-corpuscles, much altered; some pus or leucocytes, mostly largely vacuolated, with a great number of Bacilli, and spores or Micrococci; the former actively mobile, *i.e.*, they possessed the power of independent movement, while the spores or Micrococci were merely affected by the Brownian movement.*

In the lower layer of the tissues of the abdominal wall were found in places several Bacilli and some few Micrococci or spores; these occurred mostly in the connective tissue and between the muscular fibres; none were found in the skin or subcutaneous connective tissue, either here or in any case of the intra-peritoneal injection of a chemical irritant; the puncture in the skin and abdominal wall by insertion of the needle of the syringe had produced but very slight,

* Micrococci, however, are sometimes actively mobile; but in many cases where moving bodies seen under the microscope are taken for these organisms they are merely the elongated cells of bacteria or Bacilli seen endwise, in "optica section" as it is termed, *i.e.*, floating perpendicularly to the cover-glass of the preparation.

scarcely appreciable, inflammation; in some other cases the spot was not perceptible even on microscopical examination of the tissues. The occurrence of the microphytes, in the situation only here stated, in conjunction with the circumstance that the substance injected was a chemical irritant, germ-free, is important in respect to the question of their origin.

These Bacilli are in width about 1·0 to 1·3 μ (0·001 to 0·0013 millim.) : in length, the single cells vary up to about 4 or 5 μ (0·004 to 0·005 millim.) ; filaments consisting of some few of these, united end to end, and in less active movement than the individual rods, also occurred in the fluid as examined fresh under the microscope, but in dried and stained preparations these are not nearly so numerous. In dimensions these Bacilli are somewhat similar to the *B. anthracis*, but it is impossible for any careful observer to confound the two; for independently of the species here described being active, while the *B. anthracis* is invariably immobile, and though in the serous fluids the former develope into filaments of variable length, yet the segments of these show the original mature cells of about the dimensions here given, into which they again break up, *preserving their rounded ends*, and are distinctly unlike the segments which the filaments of *B. anthracis** form, as it is possible to be, for the latter are *rectangular*, pretty uniform in length, of little more than about twice the width; in cases of Anthrax, whether in the animal organism, or in artificial cultivation this formation may invariably be recognised where the cells are sufficiently developed to form filaments. In the present case blood from the heart contained no organisms, but in the spleen and peripheral portion of the kidney were found several Bacilli, though not in the other organs. In this case it is to be noted that death had occurred some, probably several, hours before examination, and decomposition was advancing with the rapidity that characterises all these cases. In other experiments where the infected animals were examined immediately after death, in no instance were any microphytes found either in the blood or organs, but only in the serous fluids and in the connective tissue as before stated.†

Of the exudation fluid in this case 0·05 cub. centim., diluted with equal parts of salt solution, was injected into guinea-pig No. 2, and 0·022 cub. centim. similarly into another, No. 3. Both these animals

* The terminal cells, however, of this form too, it must be remarked, retain their rounded ends, "the growing-point," though but few of these may be found in mature development.

† Some writers, however, in experiments upon Pasteur's septichæmia have referred to organisms found in the blood of infected cases. What the import of this is I cannot say, in the absence of a clearer diagnosis of this disease; whether some other form of specific septichæmia or septic infection had been obtained, or, in the absence of any precise statements on the subject, whether examination had been deferred until some hours after death, and septic bacteria had developed.

