Interim report on the relations of septic to pathogenic organisms. / by E. Klein.

LOCAL GOVERNMENT BOARD.

SCIENTIFIC INVESTIGATIONS.

INTERIM REPORT

ON THE

RELATIONS OF SEPTIC TO PATHOGENIC ORGANISMS.

By E. KLEIN, M.D., F.R.S.

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INTERIM REPORT

TO THE

Local Government Board, by Dr. Klein, on the Relations of Septic to Pathogenic Organisms.

George Buchanan,
Medical Department, 1884.

Section I.

There is hardly any question which to the pathologist and sanitary officer can be of greater importance than the relation of septic to pathogenic organisms. To the pathologist the life history of a micro-organism, outside and within the animal body, must ever remain an important field of inquiry; to the sanitary officer all conditions affecting the life and death of those organisms which produce, or at least are intimately bound up with, infectious diseases, such as the distribution and growth of these micro-organisms outside the animal body, the agencies which affect it in a favourable and unfavourable sense, are the points which he has particularly to consider in dealing with the spread and prevention of infectious maladies. Now, it is known of many micro-organisms, both those that are associated with putrefactive processes as well as those that are bound up with infectious disease, that temperature, the medium in which they grow, presence and absence of certain chemical compounds are capable of materially affecting them. I need not for this purpose enumerate all that is known already by direct experiment, but will only limit myself to reference to the researches of Schröter, Cohn, and Wernich on that group of micro-organisms known as pigment bacteria, *i.e.*, bacteria which only under certain conditions, notably temperature and soil, produce definite pigments (Cohn's Beiträge zur Biologie d. Pflanzen); to those of Hansen (Carlsberg Laboratory) on yeast; to those of Neelsen on the Bacilli producing

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the blue colour of milk, the Bacillus syncyanus (Beitr. zur Biol. d. Pflanzen, iii. 2, p. 187); to the works of Toussaint, Pasteur, Chauveau, Koch, and others on the bacillus anthracis; Arloing, Thomas, and Cornevin on the bacillus of symptomatic charbon; of Koch on the bacillus of tuberculosis; of Israel on Actinomyces, and many others; and particularly would I refer to the many valuable suggestions and considerations expressed by v. Nägeli in these respects in his book, Die niederen Pilze, München, 1877 and 1882.

While from these observations it would appear that both septic and pathogenic micro-organisms are capable of suffering some modifications in their morphological and physiological behaviour, it is nevertheless still an open question whether an organism which under ordinary conditions is only associated with putrefactive changes in dead organic material, and which cannot under these ordinary conditions grow and multiply within the living body, can, under certain extraordinary circumstances, become endowed with the power of growing and multiplying within the body of a living animal, creating there a pathological condition, inducing there an infectious disease.

If on the one hand it can be shown that a micro-organism at first deadly virus to an animal, can by particular conditions of growth or otherwise be so modified that, although still a living entity, *i.e.*, capable of self-multiplication, it nevertheless loses hereby its previous pathogenic activity; then the assumption becomes *a priori* not untenable that a normally harmless saprophyte might under exceptional conditions be transformed into a harmful, *i.e.*, pathogenic, organism. Now, has the first proposition really been proved? Has it been experimentally proved that a pathogenic micro-organism is capable, owing to certain conditions of growth, of losing its harmful characters, though retaining its vitality? The only case in which such a modification has been apparently effected is the bacillus anthracis. But on a critical examination it will be seen that what seems to be a modification of the bacillus, is far from well established as real.

This is what has been noted in regard of the different ability of bacillus anthracis under one and another set of conditions to kill various animals not already protected against the virus:—

Bacillus anthracis taken from the blood of an animal, say, guinea-pig, dead of anthrax, and grown for a few days under ordinary conditions, yields a crop of bacilli, which appear

fatal to any rodent susceptible to anthrax, but grown for a number of days, two to three weeks, at 42°–43° C. (of course without being allowed to form spores) lose, in most instances, their virulence for sheep and cattle (Pasteur), but not for the guinea-pig and rabbit. The same effect can be produced by heating the blood teeming with bacilli up to 55° (Toussaint), and by keeping the bacilli exposed a short time to the action of carbolic acid (Toussaint, Chauveau). Further, I myself have shown that ordinary cultures of bacillus anthracis, provided they remain free of spores, lose in from several days to a few weeks their virulent action on white mice, but retain it for other rodents (guinea pigs and rabbits). Koch (Die Milzbrandimpfung, Berlin, 1882) observed that his cultivations of bacillus anthracis, carried on at 43° for more than six days, lost their activity for rodents. Again, it is now well established that bacillus anthracis of whatever source having passed through the guinea pig loses its fatal activity on cattle (Sanderson and Duguid); that bacillus anthracis of whatever source passed through the white mouse loses its fatal activity on sheep (Klein and Roy); further, that bacillus anthracis passed through the South American rodent biscachia loses its fatal activity on cattle (Roy).

Now, in all these instances of loss of virulence of the anthrax organism, the bacilli have not lost their vitality; for, as I have convinced myself again and again by actual experiment, if *new cultures* are made with them in the ordinary manner they yield crops of bacilli which possess not only full vitality, *but also full virulence for the same species for which the previous generations of bacilli proved inactive.*

These experiments of mine, it will be noted, call in question M. Pasteur's statement that a culture of bacillus anthracis that has become inactive for sheep always yields further cultures which, although full of the typical anthrax bacilli, nevertheless are inactive on sheep, that is to say, that the bacilli anthracis, having once lost their pathogenic property, are capable of yielding crops of bacilli also barren of normal pathogenic properties. I therefore briefly enumerate the facts I have observed in this connexion—(*a.*) Blood of mouse, dead of anthrax of whatever source, does not kill sheep, but if a culture be made of the bacilli of the blood of such a mouse, the bacilli of this new culture, with or without spores, kill sheep. (*b.*) A culture of bacillus anthracis established and kept at 30°–35° C. kills mice in the first week, but kept for a longer time in the incubator, provided no spores appear, loses its virulence for mice only; but if of

such a culture a fresh culture be established, it again proves fatal to mice; or if in the culture spores appear it retains its virulence *ad infinitum*. (*c*) If a culture of bacillus of blood of guinea pig be established and kept at 42°–43° C. for two or three weeks, it loses, in many cases, its fatal action on sheep; but if the bacilli of such a culture be afterwards allowed to form spores, or if a new culture is made, it proves again virulent to sheep. The same holds good also for those bacilli anthracis which, owing to the admixture of certain antiseptics, lose their virulence, though they do not lose their vitality. Such bacilli are capable of starting cultures, in which the new bacilli, although not so plentiful, still possess virulent action.

Seemingly M. Pasteur has worked with cultures which did not contain the bacillus anthracis, but which, owing to accidental contamination at the time of starting the new cultures, developed a putrefactive non-pathogenic bacillus, and it hardly needs to be noted that if the bacilli of a culture not containing spores become, all of them, involved in a process of degeneration (*see* my Report, 1881) their virulence and their vitality have alike passed away. Blood bacilli and bacilli of cultures too that have been subjected for more than a few minutes to a high degree of heat or have been acted on by certain antiseptics are similarly inactive, for they are dead.

To sum up—all that may be confidently asserted respecting modification of bacillus anthracis by culture is this: bacillus anthracis of a particular cultivation is capable, without losing its vitality, of losing its virulence for a particular species of animal, while retaining it, however, for certain other species; and, further, the bacillus which has thus lost its virulence will regain it in a new cultivation. But surely this cannot be called a change of a pathogenic into a non-pathogenic organism, for is not the same organism pathogenic to other species, and does it not yield again pathogenic offsprings?

Section II.

I now pass to consider whether there exist naturally or can be artificially induced, varying degrees of virulence of bacillus anthracis.

M. Pasteur bids us recognise varying degrees of virulence in his cultures of anthrax bacillus, and a superficial consideration of the subject lends support to an assumption of this sort. Thus the bacillus of the blood of sheep and cattle

may be thought of as *stronger* than that of the blood of the white mouse and guinea pig respectively, because blood of sheep kills mice, but the blood of mice does not kill sheep; blood of cattle kills guinea pigs, but blood of guinea pigs does not kill cattle. Again, sheep and cattle appear to require a *stronger* virus than mouse and guinea pig, because sheep and cattle are unaffected by vaccin charbonneux, while mice and guinea pigs are killed by it. Thus considered, cattle require a *stronger* virus than sheep, because guinea pigs' blood does not kill cattle, but kills sheep.

Upon this view, too, we should have to say that virus taken from the guinea pig and passed through the mouse decreases in *strength*. And we should have to define as the *weakest* virus, that which kills mouse but does not kill guinea pig, sheep, or cattle.

Again, upon this view weak virus passed through the guinea pig becomes *stronger*, because vaccin charbonneux, which does not affect sheep, passed through the guinea pig kills sheep. But in this case it has not become strong enough to kill cattle, which it does when passed through the sheep.

It would appear then that the virus of the mouse is weaker than that of the guinea pig, sheep, and cattle, and that mouse requires weaker virus than any of these animals; further, that the virus of the guinea pig is weaker than that of sheep and cattle, and that the guinea pig requires a weaker virus than sheep and cattle; and, lastly, that the virus of sheep is weaker than that of cattle, and that the cattle require a stronger virus than sheep.

