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XXXVIII.—On the origin of bacteria and their allies by heterogenesis

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longitudinal nervures and cross-nervures just beyond the opaque part of the tegmina and to beyond the middle below the costal area rufo-testaceous, the rest of the cross-nervures white. Wings broad, as long as the tegmina, hyaline, and with white nervures, except that the longitudinal nervures and the branches of the subcostal nervure are pale brown.

Hab. Mbuyuni, Brit. East Africa (*Betton*).

This insect agrees fairly well with *Ischnoptera macra*, Stål, but is twice the size; from *Derocalymma versicolor*, Burm., it differs in the colour of the legs &c. Both these species may, perhaps, belong to the present genus. The female probably resembles *Perisphæria æqua*, Walk., or *Derocalymma Brunneriana*, Costa; but there is no reason to identify the male here described with either of these species.

PANESTHIINÆ.

Genus PANESTHIA, Serv.

Panesthia nigricans, n. n.

|| *Panesthia nigrita*, Sauss. Rev. Suisse Zool. iii. p. 317. n. 17 (1895).

Hab. Macassar.

Not *Blatta nigrita*, Stoll, Spect. Blatt. pl. ii d. fig. 6.

XXXVIII.—On the Origin of Bacteria and their Allies by Heterogenesis*. By H. CHARLTON BASTIAN, M.A., M.D., F.R.S.

[Plates XXV. & XXVI.]

IF we turn to the question of the origin of Bacteria and their allies by heterogenesis we shall find, I think, that the evidence is overwhelming in regard to its reality, though it lacks that kind of certitude which obtains in regard to the heterogenetic origin of some much larger organisms whose birth from strange ancestors we have been following in some of the preceding sections. We may, for instance, as I have shown, see the whole substance of a large Rotifer's egg segment into a number of smaller parts, and we may see such segments presently become active as Amœbæ, Monads, Peranemata, or even as Ciliated Infusoria †.

* This paper forms one of the concluding sections of the author's 'Studies in Heterogenesis.'

† See 'Studies in Heterogenesis,' pp. 31 & 46.

But this kind of proof is impossible in the case of Bacteria. Where the proof is most direct (even where the birth of Bacteria seems to take place under our eyes) it must always be a question of particles of living matter emerging from the region of the invisible—appearing, that is, where previously visible particles were absent. While in other cases, without being so directly present as it were at their birth, we may find them existing in parts of animals or plants, after these have been subjected to certain experimental conditions, where previously, in accordance with all existing knowledge and belief, neither they nor their germs are to be found. They may appear, that is, when such animals or plants, or, rather, portions of them, have been exposed to certain experimental conditions unfavourable to their pre-existing vital processes, but which yet, as we assume, allow the constituent protoplasm to die more or less slowly, and portions of it to individualize themselves and grow into this or that form of microorganism.

The assumption here is that we have, as starting-points, to do with pre-existing units of living matter. Still, in the cases where our observations are made upon protoplasm which appears to be actually structureless, or upon one or other of the fluids pertaining to an animal or plant, the actual mode of origin, so far as appearances go, may be precisely similar to that which would occur in archebiosis; in each case there would be the appearance of particles where none previously existed. The difference between the two cases would be this: in archebiosis we should have to do with the actual birth of units of living matter, with a synthesis, that is, from its elements; but in the case of heterogenesis we have confessedly to do with living matter already existing, so that we postulate only an individualization of living particles or of larger units, together with a change in their mode of life.

Where the particles of living matter so individualizing themselves are so small as to be beyond the range of our most powerful microscopes, it would be impossible to say, as they grow and become recognizable in organic fluids, whether newly appearing particles had arisen by heterogenesis or by archebiosis. On the other hand, it must be recognized that there are particles of various kinds in the tissue elements and fluids of an animal or plant which it is often impossible by mere microscopical examination to discriminate from germs of microorganisms. And until such germs begin to grow and assume specific shapes, or particular collocations, their discrimination from particles which are normal constituents of such tissues or fluids cannot safely be made.

Whenever, therefore, Bacteria or their allies appear in the midst of the tissues or fluids of animals or plants, two possibilities have to be considered in order to account for their presence. The two questions that have to be asked are these:—

(a) Are the bodies of plants and animals interpenetrated in all parts by invisible germs of microorganisms, or are they germless?

(b) Have the microorganisms which may be found in the tissues or fluids of plants and animals under various conditions been produced therein by heterogenesis (possibly in the fluids by archebiosis), or is their presence invariably to be ascribed to infection from without?

(a) During the last thirty years it has been commonly held, in accordance with the teachings of Pasteur, Lister, and others, that the tissues and fluids of healthy animals and plants are germless and altogether free from microorganisms.

In regard to animals, however, it is clear that this position is one which cannot be accepted without very important limitations. It is obvious that microorganisms may, like other particles, get taken up from the mucous membranes of the alimentary canal and the respiratory system, and pass by means of lymphatics into the nearest glands. If they should get through these and ultimately pass into the blood, the generally accepted view is that they are speedily destroyed in this fluid. This view is based upon the fact that bacteriologists are never able to get evidence of the existence of microorganisms or their germs by the inoculation of different suitable and sterilized media, with blood drawn from a healthy man or from one of the lower animals similarly free from disease.

Yet, although the blood and internal tissues of healthy animals and of man are declared to be free from microorganisms and their germs, such organisms will habitually show themselves after death, in the course of a few days, throughout all the organs, even when life has been abruptly terminated in a state of health. It cannot reasonably be said in explanation of this that the organisms naturally present in the intestinal canal have been enabled to spread through the body so as to reach the most remote parts *after death*, since many of the organisms are motionless and others exhibit mere to-and-fro movements of a non-progressive character. The blood, again, has ceased to circulate, so that this fluid, germless during life, cannot after death be considered to act as a carrier. But if the organisms themselves cannot make their way through the tissues and travel far

within brief periods, and if no carrier exists, they must naturally have been born in or near many of the sites in which after death they are speedily to be found.

If bacteriologists are right in their view that the blood is germless in healthy animals because any germs or organisms obtaining access thereto are straightway killed, there seems no escape from the foregoing conclusion.

It has, however, been held by Tiegel, Burdon Sanderson, and others that though the blood is germless, "those parts of the animal body which are in closest proximity to absorbing mucous membranes are most liable to be found pregnant with microphytic life when tested by suitable methods"* . Their experiments showed, indeed, that such organs as kidney, spleen, and liver, when removed from the body of a healthy animal immediately after its death and suitably treated, could always be made to reveal the presence of microorganisms.

