On the "Life Cycle" of Bacteria.  
A Contribution to the Study of the Granular Form.

by

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(With Plates 13-15).

The Bacteria are in no way a group of simple organisms, but rather a group displaying a high degree of morphological differentiation coupled in many cases with a life-cycle of considerable complexity.

C. CLIFFORD DOBELL (1).

The starting-point leading up to the present study was the research-work I carried out in 1910 on the cytology of the tubercle bacillus (2). Since that time, when I ascertained the possibility of the transmission of the tuberculous infection by the granular form, obtained by the filtration of tubercular pus, I made sure that the phenomenon studied must be a general one, for it would seem impossible logically to admit an exception of such far-reaching consequences in the field of microbiology and one so flagrantly at cross-purposes with the classic principles, built on and confirmed by about thirty years of research.

Many circumstances concurred to prevent my pursuing these studies, after some insuccses in posterior verifications.

Taken up by several workers, during these last years, these studies had the confirmatory evidence of KIR-SCHENSTEIN, (3) in Riga in 1922, of VAUDREMER (4), VAUDREMER and HAUDROY (5), BEZANÇON and PHILIBERT (6), J. VALTIS (7), in France in 1924, RAVETLLAT and PLA Y AR-MENGOL (8), of Barcelon in 1924 and of ALBERT PETIT (9) in Tunis in 1923.

I resolved to investigate the ultimate facts of the growth and reproduction of bacteria, convinced as I was of the unsatisfactory nature of our knowledge on the subject, and it is the results of the studies I undertook, that are set forth in this paper.

The material selected for study, contrarily to what is usually chosen by microbiologists, consisted of small bacteria, of those pathogenic for and commonly affecting man.
Reasons for this choice are the following:

a) a belief in the diversity of mode of reproduction of different species of bacteria and even of the mode of reproduction of bacteria of one species;

b) the greater significance of any conclusion reached if applying to bacteria pathogenic to man.

I therefore selected a material for study among cocci, staphylococcus, streptococcus and gonococcus and among bacilli, the B. diptheriae, representing the corynobacteria, B. coli and B. dysenteriae, SHIGA and FLEXNER types.

Observation was always carried out on different days, different preparations subjected to varying technical processes. Wherever feasible recently isolated strains and laboratory strains were both studied.

Observations made on fresh (unfixed) material were always controlled by preparations of the same material stained and fixed, for later perusal.

Observation was made by the light furnished by a metal filament lamp, with cobalt glass, 1/2 watt, 50 candle-power. Drawings on the other hand were made by natural light. Optical material used was a Zeiss microscope, comp. oculars 4, 6 and 12, homog. immersion 1/12.

Methods of investigation used were:

a) observation in life of fragments of colonies between cover-glass and slide;

b) observation of similar material after staining by methylene-blue and by SALOMONSEN stain;

c) preparations fixed in sublimate-alcohol and stained with iron alum—haematoxylin, DELAFIELD and HEIDENHAIN;

d) preparations fixed with the MAY-GRÜNWALD solution and stained over with GIEMSA;

e) preparations of colonies fixed with MAY-GRÜNWALD and stained over with GIEMSA. These were obtained by the following procedure: On perfectly clean microscope slides a layer of gelose is placed, sufficiently thin as to adhere perfectly to the slide and sufficiently thick to allow of the growth of colonies, without hindering the transparency of the slide which ought to give the same impression as blood drawn out in a thick film.

After cooling down the slide is inoculated with a platinum loop at points equally spaced. The loop is not pressed hard.

The inoculated slide is kept in PETRI dishes with a little sterilised water at the bottom (a few drops of water), so as to provide the moisture necessary for growth. The dish is then put in an incubator.

After the colonies have developed the slides can be fixed and stained by the MAY-GRÜNWALD stains, just as blood-films are stained.

The preparation if shut in balsam will keep very nicely.

The present paper will be divided in the following parts: 1) observation; 2) summary; 3) discussion; 4) conclusions.

OBSERVATION.