Certainly all this harmonizes with the general assumption of various degrees of virulence. But there is also another side to the question; it is this: from the fact that a culture does not kill mice, but kills guinea pigs and sheep (such as I have mentioned on several occasions), one would arrive at a directly contrary conclusion from that above, viz., that the mouse requires a stronger virus than the guinea pig or sheep. Again, if blood of such a guinea pig be used, it will be found very virulent for the mouse,* and if the blood of this mouse be used, it will be found to kill mice but not sheep. Consequently, while on the one hand we have a virus which is not strong enough for mouse but fatal for sheep, we know, on the other hand, that virus exists which is fatal to mouse, but not fatal to sheep; and once more, as I have mentioned before, if a culture be made of the blood of the

* "Mouse" should be read throughout this report as signifying "white mouse."

mouse at a temperature, say, 30° to 35°, we know that we get a material which (though not containing spores) is fatal to sheep, but is not fatal to mouse after some days, so that we started with a material (blood of mouse) which is fatal to mouse, but not fatal to sheep, and from this we obtained a culture which is not fatal to mouse, but fatal to sheep.

These further facts appear to me irreconcilable with the previous assumptions—(1) that the bacillus is in reality capable of undergoing a diminution of its physiological activity, *i.e.*, suffering a real attenuation, and (2) that there exist anthrax bacilli having an intrinsic virulence of various degrees.

The facts seem, however, capable of another explanation. Owing to different conditions of growth, *e.g.*, high temperature, or other artificial conditions, or owing to different soil on which they grow, *e.g.*, the body of a mouse or of a guinea pig, the bacilli, *although themselves the same*, embody or appropriate, chemically or otherwise, some new or different substance, which produces the alteration for a particular species of animals. Whether this substance is comparable to a *ferment* or not, I am not in a position to say, possibly it is some ferment produced by the new conditions. Assuming this influence of some such superadded substance, it becomes at once clear how such bacilli regain their full force when grown differently, *i.e.*, removed from this condition that produced on them this "weakening" effect, or when in a new generation this "weakening" effect has become lost. The fact that a culture of bacillus anthracis while of recent date is virulent to white mice, but becomes inactive on them after several more days, and further that such an inactive culture is again capable of starting a culture which is active while of recent date, seems to me to harmonize well with this theory, viz., a certain substance is absent in the first few days of cultivation, but it makes its appearance after further progress, and *owing* to *its presence* the bacilli are inactive on mice; starting again a new culture, this supposed substance not being yet formed leaves the new bacilli with full activity, in order again to lose it when after some more days of incubation this supposed substance is produced.

This hypothetical substance, injurious to the activity of the bacilli as regards certain species, need not necessarily be injurious to this activity as regards other species, for the bacilli, being according to this theory the same, might act with full activity on other species, and thus we can understand vaccin charbonneux being inactive on sheep, but active

on mice and guinea pigs, and anthrax blood of guinea pig being virulent to sheep, but not virulent to cattle.

What we have to suppose is that the bacilli are in all instances the same, but that there is something else present which has become associated with the bacilli in particular cases, and which hinders them from producing their fatal effects; and that it is the presence or absence of this something which determines the effect of the bacilli upon animals of one or another species.

That an inhibitory effect can be produced on the bacillus anthracis by purely chemical processes is shown in the behaviour of these bacilli under the influence of certain antiseptics. Thus Chamberland and Roux found that carbolic acid or bichromate of potash added to the nourishing medium in the proportion of 1 : 800 or 1 : 1,000 respectively, produced a distinct "attenuation" of the bacillus anthracis; and I myself have found (by experiments, which I shall state in a further report) that exposure of bacillus anthracis, both of the blood and of a culture, to weak solutions of phenyl propionic and phenylacetic acid produces a distinct inhibition of the virulence of the bacilli, but these bacilli were capable of again starting cultures of virulent bacilli. An inhibition of the function and vital activity by purely chemical means occurs in the case of many other organisms besides the bacillus anthracis. It is well established by Wernich and others that in cultures (natural and artificial) of *putrefactive* organisms certain chemical substances (chiefly belonging to the aromatic series) are brought into existence (*e.g.*, indol, skatol, phenol, and others) which have an injurious effect on the organisms, so much so, that as these substances accumulate in a given culture, in proportion to the progress of the growth of the organisms, the functional activity of the particular organisms becomes inhibited and eventually altogether extinguished. And there is *a priori* no reason why something similar should not occur in the case of *pathogenic* organisms, and certain chemical substances be produced in a nourishing material as the result of the growth and multiplication there of the organisms themselves; such substances, although owning this origin, having some such inhibitive power for the pathogenic organisms that indol, skatol, and phenol had for putrefactive organisms.

Whatever may be the nature of this inhibitive material, we may infer something about the circumstances of its presence and about its properties.

It is present after some days in a sporeless culture esta-

blished at 30–35° C., for it prevents fatal activity of that culture upon mice. It is present in a sporeless culture maintained at 43° C. for six days, for thenceforth it avails to prevent the fatal activity of the culture upon mice and rodents of every sort. Yet in both these cases its inhibitive action has been exerted without ultimate injury to the bacilli themselves, seeing that in each instance the impotent culture can be used to establish a new culture that will have all the original power of anthrax bacilli upon the particular species of animal. The influence of the inhibitive material has been to suspend, not to destroy, the potency of the anthrax bacillus.

In the next place, the inhibitive material, whatever may be its nature, which avails to suspend the power of the bacilli does not operate to suspend the power of their spores. In cultures such as the above described, but in which spore formation is taking place, the fatal activity of the culture towards the animal is never in any way prevented or suspended. We must regard the restraining matter as being absent from the spores; and there is no difficulty in believing that it is so absent, though it be present in the bacilli themselves.

Thirdly, the inhibitive material may find itself in abundance within the system of a given animal, without its having any restraining or modifying power upon fresh anthrax bacilli introduced into that animal. Thus it may be introduced, apart from disease-producing material, into a mouse (by use of an old sporeless culture in which all the bacilli are dead, but in which matter that is inhibitive qua mouse remains abundant), and we shall not render that mouse thereafter insusceptible to fresh anthrax inoculation, nor confer any modification upon the anthrax that we produce by such inoculation. Evidently the inhibitive material is something very closely associated with the bacilli themselves.

Fourthly, the inhibitive material that we believe to accrue during artificial cultures of bacilli under one condition or other, and during the growth of bacilli within the system of certain animals, cannot be always one and the same material. For the blood of a mouse dead of anthrax injected into a sheep carries with it an inhibitive matter competent to prevent fatal activity in the sheep, though it had not availed to prevent the death of the mouse; and yet there exist cultures which do contain matter inhibitory for the mouse of the effect of anthrax bacilli. Wherefore it is plain that the in-

hibitive matter is not the same in nature in the case of sheep-anthrax and of mouse-anthrax.

We have, then, to deal with a variety of substances which have in common the property of inhibiting during a particular culture the action of anthrax bacilli; by entering into markedly close association with the bacilli themselves, though not into any association with the spores of the bacilli; and which exercise their inhibitory property upon the bacilli only during the maintenance of the particular culture, having no further power of the kind upon a succeeding culture of those bacilli. Of these inhibitory substances we know that they can exercise this property within the body of an animal inoculated with anthrax, either by simple restraint of all activity of the associated bacilli, the animal keeping well after the inoculation and remaining as susceptible as ever to death by a fresh inoculation, or otherwise (as in the case of mouse's blood in sheep), by restraint of the fatal activity of the bacilli, the animal getting a modified attack of anthrax and remaining protected more or less completely against attack of anthrax when a fresh inoculation is practised. Probably some of the inhibitory substances possess the one kind of ability, whilst others act in the other way.

As far as I can see, the bacillus anthracis is the only case in which a change of activity has been experimentally shown in a pathogenic organism; as regards other micro-organisms, such as those of septicaemia (Semmer), tubercle bacilli, and others, the observations hitherto made, are too incomplete to admit of any critical review, but in any case the considerations mentioned in connexion with the bacillus anthracis are applicable in like manner to the other cases.

Section III.

We come now to the question, has any change been observed of a putrefactive or septic into a specific or pathogenic micro-organism? Three distinct septic micro-organisms have, after numerous experiments and careful observations, been mentioned, as being capable when growing under certain extraordinary conditions of assuming pathogenic properties. These three organisms are; A., the common bacillus of hay infusion is said by Buchner to be capable of transformation into bacillus anthracis; B., a bacillus subtilis, present in the air, which, although quite harmless in itself, assumes distinct pathogenic properties when growing in an infusion of the seeds of Abrus precatorius, becoming hereby endowed with the power of causing severe ophthalmia

(Sattler). C. A common mould, aspergillus, which harm, less in itself, when grown on neutral and alkaline material at about body-temperature (38° C.) assumes, according to Grawitz, very poisonous properties, producing in rabbits inoculated with it death, with metastasis of aspergillus and its spores in the various internal organs.

There are in the literature of micro-organisms other cases mentioned, in which such a transformation has been *supposed*, but without any experimental proof, and we need not therefore trouble ourselves more about them.

Let us now review seriatim the above three cases :

A. Dr. Hans Buchner in a paper, which for many reasons may be considered an important one, Ueber d. experim. Erzeugung des Milzbrandcontagiums, &c., published in the Sitzungberichte d. math. physik. Classe d. k. Bair. Akademie d. Wiss. 1880, III. Heft, p. 369, states that he succeeded in transforming the common bacillus of hay infusion, the hay bacillus, into the bacillus anthracis.

The hay bacillus and the bacillus anthracis rank together morphologically under that form which Cohn has named bacillus subtilis.