But cutting out portions of internal organs of recently killed animals, enveloping them with superheated paraffine, and then placing them in an incubator at a suitable temperature, followed by the finding of swarms of Bacteria in the central red and uncooked portions is not a method that can possibly give us certain information as to the mode of origin of the organisms found. It may be, as Burdon Sanderson and others concluded, that the organisms found came from "germs which existed and retained their latent vitality in the living tissues"; but it is equally possible, as I maintain, that they may have had a heterogenetic origin within these tissues themselves.

It seems perfectly clear that experiments of this kind, if carried no further, could teach us nothing decisively, that their results, in fact, are of no more value than those that may be obtained by the examination of the brain and its membranes three or four days after a healthy animal has been killed. There also swarms of microorganisms would be found, as I can testify; and if bacteriologists are right that organisms and their germs are, as they say, "destroyed" in the blood, we could only conclude that the organisms so found must have been produced by archebiosis or by heterogenesis.

In regard to plants—that is, fruits and vegetables of different kinds—the case is not so complicated, and Pasteur may have been perfectly right in declaring that, when healthy, their tissues are germless. Thus, in considering the interpretation of cases in which microorganisms are found in the

* Brit. Med. Journal, 1875, vol. i. p. 199; see also 1878, vol. i. p. 119.

interior of certain vegetables or fruits after they have been submitted to various unnatural conditions, the question will not be whether we have had to do with the wakening up of latent pre-existing germs, but rather whether the organisms found are results of an infection that has recently been brought about—that is, during the exposure of the vegetables or fruits to the experimental conditions. And this brings us to the consideration of the second of the two possibilities above referred to.

(b) The second possible mode of accounting for the presence of microorganisms in the tissues of healthy animals and plants is that they have resulted from some process of infection brought about antecedently or during the continuance of the experimental conditions to which they have been subjected*.

It is of great importance for the proper consideration of this possibility that we should have some definite knowledge as to the power possessed by such microorganisms as *Bacteria* and their allies of penetrating the healthy tissues of plants and animals, as to the means by which they are enabled to do so, as well as concerning the time needed for such an operation. Fortunately one such investigation, very much to the point, can be referred to.

M. C. Potter† has carefully investigated a bacterial disease of the turnip which he calls “white rot,” due to infection by an organism named *Pseudomonas destructans*. He shows that this Bacterium “secretes an enzyme which dissolves the middle lamella and causes the softening and swelling of the cell-wall,” and also a toxin. Later, he enters into details as to the mode in which the Bacterium attacks and penetrates the cells of the turnip, the latter process being actually seen‡. These later observations were made upon a small fragment of turnip inoculated with a pure culture of *P. destructans* and introduced into a hanging drop. The influence of the Bacteria was very rapidly brought about. One particular cell attacked by the Bacteria was watched; a wall common to it and the next was measured, and after half an hour it was found to be nearly twice as thick as before; in another twenty minutes it was nearly three times

* I say “antecedently,” because the explanation favoured by Burdon Sanderson of the finding of swarms of Bacteria in kidney, spleen, and liver in his experiments previously referred to is some process of infection from the alimentary canal. How the infection is brought about he does not think it necessary to explain (see Brit. Med. Journal, 1878, i. p. 120)—that is, how the Bacteria (not to speak of their germs) could penetrate all the coats of the intestine and the capsules of the abdominal organs; nor why, if Bacteria or their germs could be active enough and have the power of effecting such a migration, they should, on arrival in this or that organ, lose all their activity and pass into a “latent condition.”

† Proceed. of Roy. Soc. vol. lxvii, p. 442.

‡ Loc. cit. vol. lxx. p. 392.

as thick; in an hour and a quarter from the commencement the two parts of the cell-wall began to separate, and in another quarter of an hour there was a distinct gap between them. By this time also all the protoplasm had separated from the cell-wall and formed an irregular bag in the centre of the cell, owing to its death under the action of the toxin. "After this the changes were less rapid, and beyond a slight further separation of the cells, a *more rotten and watery appearance of the cell-wall* was all that could be observed." Contiguous cells were being attacked in the same manner.

"The Bacteria continued swarming around the cell-walls," the author says, "and next morning (by which time the cells had been destroyed several layers deep) many Bacteria had come to rest in contact with the wall, their long axis being perpendicular to its surface, and one or two had the appearance of being imbedded in the wall as if in the act of boring their way through." The actual penetration of the wall was subsequently watched on several occasions. "The time required varied with the thickness of the wall, but on an average occupied about three hours." But Potter found that this Bacterium had "no power to penetrate the cuticle of the mature epidermis of the turnip"; it was also incapable of penetrating the epidermis of mature leaves, the fully developed cuticle being proof against the action of the enzymes excreted by *P. destructans*, though young leaves from the growing points "possessing little or no cuticle" were found to be vulnerable.

The important facts made known by this research are therefore these:—the vegetable cell is only capable of being penetrated when its walls are not thick and hard originally, and when they have been extremely softened by long contact with the cytase excreted by a number of Bacteria. The need of a conjoint attack is distinctly indicated by the author, who says *:—"Very soon the number of individual Bacteria becomes largely increased, each one contributes its share of toxin and cytase, and in a very short time these products have sufficiently accumulated to kill the first cell. . . . It is not, however, until the protoplasm has been killed and the cell-wall very much softened that the Bacteria have the power of perforating the walls and passing into the cell-cavity. It would hardly be supposed that a single Bacterium, through its own excretions, could soften the wall and pierce it at one definite point after the manner of a fungus germ-tube. The extreme minuteness of the Bacteria and the rapidity of their multiplication lead them to act, as it were, in concert, and the wall becomes softened by the cumulative action of many Bacteria before the penetration of a single individual."

The mode in which a Mould infects and penetrates a vegetable cell is very similar, allowance being made for its greater size, which permits a single individual to do what can only be brought about by numbers of organisms in the case of Bacteria. This subject has been recently investigated by Nordhausen † while studying the parasitism of *Botrytis cinerea*. I quote from Potter, who says:—

"He has shown that the spore of this fungus excretes a powerful toxin in its initial stages of germination before any trace of the germ-tube can be detected. Its manner of effecting an entrance into a host-plant is first to kill the cell by the emission of the toxin; the germ-tube then penetrates the dead cell, and is nourished saprophytically upon it; with the vigour thus gained it destroys the neighbouring cells and passes from one

* *Loc. cit.* p. 395.