died in between twelve and eighteen hours with appearances similar to the first case, but less severe; the same organism occurred in the peritoneal exudation, but none elsewhere. Two other animals were also injected at the same time with respectively 0·0044 and 0·0022 cub. centim. of the same fluid diluted, but neither was appreciably affected. It thus appeared that the inflammatory exudation fluid of the first generation originating infection is fatally infective in quantities of from 0·022 cub. centim., but not in 0·0044 cub. centim. To continue the infection through subsequent generations, 0·05 cub. centim. of case No. 2 was injected into another animal, which likewise died in about thirty hours, and was examined immediately, the same hyperæmia with exudation and the occurrence of numerous organisms was found; of this serum, 0·05 cub. centim. diluted, was injected into another animal, No. 7, which survived, without being materially affected, thereby showing that the infective virulence of the fluids is variable, and that in the third generation of successive infection there is at least no gain in virulence from the first, in which it was fatal in less than half the quantity that was here without material effect. Infection being thus lost, it was again originated in another series of experiments by injecting 0·22 cub. centim. of a dilute solution of ammonia into the peritoneal cavity of another guinea-pig, which died within twenty-four hours, and was examined some time after death with very much the same appearances as in the first case recorded above, though here the serum was more deeply sanguineous and more coagulable; the same organisms were found to be present. Of this fluid 0·05 cub. centim. was injected into another animal, No. 9, without, however, producing any appreciable effect; thus again showing that in the same generation of infection, viz., here in the first, the virulence of the septic product is variable; it was therefore necessary to work with larger quantities. In a fresh series of experiments infection was again originated by the injection of a solution of ammonia into another animal, No. 10, which died in manner similar to the former. Of the exudation fluid in this case 0·22 cub. centim. diluted, was injected into No. 11, which died in between eight and nine hours after; another, No. 12, which received by injection 0·055 cub. centim., died in between twelve and twenty hours; subsequently 0·022 cub. centim. of the same fluid, of No. 11, was injected into another animal, No. 13, which remained without being materially affected, beyond, as in other cases which survived, some rise of the rectal temperature, which, however, in these animals is not sufficiently constant normally, for variations in it to give any reliable indications, unless very marked. In healthy animals I have found it fluctuate from very slight causes, such as fright, being handled, &c., between 97° F. and 101° F. Other animals which here received injections of smaller quantities than the above all remained similarly unaffected.

From the above-mentioned animal of the second generation that died infected, 0·22 cub. centim. of the serous fluid diluted was injected into another guinea-pig, No. 14, which died within four hours, but 0·22 of the exudation in it, being similarly injected into another, No. 15, of the fourth generation, this survived for twelve hours, and was found dead some hours subsequently, showing that, as judged by the period of incubation, the septic fluid was inferior in virulence to that of a prior generation. Infection was continued through another generation, the fifth, by injection of the same quantity of serum of No. 15 into another animal, No. 16, of which finally 0·2 cub. centim. was injected into No. 17 of the sixth generation in succession, which died in about the same period after inoculation, viz., four to five hours, as had the one previously of the third generation. The following successively diminishing quantities were then injected into other animals, viz.: 0·022 cub. centim., 0·0022, and 0·00022 cub. centim., and some smaller quantities still, all alike without producing any material symptom of disturbance. In every instance here the Bacilli were present in such numbers in the exudation serum from which the dilutions were made that the smallest quantities injected must have contained very many of them.*

In regard to the relation of these micro-organisms to the diseases in which they appear, it has been asserted† that they occur normally in the blood of healthy animals, and are only enabled to develop by the pathological condition caused in these cases by the injection of septic matter. As this point is of fundamental importance to the subject, to determine it experimentally, a young healthy rabbit, nearly full-grown, was killed by asphyxiation, and then placed in the incubator intact, for twenty-four hours; at the end of that time the abdomen was largely distended with gas, and putrefaction obviously well advanced. On examination there was found in blood from the heart, in rather small numbers, a Bacillus, the cells of which were of considerable width, but of no great length, nor forming long chains, mobile, and evidently in active multiplication, in some few cases apparently forming spores at one end only; there were a very few free spores, but growing cells in all stages of development; no small Micrococci nor any other species of Schizophyte, no *B. termo*, nor any one of the organisms here described as specifically distinctive of Davaine's septichæmia. In blood from the lungs and the liver the same Bacillus also occurred, as it did in the spleen, but in smaller numbers. In none of the organs or tissues examined was any other

* In the experiments here recorded "infection" was originated by the intra-peritoneal injection of a chemical irritant; in other series, by putrid matter; the results are substantially the same in both cases.