The two are, however, not quite identical in morphological respects. The hay bacillus is a minute rod or cylindrical-shaped bacillus which by elongation and division produces chains and further threads just like the bacillus anthracis, but in the latter (*i.e.*, bacillus anthracis) the bacilli and their threads are composed of cubical elements, as is shown in stained specimens, and as has been mentioned in a former report, whereas in those of the hay bacillus the elements are rods or cylinders. I have seen, however, many of the short hay bacilli which being constricted, *i.e.*, in the act of division, appear as two short more or less cubical elements placed end to end. It is generally assumed that in hay bacillus the bacilli are always rounder at their ends, whereas the bacilli anthracis are as if straight cut at their ends; but this is not universally the case, since I have seen the bacilli anthracis in cultures with distinctly rounded ends. But, speaking generally, the hay bacillus is a rod more distinctly rounded at its ends, the bacillus anthracis of the blood is not so.

The bacillus anthracis is slightly thicker than the hay bacillus. In artificial cultivations carried on in neutral broth the bacillus anthracis is about twice as thick as the hay bacillus growing in the same fluid, and when both are growing in neutralised hay infusion the two are very conspicuously different from one another, and can at a glance

be distinguished from one another; the hay bacillus being about half the thickness of the bacillus anthracis. In stained specimens, too, the latter is beautifully made up of a row of cubical cells, whereas the former consists of cylinders only.

True, the bacillus anthracis is not always of the same thickness, for, as I have shown, when growing in neutral pork broth it is decidedly thicker than in the blood of an animal dead of anthrax. And also in the blood of different animals the bacillus anthracis slightly varies in thickness, for in the guinea pig's blood it is slightly thicker than in that of the rabbit or sheep.

The hay bacillus is motile, possessed of a flagellum, and therefore capable of locomotion; the bacillus anthracis is not motile. I am quite aware that Cossar Ewart (Quarterly Journal of Microscopical Science, April 1878) states he has seen in a specimen kept under microscopic observation at artificial heating, that the at first non-motile bacillus anthracis is capable of becoming motile. At one or both ends a flagellum grows out from its body. But this observation is unreliable, since Ewart did not guard himself in any way from the accidental introduction of septic bacilli, many of which are motile. Besides he says of the bacilli, which he figures as anthrax bacilli, that they are connected with one another by two fine threads, and that they probably separate from one another and each retains one filament, which is its flagellum. But his observations, so far as they have application to anthrax bacilli, are capable of quite a different interpretation. In every specimen of blood and in every artificial culture bacilli can be seen, in which at one place or more the protoplasm is wanting, owing as I have shown to degeneration; in such places only the empty sheath is present, and of course in fresh specimens this gives the appearance as if the two protoplasmic portions of the bacillus were connected with one another by two fine threads, *i.e.*, the sheath being transparent is seen here edgways.

In no instance has the bacillus anthracis been observed to be motile. I have examined thousands of specimens of fresh bacillus anthracis in the blood and in artificial cultures and I have never seen anything that in the least would lead me to differ from this proposition.

As regards the spores they are of the same aspect and size in both the hay bacillus and bacillus anthracis. The threads in good cultures form in both cases the same bundles more or less twisted and forming convolutions, but

in certain cultures of the bacillus anthracis, *e.g.*, in broth, in which the growth is limited to the bottom of the fluid, the convolutions and the twisted condition of the threads are more pronounced, more cable like.

Hay bacillus being motile, every culture of it is uniformly turbid, the bacilli being capable of moving about, and after a day or two of incubation at 35° C. they form a distinct pellicle on the surface of the fluid, which in further stages becomes complete and thick. These pellicles are composed of a dense feltwork of threads of the bacilli, and in them spore formation is going on.

By shaking the fluid the pellicle sinks to the bottom, and if the fluid is not exhausted yet, a new pellicle is formed of the same nature.

In no culture of hay bacillus are there ever observed those cloudy, fluffy, whitish and transparent convolutions that are seen in cultivations of bacillus anthracis carried on at the bottom of fluid broth, and which have been so accurately described by Pasteur.

Both the hay bacillus and the anthrax bacillus when growing on gelatine mixtures liquefy the gelatine, both of them when growing in meat broth turn the at-first-colourless fluid in the course to incubation to an amber and afterwards to a brown tint.

The hay bacillus is capable of thriving well in acid solutions, it grows copiously in hay infusion, which is of a distinctly acid reaction ; the bacillus anthracis, although capable of making a slight progress in acid hay infusion, does not get far, for degeneration soon sets in ; it thrives best in neutral solutions. Hay bacillus thrives also very well in neutral solutions.

Buchner states, that by successive cultivation of bacillus anthracis under *constant variation of the nutritive material* he saw it assume gradually the properties of hay bacillus. Thus he saw that its mode of growth gradually changed, inasmuch as instead of forming, as the typical bacillus anthracis does, fluffy convolutions at the bottom of the fluid nourishing medium, it gradually showed a tendency to stick to the glass and to the surface of the fluid, and to form a sort of pellicle just like the hay bacillus does. This I consider to be an erroneous interpretation of an easily explained and simple fact. It does not want any of the many successive generations of bacillus anthracis, in which Buchner says he has achieved this transformation, it simply requires two nourishing fluids, in both of which the bacillus anthracis will thrive well, but which fluids differ in specific gravity. Let Buchner do as I have done, let him take two test tubes,

both containing sterile broth, but in one the broth concentrated in the other dilute. Let him inoculate the two test tubes with bacillus taken from the same blood, say of a guinea pig dead of anthrax, let him place them in the incubator at a temperature of 35°–42° C. After two or three days, and more decidedly later, he will notice this very difference in the aspect of the cultures that he lays so much stress on as indicating a change in the physiological character of the bacillus. One test tube, containing the dilute broth, shows the typical fluffy convolutions at the bottom of the fluid; while the other, containing concentrated broth, shows a distinct attempt at the formation of a pellicle. Let him now take out a droplet from this second test tube and inoculate with it two test tubes of the same nature as above, *i.e.*, one containing concentrated broth, the other dilute broth. After two or three or more days of incubation he will find exactly the same differences as above.

These differences and their causes I have explained in detail in a preceding article, and need not here enter again into this matter. So much for this part of Buchner's assertions.

Buchner states that the bacillus anthracis when carried through a large number of successive cultures, at a temperature of 35°–37° C., gradually loses its pathogenic properties. Of this assertion I have said already a great deal in my Report for 1881–1882, and I mention it here merely in connexion with Buchner's other assertions. I have shown that even assuming that Buchner has had in all his cultures the true bacillus anthracis, but for which there is no definite proof, as Koch has so ably pointed out in his critical review of Buchner's work (Mittheilungen aus dem k. Gesundheitsamte, Berlin, 1881, Bnd. I.), Buchner, having tested his cultures on white mice only, has fallen into a serious error, for, as I have shown (Reports for 1881–1882), a culture of bacillus anthracis may have become quite harmless to white mice, but be still very virulent to other animals. In fact, therefore, Buchner's results does not require for its achievement more than one culture, provided this has been kept for several days or weeks without spore formation, as was the case in Buchner's experiments.

As regards Buchner's statement that by successive cultivation of bacillus anthracis at 35°–37° C., this assumes the morphological and physiological characters of hay bacillus, I agree with Koch in regarding this as a complete error. If the cultures are quite safe from contamination nothing of the sort ever happens. I have now for several years carried on such cultures, and have not seen anything of the sort. It is of course clear that if by any accidental contamination,

say at the time of inoculating a fresh tube, a motile septic non-pathogenic bacillus, of which, or of the spores of which, the air sometimes abounds, is introduced, every new culture established from this one will abound in this bacillus, and, as it grows quicker and more easily than the bacillus anthracis, the next cultivations become barren of all the bacillus anthracis, and only the non-pathogenic motile bacillus will be found present. This criticism has been applied by Koch to Buchner's experiments, and I must fully endorse it.

But there is a much more serious statement of Buchner's, —serious, because, if true in nature, it is dreadful to contemplate to what amount of anthrax man and brute may become subject—viz., that he maintains to have succeeded in transforming the hay bacillus into bacillus anthracis, by carrying the former through many generations under ever varying change of soil. It is needless to detail here all these experiments of Buchner, since I do not attach any great value to them, and I should not have troubled myself much about them were it not that one meets in mycological literature, particularly on the part of botanists, an acceptance of Buchner's statement that hay bacillus can change into the pathogenic bacillus anthracis (see Zopf, Die Spaltpilze, Breslau, 1883).

I have repeated Buchner's experiments on rabbits, guinea pigs, and white mice. I have grown the hay bacillus in various kinds of broth, in gelatine broth mixtures, in hydrocele fluid, in peptone fluid, in Agar-Agar and peptone, at temperatures varying between 30° and 38° C., and I have, to put it shortly, never seen that it shows the least tendency to change its morphological characters, that it ever assumes any morphological or physiological character like the bacillus anthracis. I consider this a perfectly hopeless task, and I feel sure any one might as soon attempt to transform the bulb of the common onion into the bulb of the poisonous colchicum.

But Buchner states that with his cultures of hay bacillus, carried through many generations under varying conditions of soil, he inoculated white mice, which died under symptoms of anthrax, and whose blood contained the typical bacillus anthracis. I do not for a moment doubt that he really had mice dying from anthrax after inoculation with cultures of hay bacillus, but I question the admissibility of his interpretation. I believe that some accidental contamination of the culture of hay bacillus with anthrax spores or otherwise must have occurred, and have got overlooked. How liable one kind of infective material is to be invaded by foreign infective matter may be understood from the following examples of its actual occurrence.