† Jahrbücher für Wissensch. Botanik, vol. xxxiii. (1899).

to another without further difficulty. The fungus hypha has the power of penetrating the cuticle, *but only in young and tender structures*; old and hardened membranes could only be entered when the cuticle had been injured or when it had gained strength by special saprophytic nutrition."

These results will prove important as standards for comparison with others to which I am now about to refer*. We have seen that in the case of actual infection by Bacteria there is need for the co-operation of many organisms in order to bring about, by their secretions, the softening of the wall of every single cell that is penetrated, that some time is required for the operation, and that the softening produced must be considerable before any such penetration is possible. It will be seen how very different is the state of things in cases which I shall cite as instances of the origin of Bacteria and their allies in the tissues of plants and animals by a process of heterogenesis.

The presence of two characteristics, wherever they coexist, may be regarded as strongly in favour of the interpretation of heterogenesis as against infection, as the following remarks will show.

(1) The means adopted by Bacteria for bringing about the penetration of cells are such as are associated with the vital processes of adult organisms. So that there is no reason to think that invisible or scarcely visible germs of such minute organisms would have the power of secreting a cytase sufficient in amount to bring about that degree of softening of a cell-wall which has been found to be a necessary preliminary to their penetration. *Yet in multitudes of cases it is minute germs of Bacteria and their allies which may be seen developing within cells or tissues.*

(2) Again, the process of infection, as described by Potter, is one brought about by active organisms which affix themselves to a cell-wall until it becomes softened, and then succeed, by reason of this same activity, in boring their way into the cavity of the cell. On the other hand, in very many of the cases in which, as I maintain, Bacteria and their allies may be presumed to be originating by heterogenesis, what can often be seen is this—particles becoming visible in the midst of homogeneous protoplasm, such particles being invariably motionless, but followed soon by the appearance of definite Bacteria or their allies, recognizable as such by their shapes and modes of collocation. *But these Bacteria or their allies,*

* As well as with the case of assumed migration and infection referred to on p. 385, note *.

like their germs, are invariably, at first, found to be motionless. This primary motionless condition is, in fact, the rule in the case of organisms taking their origin by heterogenesis. Thus, when Monads are formed in the pellicle on a hay infusion, they are at first motionless; when *Amœbæ* or *Actinophrys* are developed from the substance of large Confervoid cells or from resting-spores of *Spirogyra*, and again when *Amœbæ*, Monads, *Peranemata*, or Ciliated Infusoria arise from the transformations of the substance of the egg of this or that Rotifer—in all these cases also the resulting organisms are at first motionless*.

These two points are therefore of great importance. For the purpose of interpretation it should be borne in mind that in cases of Infection by Bacteria and their allies we have to do with *adult organisms in a state of activity*, while in cases where Heterogenesis may be presumed to be occurring we have invariably, in the first place, to do with *germs and motionless organisms*†.

I shall now proceed to mention some instances in which it seems clear that Bacteria and their allies have arisen by heterogenesis. I make a selection for this purpose of a few typical cases out of many others which I might have cited.

Bacteria may constantly be seen developing in the way I have mentioned within the living cells and filaments of various *Algæ*. In *Vaucheria* and in *Spirogyra* this is commonly to be seen where the plants have been kept in unnatural conditions for a time—shut up, for instance, either in a cupboard or within a stone pot. In the case of *Vaucheria* they may often best be recognized in and near the growing points of the filaments, where they show themselves first as mere motionless specks, which gradually develop into Bacilli, and, after a time, take on an active existence. In the filaments presenting these appearances the wall may appear quite healthy, showing no signs of softening, nor is there any indication whatever of the penetration of Bacteria from without. The development of Bacteria within the cells of *Spirogyra* may be best seen in cases where the endochrome is not very abundant and the cells are small. Motionless, colourless particles seem to bud from the edges and surface of the bands of endochrome, and some of these gradually take

* See my 'Studies in Heterogenesis,' pp. 5, 8, 10, 31, 46, 69.

† Of course by "germs" I mean here merely minute and undifferentiated stages of the organisms in question, produced by heterogenesis, and not the ordinary acceptation of the word, viz. a reproductive unit formed in an organism of like kind.

on the forms of Bacteria and begin to exhibit swarming movements. This may be commonly seen in cases where the cell-wall presents a perfectly healthy appearance and where there is absolutely no indication of infection taking place from without.

The same kind of thing is often to be observed within the thick-walled resting-spores both of *Vaucheria* and *Spirogyra*. There is the appearance of motionless particles in some part of the spore, the appearance of Bacteria in the midst of these particles, and the gradual assumption by the Bacteria of swarming movements. Observations of this kind were not unfrequently made in cases where these resting-spores had been undergoing one or other of the changes that I have previously described.

Again, I have occasionally seen a development of motionless Micrococci and Bacilli taking place inside the thick wall of a *Nitella* cell, between it and the chlorophyll layer, such as is shown in Pl. XXV. fig. 1 ($\times 700$). Yet the normal cyclosis was still going on within this cell, showing that there could be no apertures or solutions of continuity of any kind; and all the microorganisms to be seen in different stages of development in this layer were quite motionless, so far as could be observed. Their imperfect definition in the photograph, however, makes it possible that absence of spontaneous movement was not absolute, unless the slight blurring may have been due to vibrations during the prolonged exposure needful to enable the light to pass through the whole thickness of the filament. Their appearance along a particular band of the filament only is, of course, due to the parts on either side being out of focus.

I have also endeavoured to throw light upon this question in another way, that is, by repeating, with variations, some of the experiments of Lechartier and Bellamy, by which they studied the fermentation that occurs in various vegetables and fruits when shut up within hermetically sealed vessels. They showed that the oxygen of the air was soon consumed by the vegetables or fruits, which then began to break up sugar, to give off carbonic acid, and to produce alcohol and acetic acid. They came to the conclusion in 1872 * that this fermentation might certainly occur without the production of the alcoholic ferment. They, in fact, adopted Pasteur's view that the formation of alcohol in these cases was due to the altered activity of the cells of the fruit, which, in the absence of free oxygen, act after the fashion of ferments. In a later

* Compt. Rend. 1872, ii. p. 1203.

FIG. 2.

FIG. 1.

FIG. 5.

FIG. 3.

FIG. 6.

FIG. 4.

communication, however, these investigators stated * that in their experiments with potatoes and beetroot, while alcohol and carbonic acid were produced in the same way as with the fruits, and the alcoholic ferment was absent as before, Bacteria of different sizes were invariably found in the acid fluid which impregnated the softened tissues of the vegetables in question. No details on this point were given, and the authors do not appear to have made any further observations on the subject; nor did Pasteur offer any reply to such statements, though he had, about this time, been working at the subject himself †.