† Most recently by Professor Rossbach, "Vermehrung der Bakterian in lebenden Tiere, &c.," Ctbl. f. d. Med. Wiss., 1882, No. 5, p. 82.

species found; whereas in cases of infection with this specific form of Davaine's septichæmia, the particular bacterium increases within certainly fifteen hours, so as to outnumber many times the red blood-corpuscles, here none were to be found in a materially longer period. Hence it seems to me to be clear that the specific bacterium of Davaine's septichæmia does not normally exist in the blood of healthy rabbits. Numerous preparations of blood from different parts were made, stained, and carefully examined, with the same result in all.

The experiment was repeated with a guinea-pig, similarly killed and kept in the incubator for twenty-four hours, at the end of which time it was enormously distended with fetid gases, the blood from the heart and principal organs also contained a Bacillus in some numbers; this was similar in form to that found in the case of the rabbit, but somewhat less in size, and spore formation was more advanced. To all appearances it is identically the same as that which developes in cases of peritonitis or the so-called Pasteur's septichæmia in the guinea-pig. Whether or not it is specifically identical with the Bacillus that developes in dead healthy rabbits or in septic diseases in that animal, can only be determined by interchanging inoculations or cultivations of each growth, for though there is a slight morphological difference in the organisms of the two animals, it is clearly the fact that the same species of Bacillus, growing in different media, varies appreciably in its dimensions, as is typically exemplified in the case of the Bacillus of Anthrax.

Artificial Cultivation.

In the first trials of cultivating the organisms here in question in open tubes, both those of Davaine's and the so-called Pasteur's septichæmia, I failed to obtain conclusive results with any of the nutrient fluids employed, infusions of the flesh of different animals, and with blood serum: in the latter the bacteria of Davaine's septichæmia germinated, producing a turbidity which extended very slowly and to small extent, dying out generally after about the second day; the serum used being partially solidified by evaporation in prolonged heating, though still perfectly pellucid and not in any way coagulated. The Bacilli in the other case behaved in much the same manner in different fluids.

In the last experiments made during the winter, as I failed to again originate specific infection with Davaine's septichæmia by inoculation with putrid blood, I have been unable to repeat the attempt to cultivate these organisms. As, however, it has been stated that the microphyte of Pasteur's septichæmia has been successfully cultivated *in vacuo* in France, and it seemed probable, from the conditions under which it occurs in the animal organism, that it would thrive best in the absence of oxygen or atmospheric air, the experiments were

repeated. In the former trials, where inoculations were made under antiseptic spray, both in the tubes of *bouillon* and of serum, accidental contaminations sometimes occurred. In the present experiments, inoculations were made through sterilised cotton wool, which is the only reliable method of artificial cultivation with which I am acquainted, and which I have found in numerous experiments, even with such unstable substances as blood serum, to be absolutely infallible, with moderate care in manipulation. It was communicated to me by Dr. E. Klein, and is, I believe, described by him in detail elsewhere. The infective exudation serum used for inoculation in this case was from a guinea-pig, in which it had been produced by the subcutaneous injection of a few drops of the serous fluid in infective peritonitis, artificially induced: the organisms herein were not very numerous, consisting chiefly of short rods of somewhat variable breadth, one end being frequently swollen, as in the formation of spores, distinct forms of which, however, were not apparent, though cell-rods, in a very early stage of development, were. It at first seemed as if there were here two distinct species of Bacilli present, but subsequent observations showed that they were only forms of one and the same in different stages of development. The narrower cells were, in fact, degenerating and withering, which frequently accompanies spore formation, the cell presenting a shrivelled and somewhat contorted figure instead of the usual uniformly cylindrical sharp contour. This stage is more frequently and readily seen in artificial cultivation outside the animal body. The tubes containing sterilised blood serum and beef *bouillon* being inoculated with a small particle of this fluid, were then exhausted of air, sealed, and placed in the incubator, when the next day their incipient turbidity showed that vegetation was proceeding readily; on the second they were opened, and both the serum and *bouillon** were found to be full of the same species of Bacilli originally inoculated, in various stages of development, *forming numerous and distinct spores*. More than one tube of each nutritive medium was always prepared, and it was observed in those containing serum that the more solid it had become by evaporation the more slowly did the vegetation progress in it, rendering it fluid as it proceeded. On opening the tubes