It is now admitted on all hands that the experiments of Villemin of producing what is called artificial tuberculosis in guinea pigs, by inoculating the animals subcutaneously with cheesy matter derived from human and bovine tuberculosis or from a guinea pig suffering from artificial tuberculosis, cannot be produced by any other means; it cannot be produced by ordinary, *i.e.*, non-tubercular cheesy or other pus,* nor by setons (as once thought by Wilson Fox and Sanderson) setting up chronic caseous inflammations in the skin of guinea pigs, nor by chronic mechanical irritation, *e.g.*, insertion into the peritoneal cavity of bits of gutta percha or other substances producing chronic peritonitis (as was thought by Cohnheim and Fraenkel), but, as Cohnheim now tersely put it, tuberculosis can be produced only by matter derived from a tubercular source, and anything that produces this tuberculosis is derived from a tubercular source. Dr. Wilson Fox, after the very important experiments performed by Dr. Dawson Williams, according to which chronic inflammation in the skin of guinea pigs produced by setons, is in no case followed by tuberculosis, has conceded that in his earlier experiments there must have entered some error in the use of the materials. Cohnheim has conceded the same. It is clear that these observers, while working at the same period with both tubercular and non-tubercular matter, must have had, in the course of experiments with the latter substance, accidental contamination with the former, and hence had the guinea pigs inoculated by them with non-tubercular matter nevertheless affected with tuberculosis. Dr. Williams, who had no contamination to fear, working with non-tubercular matter only, had consequently no accidental contamination. This shows us how dangerous, as regards reliability of results, it is to work in one laboratory with different infective materials at the same period.

I have myself experienced some very curious results bearing on this very point. During the last year I have seen the following cases of accidental contamination occur: I work in the laboratory of the Brown Institution, which comprises a suite of rooms. Although working extensively on anthrax, I generally limit myself to one room only. A friend of mine, who one day injected into a vein of a guinea pig blood taken out from a blood vessel of a dog suffering from distemper, found, to his great disappointment, the guinea pig dead after two days under the typical symptoms of anthrax, the blood of this animal teeming with the characteristic bacilli. The hypodermic syringe used in this experiment for injection

* Compare: Watson Cheyne, Practitioner, April 1883.

had not been previously used by me in my anthrax experiments, since I never use a syringe in my inoculations, but only glass pipettes freshly made and drawn out into a fine tube. The experiment was performed in the room adjoining the one in which my anthrax investigations were being carried on, but I was in the habit of making every day a good many specimens of anthrax cultivations and spores, so that there must have been a good many of these spores distributed on the table and floor, and probably found their way into the wound of the guinea pig at the time the above experiment was made.

Another gentleman working in the laboratory of the Brown Institution intended to inoculate several guinea pigs with human tubercles. For this end he mashed up in saline solution, in a clean mortar, a bit of human lung studded with tubercles. He did this in my room on the same table on which I was working with anthrax. One of these guinea pigs, inoculated with human tubercle, died before the second day was over of typical anthrax. Its blood was teeming with the bacillus anthracis. Such an accidental anthrax of a guinea pig inoculated with tubercular matter occurred again. In both cases freshly drawn out glass capillary pipettes had been used for performing the inoculation, and also the other instruments had been carefully cleaned before the inoculation.

I myself had the following accidental contaminations:—

A guinea pig had been inoculated with a culture of bacillus anthracis, which I did not expect would produce anthrax, the culture not being capable of starting new cultures, the bacillar threads being all in a state of degeneration. The animal, of course, remained unaffected. Some weeks afterwards inspecting the guinea pig to my surprise I found the inguinal lymphatic glands at the side of the former inoculation greatly swollen, filled with cheesy pus. The animal was killed and was found to be affected with general tuberculosis, the cheesy matter of the tubercular deposits containing the tubercle bacilli. Comparing my notes on this animal with those of my friend Lingard, we found that on the very day on which I inoculated the animal with my anthrax culture we had inoculated several other guinea pigs with tubercular matter. This tubercular matter was prepared in the same room in which I prepared the fluid for my anthrax inoculation, but the instruments in the two sets of experiments had not been the same.

A rabbit was inoculated with a culture of bacillus anthracis which I did not expect would produce anthrax. The animal remained unaffected with anthrax, but died after

four weeks with the symptoms of extremely well-marked tuberculosis—in fact, the best marked case that I have seen —of both lungs, spleen, liver, and kidney. All tubercular deposits contained the tubercle bacilli.

Also in this instance inoculations with tubercular matter had been going on at the same time, when I meant to have inoculated nothing else but a culture of anthrax bacilli.

I think all these facts taken together prove unmistakeably that working with two contagia in the same laboratory and at the same period, accidental contamination is of no rare occurrence. And this applies with equal force to Buchner's experiments. Buchner worked extensively with anthrax cultures in the same laboratory, and at the same time he had those successful cases of anthrax in mice which he thought to have inoculated with cultures of hay bacillus, and accidental contamination probably was the result. Buchner himself has experimentally shown that anthrax virus in the shape of spores can by inhalation produce anthrax, and, therefore, this is another argument against his above cases of positive results. I am assuming that his cultures of hay bacillus were really free of spores of bacillus anthracis; but, seeing that his anthrax cultures were probably contaminated with hay bacillus, I do not see why, by some chance, one of his tubes which he thought he inoculated with hay bacillus should not have been accidentally contaminated with the spores of bacillus anthracis, of which there must have been many floating about in the air of the laboratory.

If Buchner could show us that in a laboratory, in which for some considerable time anthrax cultures, anthrax animals, and examinations of anthrax bacilli had not been carried on, cultivation of hay bacillus ultimately yields a fluid which produces typical anthrax, then I should be perhaps prepared to concede his proposition of a transmutation of hay bacillus into bacillus anthracis. Such a proposition is of the widest importance, and therefore its proof ought to be beyond cavil, there ought to be no chance of a possibility of error. Such proof Buchner has not given, and I cannot therefore accept his interpretation.

B. The second instance in which the transformation of a common septic into a specific or pathogenic organism has been experimentally achieved, or I should rather say has been stated to have been achieved, is the jequirity bacillus. In 1882 the well-known opthalmologist M. L. de Wecker in Paris drew attention to the therapeutic value of the seeds or beans of Abrus precatorius, a leguminosa common in India and South America. The people of Brazil use it under the

name jequirity as a means to cure trachoma or granular lids. De Wecker after many experiments found that a few drops of an infusion made of these seeds causes severe conjunctivitis, in the course of which, no doubt, trachoma is brought to disappearance and cure, and it is accordingly on the continent and in this country now used for this therapeutic object. [I am informed by my friend Dr. T. Lewis, formerly of India, now pathologist at the Netley Army Medical School, that the people in some parts of India know the poisonous properties of these seeds, and use it for inoculating with them subcutaneously cattle; in consequence a severe inflammation is set up and the animals die of some sort of septicæmia. This is done for the sake of simply obtaining the hides of the beasts.]

Sattler, in a very important and extensive research (Wiener medic. Wochenschrift, N. 17–21, 1883, and Klin. Monatsbl. f. Augenheilk, June 1883) ascertained that when an infusion of the jequirity seeds is made of the strength of about half per cent., this infusion after some hours to a few days contains numerous bacilli, motile, capable of forming spores, and in all respects identical with a bacillus subtilis. The bacilli are about 0·00058 mm. thick, and from 0·002 to 0·0045 mm. long. They form a pellicle on the surface of the infusion, and in the bacilli of this pellicle active spore formation is going on. The bacilli grow and multiply well at a temperature of about 35° C., but also, only slower, at ordinary temperature. Sattler cultivated artificially the bacilli on blood serum gelatine and meat extract peptone gelatine, both solid media, and continued their growth through several successive cultivations. Both the infusions of the jequirity and the bacilli taken from these artificial cultures inoculated into the conjunctiva of healthy rabbits produce severe ophthalmia, leading to the production of great œdematous swelling of the conjunctiva and eyelids, and temporary closure of the latter, and to the secretion of purulent exudation. Both the exudation and the swollen lids are said to contain infective bacilli and their spores. Sattler ascertained by many experiments, that none of the bacilli and the spores distributed in the atmosphere had those specific properties, viz., to excite ophthalmia, as long as they grow in other than jequirity fluid, but having had access, *i.e.*, having entered the jequirity infusion assume here this specific power. There is no doubt that Sattler worked the whole problem with great care, worked out all points connected with it in great detail, and for this reason his work was considered to have for the first time unmistakeably established that a harmless bacillus, owing to the particular

soil in which it grew, assumes definite specific or pathogenic properties. To me this jequirity bacillus had a great interest, since I was particularly anxious to get hold of such an organism, in order to see whether and how it can again be made harmless. For if ever there was a good case, a case in which a previously harmless micro-organism had by some peculiar conditions become specific: this was a case, and therefore it must be here possible by altering its conditions of life again to transform it into a harmless being. The theoretical and practical importance of such a case must be evident to every one who has at all devoted any thought to the relation of micro-organisms to disease. The whole doctrine of the infectious diseases, I might almost say, is involved in such a case, for if in one case it can be unmistakeably proved that a harmless bacterium can be transformed into a pathogenic organism, *i.e.*, into a specific virus of an infectious malady, and if this again can under altered conditions resume its harmless property, then we should at once be relieved of searching for the initial cause in the outbreak of an epidemic. But in that case we should be forced to contemplate as floating in the air, in the water, in the soil, everywhere, millions of bacteria which, owing to some peculiar unknown condition, are capable at once to start any kind of infectious disorder, say anthrax (Buchner), infectious ophthalmia (Sattler), and probably a host of other infectious diseases, and thus to form the starting point of epidemics. And the only redeeming feature, if redeeming it can be called, in this calamity would be the thought that the particular bacterium would by and by, owing to some accidental new conditions, again become harmless.