1. PYOGENIC COCCI:

Staphylococcus, Streptococcus, and Diplococcus gonorrhoeae.

The study of staphylococcus, streptococcus and diplococcus of gonorrhoea in preparations fixed and stained by MAY-GRÜNWALD and stained over with GIEMSA show merely a condensation of the chromatic substance in the different phases of the evolution of the bactericidal cell, bringing about a clearly defined plane of division, which gives rise to newly formed bacteria. The glutinous substance joining bacteria together could be easily made out.
With the diplococcus of gonorrhoea one has the impression that its shape is that of a thick cocco-bacillus in which by the condensation of the chromatin at the poles, a clearly outlined space is left in evidence.

Similar pictures were obtained in preparations fixed in sublimate-alcohol and stained in HEIDENHAIN's haematoxylin.

2—DIPHTHERIA BACILLUS

a) Strain isolated from a case of angina in a child. First passage on serum after isolation.

Observation of fresh material in hanging drop.

Drop of condensation water from an 18 hour-old culture on serum.

Observation during two hours in a room heated at a temperature between 35° and 39°C. Observ. carried out on June 26, 1925.

Short forms like cocci predominate, some of them showing a clear central transverse plane, looking like a plane of division. Granular forms in clumps, the grains being joined by a substance which looked at times amorphous, at times reticulate and finely granular according to the micrometric variation of the focal plane.

Short rod-shaped forms with diffuse substance, without a granular appearance are seen, others in which the variation of the micrometric plane allows one to distinguish a condensation of the substance at poles and on the lateral walls with intervening clear spaces.

Forms in which this differentiation had progressed further showing the substance of the body of the bacteria already clearly granular.

I was unable to observe clearly the separation of bacteria which appeared joined together.

b) Preparation fixed in sublimate-alcohol and stained in HEIDENHAIN's haematoxylin. Twelve hours' culture on serum. First passage after isolation. Observ. made on June 28, 1925.

Short forms are predominant, with appearances varying from that of a dot to that of granular rods, with clearly discernible grains, lying isolated in the body of the rod, or else connected with each other by extremely delicate threads. In some forms the outline of the bacteria is clear, in others not.

Some forms appear with a diffuse chromatic substance, showing by the micrometric variation of the focal plane an indication of granular condensation. In a few longer forms a very delicate reticulum can be discerned connecting a set of clearly separated granules.

c) PARKES strain kept up in laboratory. Gelose culture observed after 6 hours of development. Preparations fixed in sublimate-alcohol. Stained with DELAFIELD's haematoxylin. Observed on June 30, 1925.

Granular forms connected by very delicate threads. A larger granule is always to be seen looking as if it were a centre of origin for the other smaller ones.

d) Preparations fixed and stained with MAY-GRÜNWALD and stained over with GIEHSA.

The bacteria seem to proliferate in a mass of glutinous substance, stained a pale pink. The granulations are visible and appear separated (comp. oc. 6). Observed with comp. oc. 12 they are made out to be in continuity, connected by an amorphous looking substance.

The discernible granulations appear diffusely stained, with a deeper tone than the rest of the substance which unites them.

In many of the forms one of the granulations is bigger than the others.

Isolated forms also prove to be
granular, showing two or more granules, connected by a substance stained a pale pink.

In the clumps there are forms which clearly allow one to discern a connection between parallel granulations. This connection is at times in a straight line at times in a broken one forming an angle. In some forms by the side of the big granulation, another much smaller one is seen, as if having arisen from the former, like a little bud.

e) Recently isolated strain. Serum culture, 48 hours-old. Second passage. Obs. on July 3, 1925. Examination fresh unstained material. Fragment of colony between cover-glass and slide.

Elongated coco-bacilli showing clearly an enveloping halo. Refractive median plane imitating a scission. Refractive dots at poles indicating a condensation of substance. Upon observation during 45 minutes at room temperature (22°C.), granulations appeared, which little by little increased in volume, with a progressively thicker and more oblong.