* Blood serum offers a suitable cultivating medium for probably all pathogenic organisms; it is easily sterilised, and when once this is effectually done, it may be kept for any length of time, and while remaining perfectly translucent may be rendered by prolonged heating of any consistency required. Koch's method of gelatine culture, though most valuable for particular purposes, is not for several reasons suitable for use generally in these experiments. With the exception of *B. anthracis* most pathogenic bacteria, as distinguished from the merely septic, vegetate sparsely if at all in vegetable infusions, the attempts to cultivate them in which are generally disappointing and misleading.

the strongly fetid odour caused by the development of the vegetation was remarkable and distinctive, and was observed in the case of every tube opened, both those with serum and those with *bouillon*. Their cultivation was repeated four times, in exactly similar manner, each cultivation lasting two or three days, and precisely similar appearances being observed in each. From the fifth collection, which contained the Bacillus in considerable numbers, five drops, diluted with an equal quantity of normal saline solution, were injected into the subcutaneous tissue of the abdomen of guinea-pig A, which died with the usual, though slight, symptoms of infection in about forty hours after inoculation. Another guinea-pig, B, at the same time received in like manner ten drops of the same fluid, diluted 1,000 times ($=\frac{1}{100}$ gtt. of the cultivation). This animal was perfectly unaffected, beyond a slight local and temporary irritation at the spot of injection, which, without infection, sometimes occurs with animals inoculated in the abdomen, and arises probably, as I have witnessed, from their scratching; it passed off within forty-eight hours and the animal remained unaffected. The quantity of fluid here injected must have contained many thousands at the least, and probably some millions of the Bacilli, but they do not appear capable *per se* of developing to any extent in the tissues of a healthy animal.

From these experiments I conclude that in the affections here in question, that is, in Davaine's septichæmia in the rabbit and the so-termed Pasteur's septichæmia in the guinea-pig, there is no increase of infective virulence in the septic fluids in successive generations, either in respect to the minimal quantities required to produce fatal infection, nor as to any constant difference in the incubation period, though in the latter case this period is less constant than obtains in Davaine's septichæmia; the infectivity, too, of the inflammatory product, though not comparable in virulence to septichæmic blood, is here more variable, partly owing it may be, as Dr. Sanderson originally considered, to differences in the severity of the cases affording the infective matter, and partly also, as I have above stated, to constitutional idiosyncrasy in the animal inoculated.

With respect to the nature of the contagium and the relation of the micro-organism to the disease in which they occur, I conclude that in the first case, *i.e.*, in Davaine's septichæmia in the rabbit, all the circumstances taken into consideration, the microphyte constitutes the actual contagium, and that the numbers in which it is present in the blood, both septic (putrid) and septichæmic, clearly condition its infective virulence. Its numbers alone would not account for the difference in the incubation period—so-called—of the two cases, but the purity of the growth in the latter case, and in the other the fact

of the specific bacterium being always far outnumbered by other species with which it has to contend in order to establish its growth, fully accounts for its slower development, and the consequent protraction of the incubation period.

In the second case, that of infective peritonitis, or Pasteur's septichæmia in the guinea-pig, the circumstances are materially different; in the first place, the microphyte appears to originate from within the animal organism, as shown both by the anatomical examination of the tissues, in the case of originating the disease by the injection of an antiseptic fluid; and, further, by the fact that a Bacillus, as far as can be determined, identical with the form occurring in these cases, is found to be present normally in healthy animals shortly after death, when kept at the temperature of the body. Moreover, the pathogenic matter containing these organisms when infective, is only so in incomparably larger quantities than is the case with the blood in Davaine's septichæmia, nor are cultivations of the specific organism more virulent; hence, although as shown elsewhere,* the infectivity of the pathogenic matter is destroyed by heat, I cannot conclude that in this case the microphyte is to be considered as constituting the contagium, *per se*, purely and simply as in Davaine's septichæmia; the large numbers in which it must be injected in order to develop its growth, in addition to the circumstances of its origin, seem to forbid this view, which would imply that in the one case it constituted the exciting cause of the pathological condition, to which it owed its development, that it is to be regarded in the one case as the secondary result, in the other as the efficient cause of the same thing. No doubt, however, when present in sufficient numbers, they greatly modify the characters of the pathological condition which they accompany. This, which was the opinion of Dr. Sanderson, at the time referred to, does not seem since to have received the attention it deserves, as the case of a disease in which the presence of micro-parasites originated by the injection of a germ-free chemical substance stands alone, and has the most important bearing on the relation of these organisms to disease.