Having determined to endeavour to obtain some more definite information as to the appearance of Bacteria in this way, I have, during the last two or three years, made various experiments in which, after small *Potatoes* had been carefully washed they were allowed to soak for a time in different germicidal fluids. First of all I employed a solution of mercury bichloride (1 : 2000); while later, after the preliminary washing, the potato was allowed to soak in a 5 per cent. formalin solution. The screw-top bottle in which the potato was placed was also thoroughly washed out with one or other of these fluids. In these cases organisms were found within, but also after a time on the surface of, the potatoes thus treated; so that these particular experiments and methods were rejected as not yielding trustworthy results. This was necessary because at a rather earlier date Pasteur had stated ‡ that in experiments which he had made with many fruits no ferment-organisms ever appeared. He declared again that the tissues of healthy fruits and vegetables were germless, but intimated that unless care was taken they might make their way in from without.

Subsequently I used a stronger solution of formalin, and have never since found organisms on or near the surface, though they have often been found within cells in the central portions of the potato. I will now, therefore, give brief details of some of these experiments.

In July, 1901, after well washing a small new potato it was allowed to soak in a 10 per cent. solution of formalin for ten minutes, in a small screw-top bottle, and during this time the fluid was frequently shaken so as to cover the whole inner surface of the bottle. At the expiration of the time named the top was unscrewed, the fluid poured out, and the top then tightly refixed, leaving the potato itself and the inner surface

* Compt. Rend. 1874, ii. p. 1006.

† *Loc. cit.* 1872, ii. p. 788.

‡ *Tom. cit.* pp. 788 & 981-2.

of the bottle wet with the formalin solution. The bottle was subsequently left in a cupboard for seventeen weeks, the temperature of which for a long time remained about 70° F., although it afterwards fell to 55° F.

When removed from the bottle at the expiration of this time the potato was found to be quite firm and not at all shrunk. On section it was seen to be discoloured to a pale earthy tint, with mottlings here and there of a rather darker colour. The cut surface was moist and had a distinctly acid reaction, and there was not the least sign of softening or disintegration anywhere. Thin sections having been made, they were shaken up in a small tube with distilled water, so as to get rid of the starch-grains from many of the cells, and the sections were subsequently allowed to soak in some of Westphal's mastzellen stain, diluted with 2 per cent. formalin, for two hours.

On microscopical examination of these sections groups of Bacteria were found in large numbers of the cells, and everywhere except in those near the surface. The contents of one of these cells is shown in Pl. XXV. fig. 2, B ($\times 500$); some of the Bacteria were free and others were in or lying on the primordial utricle, but, as I have usually found with micro-organisms in such situations, they were not appreciably stained. Some cells, which did not contain obvious Bacteria, showed plenty of minute cocci-like bodies on the surface of the primordial utricle, also not taking the stain, which probably represent early stages of the Bacteria (fig. 2, A, $\times 700$).

Another larger potato, about two inches in diameter, was treated in exactly the same way as the last, in September 1901, and after the bottle was finally closed it was left on the surface of an incubator at a temperature of about 80° F. for seven weeks.

When examined the potato was not found to have shrunk or to be appreciably altered on the surface. On section it was moist, of acid reaction, and showed as before a pale earthy colour with rather darker mottlings.

Sections were made and treated in the manner previously indicated, and on examination multitudes of Bacilli were seen here and there in cells in all parts of the section except for about one fifth of an inch from the surface. In places also there were fine mycelial filaments containing spore-like bodies. Some of these Bacilli took the stain fairly, as may be seen in Pl. XXV. fig. 3, A ($\times 500$), in which the two kinds of organisms are shown. In or on the primordial utricle also there were multitudes of very delicate interlacing filaments (? Bacilli), containing an abundance of spores which had

taken the stain freely, as may be seen by Pl. XXV. fig. 3, B ($\times 500$).

In November, 1900, a small new potato had been treated in the same manner that I have already described, but after pouring away the 10 per cent. formalin solution the bottle was filled with carbonic acid gas before screwing on the top*. This bottle was then placed within an incubator and allowed to remain there at a temperature of 84° F. for six weeks.

On examination the appearance of the potato externally and internally was almost exactly such as I have described in the others. There was the same mottled colour of the cut surface, with a rather deeper tint in the centre as well as in some other parts.

Sections of the central portions of the potato were made and placed for a short time in a carbo-fuchsin solution. On microscopical examination in many of the cells very small mycelial filaments (something like *Crenothrix* filaments), with spore-like bodies at intervals, were found, such as are shown in Pl. XXV. fig. 4, B ($\times 500$). The filaments were lying on the primordial utricle, and, as in many other cases, the organisms had scarcely taken the stain at all. In other cells what seemed like the beginnings of such organisms, or of others very similar, were found on the surface of the primordial utricle, as shown in Pl. XXV. fig. 4, A ($\times 500$).

At the end of March 1903 I made another slight variation in the conditions, and again obtained a rather different result. A small new potato, after careful washing, was placed in a small tin with a very tightly fitting cover and allowed to soak in 10 per cent. formalin for twenty minutes, the fluid having been shaken about several times so as thoroughly to wet the whole internal surface of the tin. The fluid was then poured off, leaving the surfaces wet with the solution as before; the tin cover was very tightly jammed down and the vessel was again placed within a copper incubator at a temperature of 75° F., and allowed to remain there for eight weeks.

When taken out and examined the cut surface of this potato presented just the same characters as in the others: the whole substance was firm throughout, there was no shrinking, and the central portion was rather darker than the other parts, which showed the usual mottling.

Sections were made, shaken up in distilled water in a small tube as before, and then placed for a short time in a dilute gentian-violet solution. On microscopical examination a

* The filling the vessel with CO_2 was a method adopted by Pasteur in his experiments on the fermentation occurring in fruits contained in closed vessels.

large number of the cells scattered throughout the sections were found to show the most delicate branching tufts of a new kind of microphyte, probably a species of *Cladothrix*, which had taken the stain slightly, and such as are shown in Pl. XXV. fig. 5 ($\times 500$). These tufts were mostly seen to be sprouting from the external surface of the primordial utricle where it had shrunk away from the cell-wall. No Bacteria and no ordinary Mycelia were found in either of the sections, though they were most carefully examined.

A few experiments have also been made with small *Turnips* about two inches in diameter, to two of which I will now refer.