Elongate forms, clearly granular with clear spaces between granulations. Movements of the micrometric screw bring out the condensation of substance at the limits of the rods, sides and poles. Some forms appear as threads with intercalate granules.

In one of these rod-shaped forms I was able to watch the rupture of the connective filament of a granulation, resulting in the formation of two cells, with the appearance of a transverse division. Some granular forms seeming to evolve towards coccus form, show a small protrusion (gemma?) which remains smaller than the mother-granulation until a phase which seems to indicate division, and is represented by the presence of a plane of marked refractive property.

Forms seemingly regressive (involutive), long, thick and flexuous, made up of refractive, non-granular substance.

f) Preparation with methylene blue without fixation. Observation which confirms the evidence given above. Preparations fixed with sublimate-alcohol and stained with DELAFIELD's haematoxylin. Differentiation with ferric alum. Observ. on July 5, 1925.

Short forms, of slight granular appearance predominate. On one or another more differentiated bacillus granulations not very condensed are to be seen. Pronouncedly regressive (involutive) forms, some with clear polar granulations others more diffuse.

In one or another form one has the impression of connection between the bacilli. Numerous free granulations.

g) PARKES strain. Eighteen hours' culture on gelose. Preparations fixed in sublimate alcohol and stained in HEIDENHAIN's haematoxylin. Differentiation by ferric alum. Observ. on July 7, 1925.

Medium forms in the shape of slightly curved rods, with one clear central granulation, diffuse and small polar granulation and another at the other pole, diffuse and oblong. The latter observed through comp. oc. 12 shows a greater condensation at one end, and continues diffuse up to the lateral wall of the rod. Between the two lesser granulations, a shadow is to be discerned which seems to correspond to the substance which connects them.

Long rod-shaped forms with clear granulations, polar and central; one of the central granulations is oblong and larger than all the others. Slight movements of the micrometric screw show up small or intermediate granulations. The body of the rod appears slightly curved.

Giant flexuous forms, with diffuse staining, with a finely granular appearance, some with a granular reticulum discernible, most however without any
clear structure. Small chromatic condensations in both the poles of the bacteria.

Short oblong forms, similar to the form of pneumo-cocci, varying in size, until they appear as centrally strangulated rods. These forms appear with a diffuse staining, some of them show clearly connection by a pediculum. In some a differentiation of chromatic substance is to be seen.

Exclusively granular forms, with or without a halo, of varying size, minute, small, medium and large. The medium-sized and large-sized forms appear slightly oblong. In these forms no differentiation of chromatic substance is to be clearly discerned. In some small granular forms, a smaller granule may be seen connected with them.

h) Preparation without fixation stained by SALOMONSEN of a fragment of culture between cover glass and slide. Obs. on July 7, 1925.

Granular forms without differentiation. Cocci forms allowing a central plane of division to be discerned, made up of refractive substance of a non-granular appearance. Rod-shaped forms short, lanceolated, which have the appearance of being connected laterally by the poles to other twin-forms.

Rod-shaped forms, clearly granular, showing other shorter forms also granular attached to them. A relative proportion is to be observed between the granulations of the smaller and the larger forms.

i) PARKES strain. Five days'old serum culture, kept in incubator. SALOMONSEN stain. Same technical procedure as in e). Observ. on July 12, 1923.

Coccus-forms, short, medium and long rod-shaped forms, torula forms, all of them clearly granular. In some forms other smaller ones are seen to be appended.

Numerous minute granular forms free. Some granular forms are juxtaposed and show a correspondence of and probable connection between granules. In certain fields of the preparation brownian motion allows one to see the movement to-and-fro of these forms round the point of connection which ties them together. This correspondence and connection are also clearly seen in the parts of the preparation in which there are no fluid currents and in which the germs remained adhering to the cover-glass.

j) PARKES strain. Gelose culture of 18 hours'development. Fragment of colony stained by phe-nicated methylen-blue, without fixation. Obs. on July 13, 1925.

Granular form easily recognised in isolated granules and in clumps agglutinated by an intermediate substance.