In both the forms of septic infection herein referred to, the important function of the lower fungi which therein occur, is clear, but it is remarkable that, notwithstanding the numerous observations and experiments that have been made on the subject, and the fact that in both cases these affections were recognised by the chief writers thereon, as being of micro-parasitical origin, yet so little attention should have been paid to the microscopical examination and description of the organisms themselves. In the one case, indeed, the bacteria which occur had been figured and described by MM. Coze

* "Proc. Roy. Soc.," vol. xxxiv, p. 150.

and Feltz, but imperfectly as above mentioned, and this, as far as I know, is the only attempt to describe them at all particularly; the terms in which the microphytes they mention are referred to by later French writers are most vague, while others have scarcely alluded to their presence, save in the most general manner, while recording experiments made with the object of investigating the ætiology of these diseases.

In the case of Davaine's septichæmia, I have been able in all essential points, not only to confirm his original statements, but to account for them, more especially with regard to the incredibly minute quantities in which the blood in these cases is infective, statements the accuracy of which has been sometimes doubted. To emphasise this is due to the reputation of one who has done so much in many directions to advance our knowledge of micro-parasitical diseases, and indeed, it may be said that almost the only points left undetermined by this observer, or the mistakes which he made,* were those dependent upon microscopical investigations, the immense advance in the appliances of which the last few years now enables this to be done. In the other case, the so-termed Pasteur's septichæmia in the guinea-pig, the relations of the Bacillus therein occurring are not so clearly shown as in the former, yet if its characters of morphology and mobility as above mentioned had been carefully observed, neither this affection, nor, still less, the former, could for an instant have been confounded with charbon or anthrax, as was the case; nor again in this latter disease could any observer have fallen into the grave error of confounding the *B. anthracis* with either the Bacillus here in question, or the hay Bacillus, the characters above briefly described being perfectly constant and sufficiently distinctive to be at once readily recognised under even moderate magnifying power.

It is on the microscope that I have relied for determining the questions herein, and it appears to me that it is on the more assiduous use of its greatly increased powers which we now possess, that the advancement of our knowledge of these subjects depends.

The observations here recorded were commenced and chiefly performed in the Physiological Laboratory of the New Museums at Cambridge, during the last winter (1881): circumstances have delayed their final completion till lately, in the meantime an article on the same subject has been published at Berlin, by one of Dr. Koch's assistants, Dr. G. Gaffky,† who has arrived at the same conclusions mainly as myself with reference to the principal point in question; but

* As in regarding the specific organism of septichæmia and the putrid ferment as one and the same.

† "Experimentell erzeugte Septikämie, &c.," in "Mittheil. a. d. Kaiserl. Gesundheitsamte, &c.," Berlin, 1881.

on the other hand, the doctrine of an increase of virulence in septic fluids in successive generations has been again affirmed by Professor Rosenberger* in relation as appears to both the forms of disease here in question, without, however, giving any detailed account of the experiments on which he grounded his opinion.

In conclusion I have to thank the British Medical Association, through the Scientific Grants Committee, for assistance in defraying the expenses of these experiments.

Table I.—Showing the Result of the Subcutaneous Injection of Septic and Septichæmic Blood in different quantities in Rabbits.