These were the reasons, and good reasons I think they were, which prompted me to inquire into the jequirity bacillus and jequirity ophthalmia, and after a very careful and extensive series of experiments, to be described presently, I have proved beyond any doubt that the jequirity bacillus, *per se*, has no more power to create an infectious ophthalmia than Buchner's hay bacillus had of creating anthrax.

The following experiments prove this conclusively :—

The seeds of jequirity (Abrus precatorius) are crushed and powdered, the perisperm is removed, and of the rest an infusion is made of about the strength of half per cent. with distilled water, previously boiled and contained in a flask previously sterilized (by heat) and plugged with sterile cotton wool. The infusion is made while the water is still tepid. After half an hour the infusion is filtered into a fresh sterile flask, plugged with sterile cotton wool, the access of

air being limited as much as possible. This is effected by keeping the cotton wool in the mouth of the flask around the end of the glass filter. The filtered fluid is of a slightly yellowish-green colour, and is almost neutral and limpid. A small quantity is withdrawn with a capillary glass pipette freshly drawn out, and from this several test tubes containing sterile nourishing material (peptone solution, broth, Agar-Agar and peptone) are inoculated; and from the same pipette, and at the same time, several eyeballs of healthy rabbits are inoculated, by placing a drop or two of the infusion under the conjunctiva bulbi. The test tubes are placed in the incubator and kept there at 35° C. After 24 hours all eyeballs are intensely inflamed, the eyelids closed and swollen, and a large amount of purulent secretion is present in the conjunctival sac, but all the test tubes remain perfectly limpid. No growth has made its appearance, and they remain so.

In a second series the infusion prepared in the above manner is used 15 minutes after it is made and used as above, for inoculation of test tubes and eyeballs. The fluid in the test tubes after incubation remains limpid, the eyeballs all become inflamed. In both series the amount of fluid inoculated into the test tubes is more than twice as great as that injected into the eyeballs. From this it is quite clear that the fluid used for inoculation of the test tubes was barren of any micro-organisms, and nevertheless it possessed a powerful poisonous principle. I do not mean to say that the infusion as a whole contained in the flask contains no organisms, but that the small quantity of the fresh infusion that was used for the inoculation of the test tubes and eyeballs contained none is absolutely certain. When such a flask is placed in the incubator, after 24–48 hours or later, there are found in it large quantities of bacilli, the spores of which must have entered from the air during the process of preparing the infusion. The bacilli are such as described by Sattler, they soon form spores in the usual way. Such an infusion is very poisonous, just like the fresh one. Sattler has shown, and this is easily confirmed, that the spores of these bacilli stand boiling for a few minutes, without losing their power to germinate. Consequently, if such a poisonous infusion full of bacilli and spores be boiled for half a minute the spores are not killed; proof for this: that if with a minute dose of this spore-containing boiled infusion any suitable sterile nourishing material in test tubes be inoculated, and then these test tubes be placed in the incubator at 35° C., after 24–48 hours the nourishing fluids are found teeming with the jequirity bacilli; *but no amount of this material produces the least symptom*

of ophthalmia. Every infusion of jequirity loses its poisonous activity by boiling it a short time, ½–1 minute, and hence the above result.

In this respect the poisonous principle of jequirity infusion comports itself similar to the pepsin ferment, which, as is well known, is destroyed by short boiling.

If an infusion is made, as above, and after 15 minutes it is filtered and then is subjected to boiling for ½–1 minute, it will be found to have become absolutely non-poisonous, but not sterile, placing it in the incubator after 24–28 hours, vast numbers of the jequirity bacillus are found in it. But no amount of this fluid is capable of producing the slightest symptom of ophthalmia.

A large per-centage of the rabbits, whose conjunctiva has been inoculated with the fresh unboiled poisonous infusion, die after several, 3–8, days. The eyeballs and eyelids are intensely inflamed, as stated above, the skin and subcutaneous tissue of the face, neck, chest, and even abdomen, is found enormously œdematous, the pericardium pleura, lungs, and peritoneum very much inflamed, their cavities filled with a large quantity of exudation. The exudations of the conjunctiva, pericard, peritoneum, the œdematous skin and subcutaneous tissues contain no infectious property and no bacilli or spores of any kind if examined in the living animal or immediately after death.

There is one point which requires careful consideration, it is this: Sattler states that he has cultivated the bacillus, taken from a poisonous jequirity infusion, through several successive generations on solid material, and with the new cultures he was able to produce the jequirity ophthalmia. I have no doubt whatever that this is really the case, but it bears an interpretation different from the one Sattler gave it. Sattler, and most pathologists, would, of course, say this: if any micro-organism taken from a soil that possesses infective properties be carried through many successive artificial cultivations, all accidentally adhering poisonous matter hereby becomes so diluted that it practically may be considered to have been lost; that is to say, that the micro-organisms of the further generations have become altogether free of it. If the organisms of these further generations still possess the same poisonous property as the original material, then we must conclude that this poisonous principle is identical with the micro-organism. I do not agree with this whole chain of propositions, although I agree with some parts. If a micro-organism be carried through several successive cultivations in a *fluid medium*, always using for inoculation of a new culture an infinitesimal dose, and as nourishing medium a comparatively large quantity of fluid, then, no doubt, carrying on the cultivations through four, five, or

six successive cultures any accidentally adhering original matter becomes practically lost, and if then the organism still possesses the same poisonous action to the same degree as the original material, then no doubt the conclusion that organism and poison are in this case identical becomes inevitable. But this is not the case with the jequirity bacillus. Taking from a poisonous jequirity infusion full of the bacilli one to two drops, and inoculating with it a test tube containing about 4 to 5 CC. of nourishing material, and using this at once *without previous incubation* we find that this so diluted fluid still possesses poisonous action. Precisely the same result is obtained when taking from a perfectly fresh jequirity infusion, *i.e.*, before any organisms have made their appearance, one to two drops and diluting them with 4–5 CC. of distilled water and using of this diluted fluid one to two drops for inoculating the conjunctiva of healthy rabbits, severe ophthalmia will be the result. Carrying on the cultivation of these bacilli, started from a poisonous infusion, for a second generation in fluid medium, no trace of any poisonous action can be now detected, any quantity of such a cultivation is incapable of producing ophthalmia. Sattler used in his cultivations solid nourishing material, on the surface of which he deposited his drop of poisonous jequirity infusion, containing the bacilli ; after some days' incubation the bacilli having become greatly multiplied, he took out from this second culture a drop and transferred it to a new culture tube of solid material, and so he went on ; every one of these cultures possessed poisonous action. Clearly it would, since he always used part of the original fluid deposited on the surface of the solid nourishing material. Part of this (being gelatine) became by the growth liquefied, but considering that Sattler started with infusions of considerable concentration—he left the seeds for many hours and days in the infusion—it is not to be wondered at that this would bear a considerable amount of dilution and still retain its poisonous properties. From all this we see, then, that the jequirity bacillus *per se* has nothing to do with the poisonous principle of the jequirity seeds, but that this principle is a chemical ferment in some respects (in its inabilityto withstand boiling) similar to the pepsin ferment.*

C. The third case, in which an experimental attempt has been made to transform a common septic into a specific or

* Since this has been in print I became possessed of a most valuable memoir by Messrs. Warden and Waddell, published in Calcutta 1884, detailing a large number of observations on the poisonous principle of the seeds of abrus precatorius. Their observations are in complete harmony with my own observations, and they have unmistakably established : that the active principle, abrin, is a proteid, closely allied to native albumin ; that its action is similar to that of a soluble ferment ; that it can be isolated ; and that it is present, not only in the seeds, but also in the root and stem of the abrus precatorius.

pathogenic micro-organism is exemplified by the common mould, aspergillus, a mycelial fungus. Grohe (Berlin Klin. Wochenschrift., 1871) was the first to show, that the introduction of the spores or conidia of some species of aspergillus into the vascular system of rabbits produces death under the symptoms of general infection and metastasis in the various internal organs, due to localised foci of growth of these spores into mycelial threads. Lichtheim (Berl. Klin Woch., N. 9 and 10, 1882) showed that such a mycosis in rabbits cannot be produced by the spores of aspergillus glaucus, but by those of aspergillus flavescens and fumigatus. Grawitz (Virhow's Archiv, vol. 81, p. 355), who made a careful experimental study of this mycosis, found that no matter whether these spores had been injected into the vascular system or into the peritoneal cavity, there are developed in the kidney, liver, intestines, the lungs and the muscles, occasionally also in the spleen, the marrow of bones, the lymphatic glands, the nervous system and the skin, minute foci, composed of spores and mycelial threads with imperfect fructification organs. Grawitz thought the spores of ordinary mould (penicillium and aspergillus) by being cultivated at high temperatures, 39°–40° C., and in alkaline media—they, as a rule, thrive only at ordinary temperature and in acid media—can by gradual acclimatisation, as it were, to those higher temperatures and alkaline media assume the power to thrive also when introduced into the body of an animal; this they ordinarily cannot do, not being accustomed to grow at blood heat and in an alkaline soil. Thus under those varied conditions of growth they are capable, thought Grawitz, to assume pathogenic properties. This view has been proved to be incorrect, for Gaffky (Mittheil aus d. K. Gesundheitsamt, I., 1881), Koch (Berlin, Klin. Woch. 1881), and Leber (Berl. Klin. Woch. 1882) have shown that the spores of those species of aspergillus which possess these pathogenic properties possess them *ab initio*, and not because they are grown under the different conditions mentioned by Grawitz. Amongst the many species of aspergillus there are some, the spores of which differ in this respect from the others, that they are capable of thriving at those high temperatures and in the animal body (rabbits), and thus are capable of producing the said mycosis in these animals; that is to say, the spores of some species of aspergillus possess pathogenic properties *ab initio*, while the majority of other species do not behave in this way, and cannot acquire such a function under any circumstances.