Short forms with the emission of clearly granular filaments. These filaments are attached either at the poles or else laterally; there seems to be no constant rule as to its site of attachment in the different forms. Longer forms with rough limits, giving one the impression of granules protruding on the surface.

Medium forms clearly connected to shorter forms by granular filaments. Minute granules tied together two by two, three by three and in greater number, making up clumps.

3. BACILLUS COLI.

Strain isolated from faces of a case of febrile enteritis.

a) Gelose spread over slide in the manner already described. Colonies studied with 2 to 4 hours' development at 37° C. Examination of unfixed material. Comp. oc. 6 and 12, homog. imm. 1/12. Observ. on July 20, 1925.

Growth and reproduction of bacilli is evinced by the appearance of granules and spots, more or less diffuse, tied to-
gether by very delicate threads.

Thus clumps are made up; the separation of bacilli is completed by the rupture of the connective threads, which gives the division an opportunity of appearing transverse or longitudinal.

In certain parts of the gelose were there are as yet no bacteria to be seen a granular dust can be made out. In this granular dust one may see here and there the appearance of well made-up granules, which give rise to new granules connected with them with other granules, with a rod or with clumps of granules.

The phenomenon comes to pass as if life arose in the midst of colloidal matter, if the term life be accepted as meaning growth and reproduction.

Between half an hour and 45 minutes an outline of the bacterial bodies could be discerned.

b) Preparations fixed with sublimated alcohol and stained with HEIDENHAIN's haematoxylin. Same strain. Twenty four hours culture on gelose. Observ. on July 23, 1925.

In these preparations uniformly stained bacterial bodies are to be seen; these forms vary between those of isolated granules (which are scarce) to those of short or slightly elongate rod-shapes, these rod-shapes being the most numerous. In some forms in which the staining has attained greater differentiation, a condensation of chromatic substance is to be seen at the poles, but granulations are not recognisable. Often condensation is more marked at one pole. In some forms, in which differentiation almost reaches the point of complete unstaining, delicate granulations, more intensely stained are to be seen.

Bacilli placed parallel in different directions reveal between them a finely granular stroma, with clearly visible larger granulations.

In fields of the preparation which are clear on account of a lesser thickness of the material, this same substance is seen stained a pale violet-brown connecting two clearly characterised little rods. In certain clumps differentiation allows one to discern clearly the ground substance joining together bacilli and granules.

In isolated forms granular differentiation is clearly seen, with a central clear area and bipolar granulations and sometimes with a larger central granulation as well.

c) Same strain. Imprint preparations of recent colonies, from 4 to 6 hours old developed on gelose. Same stain as above. Obs. on July 25, 1925.

Beginning colonies are to be seen in islets made up of few rods (about 6 to 12 to each colony), in which one may see isolated granules and granules inside the bacilli which are joined together.

The larger islets have the appearance of a map of a microscopic city with streets and squares. In some isolated microbic forms appended granulations are clearly seen.


Round, granular forms, intensely stained in deep red, with a small slightly clearer halo. Short rod-shaped forms, stained pink and having a polar granulation intensely stained and clearly visible. Other forms with the same characters, sometimes with bipolar granulations, others with a central granulation. Some longer forms display an intensely coloured granulation connected by a finely granular thread in zig-zag to other clear but smaller granulations.

Long, thick forms, uniformly stained, without appreciable differentiation, having a sharply-cut outline which allows one to presume of the rudiment of a membrane.
e) Same strain. Gelose colonies having 4 hours at 37°C. Observ. without stain at room temperature (21°C) carried out on August 1, 1925.

In some fields a reticulum is to be seen finely granular and with the threads parallely disposed in different directions.