A perfectly sound turnip of the size mentioned was, in November 1901, first well washed in water and then allowed to soak in a screw-top bottle in a 10 per cent. formalin solution for ten minutes. It was subsequently treated in exactly the same manner as the potatoes had been. After the top of the bottle had been tightly screwed on, it was left on the top of the incubator at a temperature of about 80° F. for seven weeks.

On examination at the expiration of this time, the turnip was found to be somewhat shrivelled in its upper two thirds. The odour of the bottle was disagreeable and pungent, though slightly aromatic and spirituous. The odour was so strong that it did not seem likely that the shrivelling was due to evaporation, owing to the screw-top not having been quite air-tight.

On section the rather shrivelled upper two thirds was found to be much discoloured and honeycombed, the lower third being much less so. Sections were made and soaked in dilute "mastzellen" stain; and on examination cells here and there, not continuously, but in the upper and lower portions alike, were found to be crowded with very minute Bacteria, most of which took the stain only slightly. In Pl. XXV. fig. 6, A ($\times 500$), a large aggregate of these organisms is to be seen, with others scattered about over contiguous portions of the section.

Another small turnip of the same size as the last was, at the same date, after being well washed, placed in a screw-top bottle and stood on a small earthenware pot, in order to protect it from actual contact with 6 drachms of pure formalin which had previously been poured into the bottle. The top was then tightly screwed on, and the bottle was placed on the incubator by the side of the other at about 80° F., where it

remained for eight weeks, the turnip being in an atmosphere saturated with formalin vapour.

On examination this turnip was likewise found to be slightly shrivelled, and it was rather soft and doughy to the touch. On section the colour was almost natural, except for a depth of about one third of an inch round the periphery, where it was slightly discoloured, and in the centre, where there was a small area about one quarter of an inch in diameter which had a rather gelatinous appearance.

Two sections through this central region and its neighbourhood were made, and then soaked in dilute "mastzellen" stain. On microscopical examination they were found to contain moderately large Bacteria, mostly in small groups, in a large number of the cells; though here and there larger masses of Bacteria were found, such as are shown in Pl. XXV. fig. 6, B ($\times 500$). In many of the cells the Bacteria seemed to be developing in and on the surface of the primordial utricle, and also in and on the surface of the nuclei of the cells.

I have made only one experiment of this kind with an *Apple*, and in this case a rather small, but thoroughly sound, specimen was placed in a screw-top bottle, and stood on a small earthenware pot as before, so as to remove it from contact with some pure formalin which had been placed in the bottle. The top was tightly screwed on, and the bottle was then placed in a cupboard, where it remained for eight weeks—the temperature of the cupboard varying during this time between 70° and 56° F. The apple was thus left, as the turnip had been, in an atmosphere saturated with formalin vapour*.

On examination the surface of the apple was found to be hardened, and on section irregular patches of brownish discoloration were seen; otherwise nothing abnormal was observed.

Microscopical examination of an unstained section showed, in a few of the cells, a small Fungus mycelium, such as may be seen in Pl. XXVI. fig. 7, A ($\times 250$). Specimens like this were found in cells in different parts of the section, though in the great majority of them nothing of the kind was met with.

* I have not had many successes with this method, and do not recommend it, especially as the soaking for a time in 10 per cent. formalin has proved to be perfectly sufficient to guard against external contamination. It is difficult to tell how far the formalin vapour penetrates into the substance of fruits or vegetables left in an atmosphere saturated with it for many weeks.

FIG. 8.

FIG. 7.

FIG. 9.

FIG. 11.

FIG. 12.

FIG. 10.

When present the growth seemed to start from the cell-wall close to the nuclei, if not from the nucleus itself. No Bacilli were seen; but in some cells what appeared, judging from their uniform size and mode of arrangement, to be a number of Micrococci were found on the primordial utricle, such as are shown in Pl. XXVI. fig. 7, B ($\times 375$). They scarcely stained at all with carbo-fuchsine*.

I have now to record an interesting case of spontaneous change in some apples, which occurred under the following circumstances:—Last autumn I received from a friend in America a case of very choice Canadian apples. The case had a separate cardboard partition for every apple, and they were all in excellent condition. Some of them were kept as late as the second week in January of this year. From about the middle of December I noticed that many of these apples, when cut through the centre, showed a brown discoloration which seemed to begin at a number of separate points around the periphery of the core, as may be seen in Pl. XXVI. fig. 8, A & B ($\frac{1}{2}$ nat. size). An early stage of the change is shown in A, and a more advanced stage in B. In all other respects the apples were perfectly sound and of a delicious flavour, and none of those eaten anterior to the date mentioned showed any unusual appearance. Towards the end of December the above photographs were taken; and, on consulting Lindley's 'Vegetable Kingdom,' it became plain, from a figure there shown (p. 559), that these points of change occurred at the junction of the ovarian and the calycine portions of the fruit.

I examined portions of the altered tissue under the microscope, fully expecting to find some Mould as the cause of the change. But, much to my surprise, after a tolerably careful examination, I was unable to feel sure that organisms of any kind were to be found in the tissue which had become thus altered. Subsequently I tried to stain some sections, and made a still more careful examination, with the result that I found on or in the primordial utricle of many cells cocci-like bodies looking like the germs of microorganisms. But as their nature seemed doubtful I took two of the apples to Dr. Allan Macfadyen and asked him kindly to see whether any microorganisms could be developed from this altered tissue of the apple. On January 5th he wrote to me as follows: "I was unable to detect the presence of Bacteria in the Canadian apples you left here, by microscopical exami-

* As the magnification is low, the use of a pocket-lens will make these organisms more distinct, especially those near the centre of the figure.

nation. I accordingly made a number of subcultures, but in no instance have I succeeded in obtaining a growth"*.

I had by this time only two or three of the apples left, so I placed them in the incubator at a temperature of 76° F. and there left them for eight days. On section two of them were found to present the brown discoloration in the usual situations to a well-marked extent. Some portions of the brown tissue were broken up with needles, placed in a dilute solution of the "mastzellen" stain, and were afterwards submitted to careful examination with the microscope. There was certainly a very distinct increase of the cocci-like bodies in the primordial utricle, remaining unstained, as in Pl. XXVI. fig. 8, C ($\times 700$), though all the other granules in the cells had become strongly stained. In some places the cocci were seen in distinct rows, branching and crossing one another, as in fig. 8, J ($\times 375$) in the neighbourhood of the letter, so that they looked like spores within minute filaments†.