Thus also this third case of a transformation of a common into a specific organism due to altered conditions of growth falls to the ground.

Section IV.

It may be now asked, how about those cases in which by injection of very small quantities of putrid organic substances, pyæmia and septicæmia, has been produced in rodents? Take the case of Davaine's septicæmia in rabbits. This disease has been produced in rabbits by Davaine, Coze and Feltz, and by many other observers by injecting into the subcutaneous tissue of healthy rabbits small quantities of putrid ox's blood. The rabbits die in the course of a day or two, and their blood is found teeming with minute organisms, which prove to be bacterium termo; every drop of this blood possesses infective properties, when inoculated into a rabbit it produces septicæmia with precisely the same appearances as before. Pasteur and Koch have succeeded in producing septicæmia in mice and rabbits, and especially in guinea pigs, by inoculating them subcutaneously with garden earth or with putrid fluid. This is Pasteur's septicæmia, or Koch's malignant œdema; it is characterised by œdema at the seat of inoculation, and spreading hence into the subcutaneous tissue of the surrounding parts. The animals die generally in 24–72 hours.

Koch has produced by injection of small quantities of putrid fluids into the subcutaneous tissue of mice a peculiar septicæmia; the animals sometimes die in 40–60 hours and the white corpuscles of the blood are found crowded with exceedingly minute bacilli. Koch succeeded also in producing a pyæmia in rabbits by injection of putrid fluids, and this pyæmia is characterised by zoogloœa of minute micrococci being present in the blood vessels. Further, a progressive necrosis in mice by inoculating them with putrid fluids, the necrosis being due to the growth of micrococci and spreading from the seat of inoculation, and destroying as they spread all the elements of the tissue. All these cases have been minutely described by Koch in his classical work, Die Aetiologie der Wundinfectionskrankheiten, Leipzig, 1879.

Now do these cases prove that septic micro-organisms, living and thriving in putrid organic fluids, can when introduced into the body of animals, owing to some peculiar unknown condition, so change as to produce a fatal infectious disease? I must say with Koch, who has very ably discussed all these points, no. Those organisms which are connected with the above morbid processes possess this pathogenic power *ab initio*, not due to any peculiar condition of growth.

Amongst the legion of different species of micrococci and bacilli occurring in putrid substances, the greater majority are quite harmless, when introduced into the body of an animal they are unable to grow and to multiply, and there-

fore are unable to produce any disturbance. But some few species there are, which although ordinarily growing and thriving in putrid substances possess this power, that when introduced into the body of a suitable animal set here up a specific disease.

One of the best studied cases is that of the bacillus anthracis. This organism is capable of growing well and copiously outside the body of an animal, it thrives well wherever it finds the necessary conditions of temperature, moisture, and nitrogenous material; when it finds access into the body of a suitable animal it produces the highly infectious fatal malady known as anthrax. The micrococcus of erysipelas is now well known through the admirable researches of Fehleisen to be capable of existence and multiplication outside the animal body; it grows well in artificial cultures, so does the tubercle bacillus of Koch, so does the bacillus which I described of swine plague and of which more in a succeeding article, and so do other micro-organisms. Davaine's septicæmia in rabbits, Koch's septicæmia in mice, &c., &c., cannot be produced by every putrid blood or putrid organic fluid, only by some, only now and then, *i.e.*, when the particular micro-organism, capable of inducing the disease, is present in those substances, and then only when it finds access into a suitable animal. Davaine's septicæmia of rabbits cannot be induced in guinea pigs, Koch's septicæmia of mice cannot be induced in guinea pigs, anthrax bacilli cannot induce the disease in dogs, and so with the other micro-organisms.

We conclude then from this that some definite micro-organisms, although as a rule existing and growing in various substances of the outside world, have the power when finding access into the body of a suitable animal to grow and thrive here also, and to induce a definite pathological condition. But this power they have *ab initio*. Those that do not possess this power cannot acquire it by any means whatever. Just as there are species of plants which act as poisons to the animal body, and other species of plants which, although belonging to the same group and family, and although very much alike to the others, have no such power and cannot acquire such a power by any means, so there are micro-organisms which act pathogenic and others which are quite harmless. The latter remain so no matter under what conditions and for how long they grow.

Section V.

I have made a series of experiments with the view to obtain pure cultivations of definite septic micro-organisms, of

which the morphological characters could with precision be ascertained and which at starting were tested to be barren of any power of inducing disease. I have cultivated these in pure cultivations for many generations, and under varying conditions, and then I have tried to find whether they have or have not acquired any pathogenic property; these experiments and their results I will now proceed to describe.

1. *Small spherical micrococcus.*—Blood of healthy rabbit had been withdrawn from the vein of the ear, and with it inoculated sterile neutral pork broth contained in sterilized test tubes plugged with sterilized cotton wool. The inoculation was performed in the manner usually followed by me and described in my Report for 1881–82, viz., a recently made glass capillary pipette is drawn out at one end in a fine point, as fine as the point of a needle, the vein is opened and this end of the tube is introduced, and a drop of blood is allowed to ascend, which it readily does by capillary attraction. With clean forceps the plug of the test tube is drawn up, but not lifted up altogether; the pointed end of the pipette is then pierced through the plug and pushed down till it touches the fluid in the test tube, and a trace of the blood is allowed to flow out, the pipette is then altogether withdrawn and the plug pushed down into its former position. The test tube is then placed in the incubator and kept at a temperature of about 30°–35° C. In some instances, no doubt in consequence of accidental contamination at the moment of receiving the blood into the capillary pipette, I have thus obtained a micrococcus possessed of these definite characters :—(*a.*) It grows well at the above temperatures in the pork broth, rabbit's broth, beef broth, and chicken broth; it grows less well, but still tolerably good, in the peptone sugar solution, in hydrocele fluid, and in the Agar-Agar peptone mixture. (*b.*) The fluids inoculated with it become after 24–48 hours uniformly turbid, which turbidity increases during the following days. (*c.*) It forms after several days' growth in the incubator a whitish powdery sediment, but not of great amount. (*d.*) After exhaustion of the fluid, which occurs the sooner the higher the temperature is, this latter fluid becomes quite clear, the growth all settling down as a small mass of precipitate. The colour of the fluid does not become altered by the growth. (*d.*) The micrococcus is very minute, not more than 0·0005 mm. in diameter, isolated or in dumb-bells, and in smaller or larger groups, zoogloea; it does not form at any time a pellicle. (*e.*) When sown on to the surface of solid nourishing material, Agar-Agar peptone mixture, or gelatine broth mixture, it does not grow well or not at

all, if limited to the surface, or only slightly if at the time of inoculation it was placed into a canal extending from the surface into the depth a little way. In the latter case a slight growth will be noticed extending into the depth. It is quite clear from this that exposure to air is detrimental to the growth of this micrococcus.

With this micrococcus were made a large number of cultures in pork broth, beef broth, rabbits' and chicken broth, hydrocele, peptone solution, Agar-Agar and peptone mixture, through many generations, and the cultures were used for the inoculation of mice, guinea pigs, and rabbits. The first two kinds of animals were absolutely refractory, but in rabbits I had out of a large number of animals, amounting to several dozens, three fatal cases. These cases were:—

Case 1. A rabbit, we will call it A., was inoculated into the subcutaneous tissue of the ear on June 18 with a VII. culture in pork broth.

On July 29, *i.e.*, 41 days after, it died. At no time was there any inflammation noticed at the point of inoculation; after death this latter could not be seen. The left lung was much inflamed, almost entirely solid; both kidneys were enlarged and showed parenchymatous nephritis; other organs appeared natural. The microscopic examination of the blood and diseased organs did not reveal any trace of micrococci or any other organisms.

Case 2. Inoculated a rabbit B. subcutaneously in the ear, on July 6, with a X. cultivation in beef broth. The place of inoculation showed much swelling and inflammation after a day or two, and spread gradually in the next days towards the root of the ear. The animal died on August 2, *i.e.*, 27 days after. At the place of inoculation there was a hole of the size of a sixpence, passing through the whole thickness of the ear, and the hole was plugged by a dark scab, easily taken out; the tissue at the margin of the hole was much swollen and infiltrated. From here towards the root the tissue was swollen and inflamed; spleen and liver normal. The small intestine filled with mucous. Both lungs showed much hyperæmia, and there were in them pneumonic patches. Peritonitis with clear exudation, both kidneys large and pale. With juice of the swollen margin of the defect of the ear inoculated test tubes of sterile broth and two rabbits C. and D. Neither the tubes nor the rabbits showed any result. The swollen ear was examined in microscopical sections, but no trace of micrococci or any other organisms could be detected.

Case 3. A rabbit E. was inoculated, subcutaneously in the ear on July 6, with the same culture X. as used in the rabbit B. The place of inoculation appeared normal after a few days and remained so. The animal died on August 3, *i.e.*, after 28 days. On post-mortem examination the place of inoculation could not be noticed. The only change in the internal organs was hyperæmia of the small intestine, its cavity distended by mucous; both lungs showed much hyperæmia. No micrococci or other micro-organisms could be detected in the blood or the tissue of the lungs.

From this we see, then, that, although death ensued in these animals, no micrococci could be found in any of them, and we must therefore consider their death as in no connexion with the inoculation. I have to state here that about this time I had several deaths of rabbits that had not been subject of any experiment.