Granular forms, isolated or connected by very delicate threads so as to make up clumps, in which little by little there form granular rod-shaped forms, which remain connected during 2 hours of observation. Under separate observation one of these forms proved to be like a rod with polar condensation and lateral condensation of the chromatic substance. Half an hour later there was seen to arise from the pole that contained the granulation a small thread which 15 minutes later was clearly visible. Meanwhile the chromatic substance became more diffuse; becoming clearer in the centre, the while it became granular in the periphery and outlined the formation of two branches starting from the primitive granulation. In the filament appended to this there arose a new granulation. At the end of 2 hours, separation was not yet complete.

f) Same strain. Six hours' colony on preparation made by spreading gelose on slide. Development at 37°C, MAY-GRÜN WALD fixation with superstaining with GIEMSA. Obs. on Aug. 8, 1925.

Rods place parallel in different directions, allowing an intervening clear space; this clear space found all over the space occupied by the colony contrasts with the pink ground of the preparation made up by the culture medium.

The rods seen in groups are seen to be continuous, joined as they are by a substance weakly stained a pale blue.

In the forms which appear separated the body of the bacterium is stained violet purple and is of more or less condensed substance. Some of the bacteria in which the differentiation was more perfect allow one to see a border (halo) stained a pale blue inside which the chromatic substance condensed in the form of granulations is stained purple violet. In some forms with a brilliant illumination granulations stained red are to be seen. Chromatic condensations affect parallelism. Isolated granular forms without differentiation are to be seen outside the colonies. In some points of the preparation irregular spots stained a weak violet blue or a pale blue are to be seen against the pink background of the culture medium. Inside these, placed parallel in different directions there appear chromatic condensations, at times as isolated granulations, at times as dust, stained red or purplish red. In some points of the same quite clearly constituted forms are to be seen.

*This picture seems to correspond to that seen in a fresh preparation and already reported as a finely granular substance in which are to be seen refractive dots appearing to give origin to bacterial forms.*

In some forms an irregular distribution of the chromatic substance is to be seen; in others this substance appears uniformly stained, and takes up the body of the bacterium (short forms).

In clumps of bacteria, the correspondence of bacteria is to be seen: they are placed parallel, at a lateral angle or at the extremities and indicate a continuity of the bacterial bodies.

In the clumps which appear to indicate the initial colonies the presence of a granular dust is constant.

**4—B. DYSENTERIAE**

a) SHIGA type. Strain kept in laboratory. Observation of a 10 hours'
culture on gelose spread over a slide, on September 10, 1925. MAY-GRÜNWALD—GIEMSA fixation and stain.

Isolated granular forms with an appendage of a more faded color. Larger granular forms slightly oblong, isolated or in clumps, the well defined appendages of which seem to indicate a continuity of the forms.

Clumps seen isolated display these forms placed parallel or at angles. Long forms, some of them clearly granular, with polar and central granulations, others with a uniformly stained chromatic substance.

In colonies the bacteria are disposed parallely and at angles forming an irregular mosaic. In some preparations containing flexuous forms and in which differentiation is more perfect, fine granulations are to be seen in their interior.

b) Same strain. Fragment of colony between cover-glass and slide. Four days culture kept at 37°C. Observation of fresh material on September 12, 1925.

Short forms in the shape of cocci and coccobacilli, very short, with two refractive granulations. Similar forms slightly more elongated. Rod-shaped forms with 3 or 4 conspicuous granulations placed parallel with an exact correspondence of granulations. Rod-shaped forms connected with one another so as to form acute angles, right or obtuse angles, and showing correlation between the granulations of one and another. Long, flexuous form clearly granular.

c) Preparation stained with methylene blue, without fixation.

Free granular forms, without visible appendage and with a small appendage stained a paler blue. In the case of the rod-shaped forms, the outline of the cell is clearly seen, the isolated granulations being in its interior. Rod-shaped forms showing clearly the connection between its granulations and those of other forms appended.

d) Preparations stained by the processes described above.

Nothing besides what has been described was observed.

FLEXNER type. Recently isolated strain.

The description of the different preparations is entirely similar to that given for the SHIGA type. It is not given as nothing was found to make it differ from the indication for the SHIGA type.