A further careful and prolonged examination revealed the fact that very many of the cells showed, in whole or in part, on or in the lining membrane, the cocci-like bodies as in C, though in other cells there were none of them. There was often a tendency for these bodies to arrange themselves in rows (as in E and I), and in places to grow into delicate filaments (as in D and F). Such filaments were also seen occasionally crossing the cavity of the cell, and having spore-like bodies at intervals. A few larger filaments or hyphæ, such as G, were likewise seen, together with toruloid corpuscles, as in H. The spore-like body in G, from which the hypha has developed, is only a little larger than one of the cocci-like bodies to be seen near the lower left corner of C. Any doubts as to the reality of these latter bodies being embryo Bacteria may be set at rest by comparing them with what is shown in Pl. XXV. figs. 2 and 4. All the organisms found here, as with these shown in the figures above mentioned from other vegetable cells, were similar in refusing to stain with all ordinary dyes.

Although the first examinations of these apples showed, therefore, only very doubtful organisms or none at all, a

* Some time previously Dr. Nabarro, of University College, had been similarly unsuccessful in obtaining growths from a potato which had been treated in the manner I have detailed on p. 390, and in which organisms seemed to be present in an early stage. Such lack of success after trials with a few culture media is, of course, far from disproving the presence of microorganisms.

† Unfortunately this particular photograph was taken at a low magnification, but C and each of the others were taken at 700 diameters.

prolonged search has made their presence abundantly clear, and has shown that the "spontaneous" changes occurring in so many distinct foci in the very midst of their tissue has been correlated with the origin and development of micro-organisms.

I have also made a very few observations on *Tangerine Oranges*, to two of which I will refer. In February, 1901, two of these oranges were placed in a screw-top bottle and soaked in a 10 per cent. solution of formalin for fifteen minutes, the fluid being also shaken about several times so as to wet the whole inner surface of the bottle. After the fluid was poured off and the cover tightly screwed on, the bottle was placed in a cupboard for fifteen days, the average temperature of which was about 50° F.

When the first of these oranges was cut through in a longitudinal direction, a slight mouldy odour was at once perceived, and in the central white tissue and around the pips there was a greenish-black mass of mould. This was strictly confined to the central parts of the orange, and nowhere came within three fourths of an inch of the surface. The skin generally was perfectly sound, though it had become hard and was of a slightly brownish colour from the action of the formalin. Microscopical examination of the more peripheral parts of the orange also showed no mycelial filaments or organisms of any kind.

The other orange in the same bottle showed no organisms either to the naked eye or on microscopical examination.

Soon afterwards two other *Tangerine* oranges were treated in the same way, and subjected to similar conditions, except that they were left in the bottle for a much longer period—that is, for five and a half weeks instead of only fifteen days. About five days before the bottle was opened, one of the oranges was seen to show a patch of dark colour on one side, and when it was subsequently cut open longitudinally all the central white tissue was found to present an altered appearance, being of a rather dirty white colour; and on microscopical examination it was found to be densely infiltrated with a delicate *Fungus* mycelium. The seeds were discoloured, and the mycelium was also found to extend into the yellow substance of the orange. In one place there was a patch of a blackish colour, and this was found to have grown into the rind of the orange at the point where the discoloration on the surface was seen. It had not, however, actually reached the external surface; it had evidently grown from

within outwards, and the surface of the orange here and elsewhere showed no trace of Mould of any kind.

The companion orange again showed no organisms, either internally or externally.

There is no means of accounting for Mould springing up in the interior of an orange by infection from without. In a memoir entitled "*Recherches sur la Pourriture des Fruits*"*, Davaine points out that in fruits, such as the apple, the pear, and the medlar, in which there is an open calyx, "*le tube calicinal peut conduire les spores ou leurs filaments jusqu'au centre du fruit. C'est ainsi que se produit le blettissement †, qui n'est autre chose qu'une pourriture*"; but the process of rotting, he says, *is always external* "*chez les fruits qui sont partout recouverts d'un épiderme, tels que le citron, l'orange, et les fruits à noyau.*"

In the case of the apples to which I have referred, there was clearly no such process of infection from within as that to which Davaine refers. In the Canadian apples the change occurred simultaneously in many points almost as much removed from the seed-cavities as from the surface of the apples, and a comparison of what was found in the primordial utricle of the cells with what has been found in similar situations in the potatoes that have been referred to, leaves little room for doubt that what are shown in Pl. XXVI. fig. 8, C, D, are really germs of microorganisms; while in the other apple delicate Fungus mycelia (fig. 7, A) were found springing up within various isolated cells in the midst of the substance of the fruit. Again, the presence of the Bacteria and other organisms within the cells of the two small turnips and the different potatoes that have been referred to are equally incapable of being accounted for by any process of infection from without. There is absolutely no relation between what I have found in these cases and an actual process of infection such as M. C. Potter has described (see p. 385). We have to do, in fact, in the cases that I have cited, with motionless germs of microorganisms arising *de novo* in or on the substance of the primordial utricle of isolated cells, having intact walls, and scattered throughout the substance of the potatoes and the turnips in question—in all parts, that is, except in the superficial

* *Compt. Rend.* 1866, pp. 277-344.

† That is, the mellowing process that occurs in pears and medlars more especially. Further on in his paper Davaine says he has "recently recognized that this latter process (blettissement) may take place where spores are excluded, and in the absence of any mycelium."

portions that have been saturated with the germicidal fluid in which they had been for a time soaked*.

As I have previously pointed out, the existence of "latent germs" in the substance of healthy fruits and vegetables is not assumed—it is, in fact, expressly denied (pp. 383, 390). Hence the great weight to be attached to the preceding observations as evidence that the various microorganisms found within the cells have actually originated there by heterogenesis.

It remains to be seen what evidence of similar cogency can be obtained in regard to the origin of microorganisms within the tissues of animal organisms.

It would be useless to multiply instances. I will therefore first cite a single case in which the origin of Bacteria may be actually watched within the body of a low animal organism, and then turn to their mode of appearance within some of the tissue elements of different vertebrates.

Evidence of a particularly convincing nature is to be obtained from the examination of a little creature low in the scale of animal life, namely, *Cyclops quadricornis*, one of the Entomostraca so commonly to be found in ponds. It may be seen from pl. xxiv. of Baird's 'Natural History of the British Entomostraca'† that the four pairs of abdominal feet and also the tail are furnished with a number of "plumose spines or setæ."