2. *Large spherical micrococcus.*—In the same manner as above, *i.e.*, from blood of healthy rabbits, I have obtained a micrococcus which is altogether of a different nature; (*a*) it is larger than the former, its diameter is about twice as great or greater, it occurs singly, in dumb-bells, and in small groups; (*b*) it grows more copiously in broth (pork, beef, rabbit, and chicken), the fluids become turbid and the growth after several days' incubation is much more copious than in the case of the former micrococcus; (*c*) it forms after several days' incubation at 35° C. a distinct pellicle, composed of continuous masses of zoogloœa. It forms also a large amount of sediment at the bottom of the fluid, but this sediment is not powdery but tenacious, is more copious, is not whitish as in the former micrococcus, but of a distinct orange colour, so is also the pellicle; (*d*) during the progress of the growth the at first colourless nourishing fluid (broth) assumes a distinct yellowish or light orange colour; (*e*) this micrococcus grows well when exposed to the air as might be expected from the fact of its forming a good pellicle, hence it grows copiously when sown on the surface of solid nourishing material, as gelatine broth mixture which it does not liquify, or Agar-Agar peptone mixture, and the growth remains limited to the surface. (*f.*) It does not grow at all or only slightly in peptone sugar solution.

With this micrococcus I have made a very large number of experiments, growing it through a great many successive cultures in various fluid and solid media, pork broth, beef broth, rabbit's broth, peptone solution, hydrocele, Agar-Agar mixture, gelatine broth mixture, at various temperatures, some at ordinary temperatures, others at a

temperature of 25° C., still others at temperatures varying between 30° and 38° C., and I have inoculated with them a large number of rabbits (over 40), and out of all these I had death in three animals only. These were (1) a rabbit that had been inoculated on June 16 with a I. culture of this large micrococcus in pork broth, and again on June 21 with the second culture of the same in pork broth. It died on June 28, *i.e.*, after one week. There was no change at the place of the inoculation. The animal was much emaciated.

On post-mortem examination the following changes were found in the internal organs: peritonitis with copious clear exudation; bladder distended with sanguineous urine, in the fundus the mucous membrane showed a large number of hæmorrhagic specks; small quantity of clear pleural exudation; pneumonia of both lungs; liver and spleen appear normal.

No micrococci were found in the blood or any of the exudations, none in the lungs, none in the mucous membrane of the fundus of the urinary bladder.

We cannot therefore consider this as a successful case. What the cause of the disease was I cannot say, but must mention that at about this time, *i.e*, about Midsummer, and also later in the summer, I had several rabbits dying with similar symptoms, but without any cause being ascertainable, they had not been touched by any experiment.

(2.) A rabbit that had been inoculated on June 21 with the same II. culture of the large micrococcus used in the preceding case. The animal died on July 27, *i.e.*, about five weeks after inoculation. The appearances were similar to those of the preceding rabbit. There happened to die on this same day a rabbit under the same symptoms, but on which no experiment of any kind had been performed. In neither case was there found any trace of a micrococcus.

(3.) A rabbit was inoculated into the ear on July 6, with a VI. culture of the same large micrococcus in pork broth. It died on July 23, *i.e.*, after 17 days; skin about the seat of inoculation is much swollen, dark red, and the wound at the place of inoculation is covered with a scab. Both lungs show under the pleura numerous minute hæmorrhagic spots; the mucous membrane at the pyloric end of the stomach, and at the commencement of the duodenum, shows numerous small hæmorrhages; the left cornea shows near its upper margin a small patch of opacity. All other organs appear natural.

No micrococci could be found anywhere, except in that opac patch of the left cornea. This patch was on careful

examination found to be quite superficial, involving only the anterior epithelium; it consisted of dense masses of micrococci, and swollen and disintegrating epithelial cells. These micrococci were used for inoculation of the cornea and also of the skin of fresh rabbits but produced no result. In aspect this micrococcus could not be distinguished from the large micrococcus, but since no trace of micrococci could be detected in the blood and in the diseased organs, I am unable to say whether its presence in the cornea is of any special significance. For these reasons I am inclined to think it is accidental, and also not pathogenic on account of its being superficial, and not capable of producing any pathological change when inoculated into fresh animals.

With the blood of each of these three animals fresh animals were inoculated, but without any result.

These are, then, the only cases in which death followed after the introduction of the above large micrococcus, and it is almost certain that the cause of the death did not stand in any immediate connexion with the micrococcus, for if it did we ought to have been able to find them in the blood or the diseased organs.

3. *Micrococcus in serum of blood.* Serum of sheep's blood solidified after Koch's plan (viz, by heating it gradually up to 60°–65° C. till solid) and then kept in a flask in the incubator at 35° was seen to contain, after several days, crowds of small spherical micrococci of about the size of micrococcus 1, singly, in dumb-bells, and in small zoogloœa, and in fine chaplets of various lengths, more or less curved and wavy. This micrococcus grows best in broth, it grows tolerably well in peptone solution and in Agar-Agar and peptone mixture, but does not grow well in hydrocele fluid. The fluids inoculated with it and exposed to a temperature of 30°–35° C. become uniformly turbid after 24–48 hours. In the next days this turbidity increases, and when after some days the fluid has become exhausted the growth settles at the bottom of the fluid as a very copious loose whitish precipitate. The fluid itself remains colourless and clear. It does not form a pellicle and therefore differs, taking all these conditions together, both from micrococcus 1 and 2.

With this serum micrococcus taken from the above serum several tubes of sterile pork broth were inoculated. Kept in the incubator at 35° C. for three days, they contained a copious crop of the micrococcus. From this culture two rabbits were inoculated, both into the subcutaneous tissue of the ear and of the thigh. These rabbits we will call Nos. I. and II.

Rabbit I. died after 20 days. The animal was very

emaciated, and on post-mortem showed the following appearances:—

Place of inoculation in ear and thigh not visible; no swelling of inguinal glands; spleen is much enlarged and permeated by numerous firm white nodules, easily separating from the surrounding tissue; their size varies between that of a millet grain and that of a small pea; liver contains the same nodules but less numerous; kidneys large, contain also a few of the nodules; both lungs contain a few of the same nodules; mesenteric glands large, firm, white; cœcum contains in its wall the same white nodules.

The appearances were exceedingly similar to those one meets with when rabbits are affected with artificial tuberculosis; in fact, one would, without hesitation, pronounce it a case of very marked artificial tuberculosis, the more so since another rabbit affected with tuberculosis, and dead after four weeks, showed precisely the same appearances of the spleen, liver, kidneys, and lungs, except that the lungs were more affected than in rabbit I. In this rabbit (tuberculosis) the microscopic examination of the diseased organs, fresh and after hardening, stained with Weigert's mixture of anilin dyes, revealed the presence of innumerable masses of tubercle-bacilli and cultures made in Agar-Agar peptone mixture (solid) from the nodules of the fresh lung yielded, after 12–14 days' incubation at 38° C., a good crop of the typical tubercle-bacilli. But in the above rabbit I. no such bacilli could be detected, neither the examination of the fresh nor of the hardened organs, nor cultures made from the spleen, yielded a trace of the tubercle-bacilli or of any other micro-organisms. Nor did the inoculation of rabbits with the nodules of the fresh spleen produce any result.

Rabbit II. remained perfectly well. Other rabbits inoculated with the serum micrococcus of the same and of many further cultures remained quite unaffected.

We see, then, that also this serum micrococcus remained harmless after many generations.

4. *Micrococcus* derived from rabbit's blood as in the first two cases. Is as large as micrococcus 2, but differs from this, that it forms beautiful chains, that it does not grow well on the surface but that it grows copiously in the depth of the fluid broth. It grows well in pipettes filled with broth and hermetically sealed. It has no colour and does not produce any change in colour of the fluid in which it grows.

It forms a copious loose precipitate at the bottom of the fluid after several days' incubation.

A very interesting appearance is noticed in the chains

of this micrococcus, consisting in this: (*a*) that the individual micrococci are connected with one another by a bridge of a hyaline substance; (*b*) that some few members of the chain are elliptical and twice or thrice as big as the spherical ones.

This micrococcus 4, obtained from the blood of rabbit, was cultivated in test tubes with broth for many generations; it was used for inoculation of a great many rabbits, but in no case did it produce the slightest effect, locally or generally.

5. *Bacterium termo.*—From a glass vessel, in which in the laboratory of the Brown Institution we keep our distilled water, I am always able to obtain this organism, by inoculating broth with a drop of the distilled water, used unboiled. Boiling destroys it. This bacterium termo has the ordinary size, has the motility and grows in the way as described by Cohn in his Beiträge zur Biologie d. Pfl. II. It is a minute rod, about 0·0015 mm. long, in thickness about half the long diameter, sometimes slightly constricted in the middle (first stage of division into two), rounded at the ends, possessed of motility, *i.e.*, of a flagellum. It grows well and copiously in any broth, Agar-Agar peptone mixture (solid); it does not liquify the Agar-Agar peptone mixture, but there appear in it numbers of gas bubbles. In fluids it makes the medium uniformly turbid and thickish. Kept for two weeks in the incubator at 30°–35° C. it altogether dies, no new cultures can be started with it.

Many cultures were made with this bacterium termo, carried on from one culture to a next, and they were tested on rabbits and mice. No result of any kind was obtained. Neither a first, nor a second, nor a tenth, twelfth, and so forth generation, carried on in the same nutritive material or carried through several culture media, produced any local or general disturbance in the rabbits inoculated with them, *except in one instance*, when a rabbit was inoculated with a second cultivation. This culture was started in rabbits' broth from a culture tube of rabbits' broth in which, owing to inoculation with the distilled water above mentioned, a good growth of bacterium termo had taken place. With this second culture, after three days' incubation at 35° C., two rabbits, which I will call here for distinction *a* and *b*, had been inoculated in the subcutaneous tissue of the thigh. One animal, rabbit *a*, died 12 days after; it was found very emaciated; no trace of the place of inoculation could be seen; the first part of the ileum was distended by and filled with mucous; all epithelium was fallen off from the surface of the mucous membrane; the

heart was distended, especially the right, and filled with blood. The pericardium was distended with a copious yellowish fluid; both lungs were found dotted with grey points. No bacteria of any kind could be detected in the blood.