SUMMARY

PYOGENIC COCCI

The study of the pus-provoking cocci was of little avail. Reproductive activity is by division of the chromatic substance which seemed to me direct. This process begins with the condensation of the chromatic substance at the poles, taking along a small amount of the cytoplasm. After this there is a narrowing of the central part in which the plane of division is sketched out. This division takes place the while the cocci remain enveloped by the glutinous substance.

B. DIPHTHERIAE, B. COLI AND B. DYSENTERIAE.

Pleomorphism was constantly observed in recently isolated strains as in those kept in the laboratory. Under the same conditions of the chemical composition of culture-media and the same eugenic conditions of observation, the preparations revealed a pronounced diversity in the morphology of the bacteria which varied from the form of a simple granulation to that of long and flexuous bacteria.

Observations made under varied technical conditions, on fresh unstained preparations, on preparations stained
without fixation, on preparations fixed in sublimal-alcohol or in methylic alcohol and stained with iron-haematoxylin or with MAY-GRÜNWALD—GIEMSA fluids, made it possible to recognise, in the bacteria studied, a differentiation of the chromatic substance indicating a process of division, expressed in most cases by the organisation of granulations, variously disposed inside the bacterial cell. It was not possible to establish a clear sequence in the formation of these granulations. The possibility of some of them being given origin to by others, by the transference of chromidal currents, giving rise to the increase in volume of the granulations observed in fresh preparations, was observed. In some cells the transportation of chromidal substance took place towards the periphery of the bacterial body, in the shape of fine, granular thread; from the visible granulation, a new thread would start out, forming the axis of formation of the new bacterium or cluster of bacteria.

These pictures seen in the fresh preparations, with or without staining, were confirmed by preparations stained after fixation. Similarly there were seen in certain fields of preparations examined unfixed with oblique illumination from a strong light (metal filament-lamp, 1/2 walt, 100 candle-power) agglomerations of finely granular substance, at times with reticular disposition, in which one may observe the sudden appearance of granulations at different points. The granulations become more and more refractive and after a certain time (1/2 hour to 45 minutes) showed the beginnings of bacterial form.

These pictures were still confirmed by preparations stained without fixation (granular dust) and by preparations stained after fixation.

The separation of two bacterial cells joined laterally, simulating longitudinal division, was seen just once. The presence of the granular form was constant.

DISCUSSION

I do not propose to summarise the work that has been carried out on a subject so discussed, nor to criticise other worker's observations and conclusions. It would suffice to cast a glance on the bibliographical index appended to see that this would be no light task. This index I have compiled only for the use of those whom it might in any way interest.

It is evident however from what is known that our present knowledge as to the cytology and reproductive processes of bacterial life is still in a state of considerable confusion.

The fact is that, owing to the insufficiency of our present methods of research or to incapacity of the workers engaged, which is less likely, careful investigation leads one to the certainty of phases of the reproductive activity of the bacterial cell which pass unperceived and allows to presume of the complexity of the life-cycle of these organisms.

The supposition that bacteria are cells without nuclei must be cast aside once and for all, in spite of the fact that the morphology of these organisms cannot be compared to that of the superior cells which are clearly differentiated in cytoplasm, nucleus, nucleolus and reserve-bodies.

In the bacterial cell, and a fortiori in bacteria of small size, these elements exist in reality mixed in a structure which cannot be made morphologically evident by microchemical and staining tests for chromatic substance. In these cells we are unable to make out a typical little nucleus, rather do they seem to have a dissolved nucleus (aufgelöste Kerne) (10) or rather nuclear substance dispersed like a dust, or a chromidial system equivalent to a nucleus.
The observation of granulations seen within the bacterial cell would seem to indicate that some preside over reproductive activity, as these remain larger, and as from these may be seen a transfer of material which is going to build up others; and yet no reagent or method is to be found that would make it possible to assert categorically this function.

On the other hand, exclusive direct reproduction by division of a cell in two, seemed to me difficult to understand, in an activity that reasoning a priori would have to be extremely fast and which I was only enabled to observe in one or another cell and which was so slow that doubts arose in my mind as to cell that was being watched might not be dead!