No.	Generation.	Matter injected.	Quantity.	Result.
			gtt.	
1	1	Putrid bullock's blood dil...	3·0	Died within 40 hours.
2	2	Blood of No. 1 diluted.....	1·0	„ „ 24 „
3	3	„ „ 2 „	$\frac{1}{10}$	Died in 25 hours.
4	„	„ „ 2 „	$\frac{1}{100}$	„ 24 „
5	„	„ „ 2 „	$\frac{1}{1000}$	„ 25 „
6	„	„ „ 2 „	$\frac{1}{10000}$	Died within 40 hours.
7	„	„ „ 2 „	$\frac{1}{100000}$	„ „ 27 „
8	4	„ „ 3 „	3·0	Died in 23 hours.
9	5	„ „ 8 „	3·0	Died within 48 hours.
10	6	„ „ 9 „	3·0	„ „ 20 „
11	7	„ „ 10 „	$\frac{1}{100}$	Died in 20 hours.
12	„	„ „ 10 „	$\frac{1}{10000}$	„ 26 „
13	„	„ „ 10 „	$\frac{1}{100000}$	„ 25½ „
14	„	„ „ 10 „	$\frac{1}{1000000000}$	„ 20 „
15	1	Putrid bullock's blood dil...	5·0	Died within 48 hours.
16	2	Blood of No. 15 diluted....	$\frac{1}{1000}$	„ „ 24 „
17	„	„ „ 15 „	$\frac{1}{100000}$	„ „ 35 „
18	„	„ „ 15 „	$\frac{1}{1000000}$	„ „ 35 „
19	„	„ „ 15 „	$\frac{1}{10000000}$	„ „ 48 „
20	„	„ „ 15 „	$\frac{1}{100000000}$	Survived, abscess formed.
		Infection continued through successive generations up to the 10th.		
21	11	Blood of rabbit of 10th generation diluted.....	$\frac{1}{1000}$	Died in 21 hours.
22	„	„ „ „	$\frac{1}{1000000}$	Died after 27 hours, within 40 hours.
23	„	„ „ „	$\frac{1}{10000000}$	„ „ „
24	„	„ „ „	$\frac{1}{100000000}$	„ „ „

Note.—The quantities employed are here given in drops (minims), in order that they may be comparable with the experiments of others on the same subject.

* "Ctrlb. f. d. Med. Wiss.," 1882, No. 4, p. 66.

Table II.—Showing the Result of the Intra-peritoneal Injection of different matter in Guinea-pigs.

No.	Generation.	Matter injected.	Quantity.	Result.
			c.c.	
1	1	Solution of ammonia diluted..	0·3	Died within 20 hours.
2	2	Exudation fluid of No. 1 dil..	0·05	Died in between 12 and 24 hours.
3	„	„ „ „ 1 „ ..	0·022	„ „ „
4	„	„ „ „ 1 „ ..	0·0044	Survived.
5	„	„ „ „ 1 „ ..	0·0022	„
6	3	„ „ „ 2 „ ..	0·05	Died in 30 hours.
7	4	„ „ „ 3 „ ..	0·05	Survived.
8	1	Solution of ammonia diluted..	0·22	Died within 24 hours.
9	2	Exudation fluid of No. 8 dil..	0·05	Unaffected.
10	1	Solution of ammonia diluted..	0·22	Died within 24 hours.
11	2	Exudation fluid of No. 10 dil..	0·22	Died in between 8 and 9 hours.
12	3	„ „ „ 10 „ .	0·055	Died in between 12 and 24 hours.
13	2	„ „ „ 10 „ .	0·022	Unaffected.
14	3	„ „ „ 11 „ .	0·22	Died within 4 hours.
15	4	„ „ „ 14 „ .	0·22	Died after 12 hours.
16	5	„ „ „ 15 „ .	0·22	
17	6	„ „ „ 16 „ .	0·22	Died in about 4 hours.
18	6	„ „ „ 16 „ .	0·022	Unaffected.
19	6	„ „ „ 16 „ .	0·0022	„
20	6	„ „ „ 16 „ .	0·00022	„

HARRISON AND SONS, PRINTERS IN ORDINARY TO HER MAJESTY, ST. MARTIN'S LANE.