Examination of one of these organisms will show that within the chitinous envelope of these slender spines, which taper away to sharp points, there is nothing but structureless protoplasm to be seen (Pl. XXVI. fig. 9, A, $\times 700$). If we take one of these little creatures, put it in a drop of distilled water on a glass slip, with a tiny fragment of a No. 2 cover-glass on each side of it, and place over all a cover-glass, it will be found that the animal is soon killed by the weight of the latter, though the fragments of glass prevent rupture of the body. We may then place the microscope-slip in a Petri dish containing a thin stratum of water (so as to prevent evaporation from beneath the cover-glass), and fixing upon one of the tail-setæ, which are larger than those on the abdominal feet, we may examine it from time to time. What may be observed is this:—

After an interval of two or three days (the duration depending upon the temperature of the air at the time), we

* In these cases the organisms often have to be long and carefully searched for. A perfunctory examination would almost certainly lead to the statement that no organisms were present.

† Ray Society, 1850.

may see, under the highest power of our microscope, scarcely visible motionless specks gradually appear in increasing numbers in the midst of the structureless protoplasm; and still later we may see some of these specks growing into Bacteria, as in Pl. XXVI. fig. 9, B, $\times 700$, which is a representation of A after four days. At last the whole interior of the spine becomes filled with distinct Bacteria, as may be seen in C ($\times 700$), which is from a photograph of the same spine on the sixth day—the temperature during these days varying from 70° – 75° F. Later still, all the Bacteria, previously motionless, began to show active swarming movements.

In such a case it is clear that we have to do with no process of infection from without, but with a *de novo* origin of Bacteria from the protoplasmic contents of the spines or setæ. The fact that they appear in these situations as mere separate motionless specks, and gradually take on the forms of Bacteria (also motionless at first), is, as I have previously indicated, just what we might expect if they had actually taken origin in the places where they appear. On the other hand, such a mode of appearance is totally opposed to what might be expected if the microorganisms had obtained an entry from without, through the chitinous envelope of the spines.

I pass now to what may be regarded as another absolute proof of the heterogenetic origin of Bacteria, as convincing as that which I have shown to occur within the closed cells of certain vegetables.

I have already pointed out that in many parts of the bodies of man and of higher animals microorganisms are known to exist in abundance. This is the case, for instance, throughout the whole length of the alimentary tract, and throughout almost the whole extent of the mucous membranes of the respiratory tract. It is clear also that some of the microorganisms may be taken up from these mucous membranes by lymphatics, and if they pass the nearest lymphatic glands some of them would ultimately find their way into the blood. When there, the view generally accepted is that the Bacteria and their allies are at once “destroyed.” The blood of healthy animals is declared to be germless, and much importance is attached to the germicidal qualities of this fluid.

On the other hand, it has been found, as I have previously intimated (p. 384), that in organs contiguous to the alimentary canal—such as the kidney, the pancreas, the spleen, and the liver—taken from a healthy animal immediately after

death, with every precaution needful to prevent contamination from without, swarms of microorganisms can be made to appear therein at will.

Speaking of such experiments made by himself, Burdon Sanderson wrote as follows* :—"Under the conditions I have described to you, it seems to me quite impossible to suppose either that germs could penetrate to the organ from the outside or that any germ encountered by the organ in its transference from the body of the animal to the basin could escape destruction. If, therefore, Bacteria be found, they or their germs must have been there before the organ was plunged into the hot liquid. . . . The results of all the experiments, whether with liver or kidneys, was the same. The soft red kernel of uncooked tissue at the middle of the organ always contained Bacteria, the vigorous development of which was indicated by their large size, countless numbers, and active movements. To my mind the experiment is conclusive."

In reference to this it is right to say that similar results have been obtained by other investigators using either similar methods or methods equally trustworthy. Such experiments have been made by Tiegel, Billroth, Nencki and Giacosa, Horsley and Mott, as well as by myself.

As I have previously intimated, the finding of organisms under these conditions is a fact so important in view of the theories of Pasteur and Lister, and the general belief as to the germicidal qualities of the blood, that the results require to be most carefully scrutinized.

To postulate the presence of "latent germs" in these abdominal organs and to assign as a reason the close "proximity to absorbing mucous membranes," and nothing else, surely cannot be regarded as a full and satisfactory explanation. It assumes, without proof of any kind, that microorganisms, even in healthy animals, are constantly making their way out through the walls of the intestine, and wandering promiscuously into this or that organ, only in the end to lapse into a condition of "latent vitality." Could anything in the way of explanation be more gratuitous and unsatisfactory?

With a view to throwing light upon this important question by the production of actual evidence I obtained a sheep's kidney from a recently killed animal, and saw a coating of fat nearly an inch thick stripped from it. The whole organ was then left to soak for four hours in a two per cent.

* Brit. Med. Journ. 1878, vol. i. p. 119.

solution of chromic acid, when it was removed and placed in a bottle still wet with a 10 per cent. solution of formalin. The screw-top having been fixed so as to prevent evaporation, the bottle was transferred to an incubator and left at a temperature of 76° F. for thirty-six hours *. When the organ was cut the chromic acid was found to have discoloured it to a depth of about a quarter of an inch, but within that margin the kidney-substance was red and only slightly softer than natural. There was no distinct odour of putrefaction. A small portion of the organ was cut out and teased in a drop of a weak solution of gentian-violet, and fragments, after a short interval, were carefully examined under the microscope. A comparatively small number of Bacteria were found free, between the separated and broken up cells, and the cells were densely filled with granular matter, as may be seen in Pl. XXVI. fig. 10, A ($\times 700$), but it was impossible to identify with certainty any of the granules as germs of Bacteria. Sections that were made and carefully stained gave no more definite results.

Another sheep's kidney from a freshly killed animal was therefore obtained and treated in the same manner, except that it was left in the incubator at 76° F. for three and a half days. When the organ was cut through it was deeply stained at the circumference as before with the chromic acid; but the red tissue within was much softer, and the odour was most offensive and putrid. Portions of the organ were at once put into a 10 per cent. formalin solution with a view to obtaining sections therefrom, but a minute portion was cut off as before and teased in a drop of gentian-violet. On examination with the microscope after a brief interval I found the fragments of the tubules and kidney-cells full of Micrococci which had taken the stain well, together with numbers of figure-of-eight organisms and short chains (Streptococci) such as are shown in Pl. XXVI. fig. 10, B ($\times 700$).

In a few days Dr. J. S. Collier kindly sent me a number of sections, stained and unstained, which he had been good enough to cut from the portion of the kidney in the formalin solution which I had handed over to him. Some of the specimens sent to me had been stained with methylene-blue or with logwood, and then mounted in balsam; while I stained some of the plain sections with gentian-violet, and subsequently mounted them in glycerine. On the whole, rather more details could be made out with these latter sections than with the specimens mounted in balsam.