From this animal I inoculated two test tubes of rabbits' broth with heart's blood, and two test tubes of rabbits' broth with pericardial exudation; further, I inoculated with heart's blood one rabbit, *c*, with pericardial exudation one rabbit, *d*.

In the test tubes, subjected to a temperature of 35° C. for several days, no growth whatever made its appearance.

Rabbit *c* was found dead 19 days afterwards. It was much emaciated. No trace of the place of inoculation could be seen. Liver contained several white patches; fluid exudation in the pericardial cavity; both lungs showed a few hæmorrhagic spots, some parts of the lung hyperæmic; no micro-organisms could be detected anywhere.

Started with heart's blood and pericardial exudation of this animal cultures in rabbits' broth, two with each, and inoculated with the pericardial exudation two rabbits, *e* and *f*.

All test tubes showed after several days' incubation good growth of *micrococcus*. This micrococcus was used for direct inoculation of fresh rabbits, and for starting new cultures. And also these were then also used for inoculating rabbits. But neither produced any effect.

Rabbit *e* died four days after inoculation. On post-mortem being made there were found few white spots in the liver, much hyperæmia and inflammation of both lungs, especially of the right lung, which was almost totally consolidated. No trace of the place of inoculation could be seen.

No micro-organisms could be anywhere found, and no growth could be produced in nourishing fluid with the blood.

With heart's blood of this animal inoculated one rabbit, *g*. No result followed.

Rabbit *f* died 11 days after inoculation with the same appearances as rabbit *e*. The other rabbits (*c*, *d*) remained well.

The result, then, is this: In none of these animals could there be found and could there be produced by cultivation the bacterium termo originally employed, and therefore the conclusion is justified that the bacterium termo employed did not produce the death of animal *a*. The pericardial exudation of rabbit *c* was free of any bacterium termo, proof it did not start a growth of bacterium termo but of micrococcus. It had, however, powerful poisonous action on two animals,

e and *f*, in which it produced death. That the micrococcus cultivated from the pericardial exudation had nothing to do with the death of these animals is proved by the fact that it was quite powerless on several other rabbits.

In these cases in which the animals died we had not to deal with Davaine's septicæmia; this is proved by the absence of the bacteria from the blood.

I cannot say what the real nature of the disease of these rabbits *a*, *c*, *e*, and *f* was; but it is quite clear that the bacterium termo originally employed had nothing to do with it.

6. *Bacillus subtilis*, in most respects similar to hay bacillus, was obtained from pork broth that had become teeming with it on short exposure to the free air. The bacilli in their size, motility, mode of growth (singly, short chains, long filaments), formation of pellicle, formation of spores in the bacilli of the pellicle is in all respects identical with what is known of the hay bacillus, mentioned on a former page.

The only difference between the hay bacillus and our bacillus that I can perceive lies in the fact that in stained preparations the bacillus and its threads are composed not of cylindrical elements, but in many places of cubical and short cylindrical elements, similar to those of bacillus anthracis. But our bacillus is thinner than the latter.

Our bacillus grows well and copiously at 30°–35° C. in broth, and forms the characteristic fine pellicle with spores already after a few days, and after some days' growth turns the at first colourless fluid into a light yellowish and further into a yellowish brown colour. Owing to the innumerable spores the growth keeps its vitality *ad infinitum*.

It is very curious to notice in how definite a manner this gradual change of the colouration of the broth takes place in a series of test tubes in which this bacillus is growing, all being kept at the same temperature; it is possible at a glance to say in which tube the growth is oldest and in which it is of more recent date: *the more colour in a culture the older it is.* This to a certain extent occurs also in tubes of broth, in which the hay bacillus or the anthrax bacillus is growing, but it is not by any means so definite and regular as with our bacillus.

This *bacillus subtilis* was cultivated for many generations in broth, in peptone-sugar solution, in mixture of Agar-Agar and peptone. In every culture I waited till the pellicle with spores had been formed, and from this I took spores and inoculated a new culture. In many instances the bacillus was grown for one or two generations in one

medium, then for one or two generations in another, then in a third and so forth medium.

Many of these cultures, beginning with the original culture, were tested on rabbits, on mice, and on guinea pigs, inoculating a small quantity—one or two or three drops—subcutaneously. The temperature of the rabbits and guinea-pigs was taken daily. In no single instance have I seen the slightest effect in rabbits or guinea pigs, either locally or in the general health of the animals.

In mice, however, I have had what at first appeared successful result, but which on analysis did not prove such. These cases are:—

Two white mice, which we will call 1 and 2, were inoculated on August 30 into the subcutaneous tissue of the tail with a first cultivation of the bacillus subtilis in pork broth. Mouse 1 was dying on September 9. On post-mortem examination it was found: the tail, beginning with the place of inoculation, was much inflamed, at the root of the tail was an abscess, filled with thick pus, in it were numerous micrococci, chiefly in dumb-bells but also some in chains; the right lung was entirely occupied by an abscess filled with thick pus; in this pus were present vast numbers of dumb-bells of micrococci; the left lung was much inflamed, and in its upper lobe solid.

Mouse 2 had much inflammation of the tail; on September 10th the distal portion of the tail was almost gangrenous. On September 15th the mouse looked all right, the distal portion of its tail having become altogether detached. When killed nothing abnormal was found in its internal organs.

With the pus of the abscess of the right lung of mouse 1 inoculated on September 9th in the tail two fresh mice, 3 and 4.

These two mice both showed severe inflammation on September 12th, *i.e.*, after three days, at the seat of inoculation, spreading thence to the root of the tail.

This inflammation steadily increased until September 15th, when the tail was found much swollen and discoloured. The tails of both mice were cut off in narcosis and prepared for microscopic examination. The animals soon recovered, and remained well.

The microscopic examination of these inflamed tails proved the presence of large numbers of micrococci, chiefly in clusters, filling and permeating all crevices of the inflamed tissues, particularly the skin and subcutaneous tissue, and spreading from the inflamed parts into all the lymph-spaces of the skin and subcutaneous tissue of the non-

inflamed parts. In all layers of the skin, from the superficial layer of the corium down into the layer of the muscles and tendons the lymph-spaces of the connective tissue were all filled with clusters of micrococci. These micrococci could be traced on longitudinal sections of the tail, from the seat of the inoculation, which was occupied by dry pus and a scab, containing the micrococci in great masses, through all the lymph-spaces and clefts of the connective tissue towards the root of the tail.

With the pus of the abscess at the root of the tail of mouse 1 inoculated two test tubes of sterile pork broth and placed them in the incubator at 35° C.

These two tubes contained, after 24 hours, a copious crop of micrococci isolated, in dumb-bells, chains, and small groups. With these inoculated subcutaneously in the tail two fresh mice, 5 and 6.

Mouse 5 (inoculated with 1 cult. of micrococcus on September 10) was found dead on September 13. Both lungs were much inflamed; there were in them few small abscesses; the pleura was covered with pseudo-membranes; the pleural cavity contained purulent exudation.

Mouse 6 was on this day, *i.e.*, September 13, very quiet, the tail much inflamed, which inflammation increased until September 15, when it was found swollen and discoloured; there were underneath the skin several small abscesses, pus oozing out in many places. The animal, after removal of the tail, recovered completely. The microscopic examination of sections through the tail of this mouse 6 showed exactly the same appearances as those made of the tails of mice 3 and 4.

We see, then, that mice primarily infected with the bacillus subtilis were subject to disease, and that the bacillus could not be discovered in the diseased tissues, but in its stead we find a micrococcus which, as cultures and direct inoculation prove, possesses pathogenic properties, producing by multiplication severe inflammation about the seat of inflammation, and if the disease is allowed to spread, causing severe inflammation and abscess in the lungs, and subsequent death. Now, it would be quite unwarranted by any previous experience to assume that the bacillus subtilis originally introduced into the animals became transformed into the micrococcus; on the other hand, it seems probable that on the inoculation with the bacillus of mouse 1, that is the mouse in which the process was started, the wound became accidentally contaminated with one of those peculiar species of micrococci, which, as I said on a former page, are, *ab initio*,

pathogenic, which sometimes are present and thrive in various decomposing organic substances in the outside world, but on accidental introduction into a wound of a living animal are capable of manifesting pathogenic properties. I refer in this respect particularly to Koch's micrococcus, causing progressive necrosis in mice, and to Koch's micrococcus causing progressive abscess in rabbits. (*Untersuch. über d. Aetiologie d. Wundinfections Krankheiten. Leipzig,* 1879.)

In our case we have not to deal with the first, nor with the second. Our micrococcus has neither the morphological nor physiological characters of Koch's micrococcus of progressive necrosis of mice; it does not form those regular wavy and curved chains which Koch's micrococcus does, nor does it produce necrosis as far as it extends, as is the case with Koch's micrococcus.

Our micrococcus is not identical with Koch's micrococcus, producing progressive abscess in rabbits; it is larger, forms smaller clusters, and extends far beyond the inflamed part, so that we have to consider this micrococcus as a new form of micrococcus, causing severe inflammation in the skin of mouse and spreading hence into the system, causing ultimately inflammation and abscess in the lung. This micrococcus I have grown artificially, and with it I have produced the same disorder. We may call this *micrococcus of pyæmia of mice.*

E. KLEIN.

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