* I was using the incubator for other purposes at this temperature, and therefore did not alter it.

Every section, however, showed, inside the area which had been affected by the chromic acid, that almost every cell within the renal tubules was full of developing or actually developed Bacteria. The former appeared as mere cocci-like particles which had taken the stain like the developed Bacteria, both being situated in the midst of cell-granules comparatively unstained. The organisms were distributed through the substance of the cells, but often seemed to be most abundant in the half of the cell next the wall of the tubule, as may be seen in the transverse and longitudinal sections of tubules shown in Pl. XXVI. fig. 11, A, B ($\times 700$). In a few of the tubules in the pyramidal portions of the kidney the organisms had developed more abundantly, so that the cells were filled with a dense mass of Micrococci such as may be seen in fig. 12, A ($\times 700$). In sections through blood-vessels also a moderate number of Bacteria were seen mixed with the blood-corpuscles.

Here, then, it is clear that we have again the kind of appearances which we have a right to expect if the micro-organisms had developed in the epithelial cells of the kidney by heterogenesis. We have the cells full of particles developing into fully formed Bacteria, and, what is more important still, we have them within almost every epithelial cell to be seen in the sections.

From the point of view of the really absurd suggestion as to organisms being found in such an abdominal organ as the kidney, by reason of its "proximity to absorbing mucous membranes," it may be well to recall the fact that the sheep's kidney is encased in a mass of fat from half an inch to an inch in thickness, and that, as an additional barrier separating wandering microorganisms from the epithelial cells of the organ, there is still the thick and tough capsule which I have thought it not useless to show in fig. 12, B ($\times 700$). Let anyone compare M. C. Potter's description (p. 385) of the mode in which Bacteria are enabled to effect an entry into a single vegetable cell, and then let him imagine what an army of Bacteria would be needful, with all the cytase that they could excrete, to get through such a tough and thick layer as that presented by the fibrous capsule of the kidney. But surely the whole notion as to such a mode of infection of the kidney and other abdominal organs is too absurd for serious consideration.

We are then driven back to enquire whether it is true that the blood is germless, and whether it has in reality the bactericidal qualities with which it is credited. I have no evidence whatever to oppose to these beliefs, nor is it easy

to see, even if bacteriologists generally should be wrong in their views as to these points, how it would suffice to explain the development of Bacteria within all the cells of a kidney treated in the way I have mentioned.

It is perfectly certain that the blood of healthy persons does not contain any appreciable number of active Bacteria. But are bacteriologists right in supposing that such Bacteria as get into the blood-stream are "destroyed"? May they not rather be reduced to a condition of latent vitality? Their answer to this is that, if it were true, the organisms would be capable of revealing their presence when suitable media were inoculated with them and subsequently exposed to proper incubating temperatures. And it is the negative results of all such experiments with the blood of healthy animals that confirm them in their belief as to the germicidal qualities of the blood.

Nor, in fact, if the blood were assumed to be full of latent germs of Bacteria, would it be easy, as I have intimated, to see how that would enable us to explain the development of Bacteria within almost every epithelial cell in the kidney referred to. Could organisms reduced to a condition of "latent vitality" penetrate the walls of the capillaries, and thence migrate into all the cells of a kidney-tubule? The notion is again too preposterous to be entertained; so that we are compelled by evidence of a most convincing character to admit that the Bacteria have in reality been born in the individual cells of the kidney—we are compelled to believe that heterogenesis has, in fact, been taking place here as in the other instances previously cited.

EXPLANATION OF THE PLATES.

PLATE XXV.

- Fig. 1.* Development of Bacteria between the cell-wall and the chlorophyll layer in a living Nitella cell. $\times 700$.
- Fig. 2.* A. Early stages of Bacteria developing in primordial utricle of Potato cell. $\times 700$.
 B. Early stages and developed Bacteria within a neighbouring cell. $\times 500$.
- Fig. 3.* A. Bacilli and Mycelia filaments within a cell of another Potato. $\times 500$.
 B. Interlacing spore-bearing Bacilli in or on the primordial utricle of another cell from the same Potato. $\times 500$.
- Fig. 4.* A. Microorganisms developing in or on the primordial utricle of a small new Potato. $\times 500$.
 B. More developed form of similar *Crenothrix*-like filaments in another cell of the same Potato. $\times 500$.

Fig. 5. Tufts of a form of *Cladothrix* growing from the primordial utricle in the cell of another new Potato. $\times 500$.

Fig. 6. A. A large aggregation of Bacteria together with other scattered specimens within a cell of a small Turnip. $\times 500$.

B. Numbers of Bacteria developing in and on the surface of the primordial utricle in a cell from another Turnip. $\times 500$.

PLATE XXVI.

Fig. 7. A. Minute Fungus mycelium within the cell of an Apple. $\times 250$.
B. Micrococci or Bacteria developing in or on the primordial utricle in another cell of the same Apple. $\times 375$.

Fig. 8. A and B. Early and later stages of change in a Canadian Apple. $\frac{1}{2}$ nat. size.

C and E. Micrococci-like bodies developing in or on the primordial utricle in cells of one of these Apples.

D and F. Similar bodies in other cells giving rise to thread-like growths, after the fashion shown more plainly in fig. 4, A.

G. Larger body of the same kind from which a small hypha is developing.

H. A group of small *Torula* cells.

J. Minute branching and interlacing filaments containing rows of spores or cocci-like bodies in or on the primordial utricle of another cell.

C, D, E, F, G, H, each $\times 700$; J, $\times 375$.

Fig. 9. A. Caudal plumose spines of a recently dead *Cyclops quadricornis* showing homogeneous protoplasm within.

B. One of the same spines after four days showing motionless Bacteria developing within the protoplasm.

C. The same spine after two more days showing motionless Bacteria much increased both in size and in number.

Each $\times 700$.

Fig. 10. A. Granules and nuclei from disintegrated cells of a sheep's kidney after exposure to conditions described in text for thirty-six hours.

B. Disintegrated portions of cells from another sheep's kidney which had been exposed to similar conditions for three and a half days—now swarming with Micrococci and Streptococci.

Both specimens had been teased in a weak solution of gentian-violet, and both were $\times 700$.

Fig. 11. A and B. Transverse and longitudinal stained sections of tubules from the same kidney, showing the cells crowded with Micrococci and Streptococci.

Each $\times 700$.

Fig. 12. A. Dense masses of Micrococci in pyramidal portion of same kidney.

B. Stained portion of the same kidney, showing the thickness of its capsule as seen under the same magnification.

Each $\times 700$.