One would in reality be led to foresee in the growth and reproduction of bacterial cultures such speed that it would be difficult if not impossible to follow separately the evolution of 1 cell; observation, during two hours, however, of one sole element in a marked microscopic field did not allow me to see in the majority of cases any perceivable progress and when there appeared to be any the doubt would arise that I might be subject to a peculiar state of suggestion that would lead to a wrong conclusion. It might be objected that I did not in all cases observe at favourable temperature for cultures (37-38°C.). I did not do it for lack of a chamber that would permit it, for the one I had in use (ZEISS, based on the circulation of water), heavy to work and difficult to install, would have been cumbersome under the conditions in which this work was carried out.

This objection, however, loses a great part of its weight if it be remembered that observations were made on preparations kept in the incubator and which where examined soon after being taken out. Observation was then carried out at the ordinary temperature (21 to 25°C.). However great the slowing down of development at these temperatures, it is reasonable to suppose that they could not have been so marked as to have the appearance of a paralysis of two hours of all vital phenomena; at times even the transfer of chromidial substance was seen which warrants the state of life of the elements studied.

I therefore believe that in the life-cycle of bacteria there is an ultra-microscopic phase which is not to be revealed by our present methods of investigation.

The observation which I undertook in this direction, using the Reichert dark-field chamber, did not lead me to any conclusion, on account of the difficulty of distinguishing particles belonging to the culture media from those presumably of bacterial origin.

Reasoning would however lead one to foresee the reality of this phase. It is known that the transplantation of any bacterial germ to a new culture-medium causes, until there is an adaptation of the transplanted elements to the new conditions of life, a lysis of a great number of the bacteria and a consequent freeing of the chromidial substance over the medium. In a later phase experimentation showed me in stained preparations which confirm those made without fixation, that there are zones of culture in which morphological elements are organised in the midst of a finely powdery mass and are already recognisable by the usual technical methods.

TWOHT's experiments in 1915 (11) showing the presence of a lytic principle in a culture on ordinary gelose of glycerined vaccine, recognisable by the microscopic examination of the colonies which appeared as if made up of the débris of the bacterial bodies, also comes in support of the view of mainte-
nance of life in the chromoidal body incompletely attacked by the lytic principle.

These colonies transplanted gave rise to new colonies, some of them modified, others with a normal opaque-white appearance.

This would be a key to the explanation of bacterial mutations, due to endogenous principles, i.e. principles belonging to the culture, independently of dysgenetic influences brought about by extraneous agents, which have already been admitted by everyone.

Hence the pleomorphism seen in growing cultures even of recent ones.

That this variability is not fortuitous and that it is transmitted hereditarily, and may even be atavistic, is indicated by the works of TAENISSEN, BURRI, EISENBERG, BERGSTRAND and HANSEN among others whose work is so well reported in the brilliant paper of BERGSTRAND, of Stockholm (12).

The study of the bacteriophage or TWORT-d’HÉRELLE phenomenon takes us instinctively to the limits of live substance=live colloid, both in the case of the lytic agent as in that the cell having undergone lysis.

Whether an ultramicroscopic parasitic agent, a ferment or catalyst the bacteriophage has its power of action intimately connected with life, dependent of the equilibrium of it its component molecules. This equilibrium is easily overthrown by a variation of the electrolytic conditions of the medium, as is shown by COSTA CRUZ’ experiments, confirmed by LISBONNÉ and CARRÈRE (13).

As to the cell having undergone lysis, provided the dissociating ferments, whether autolytic or coming from outside the cell, do not disturb intragranular equilibrium, a new morphogenesis allows of the rebuilding of the cell-body.

This is what may be inferred from the filterability of the tuberculous and dysenteric virus and also from the demonstration that the cholera bacillus, reduced to a granular form, after having undergone bacteriolysis, still remains alive, according to METCHNIKOFF (14) and CANTACUZÈNE (15).

From all that has been said one may conclude that the granular form, whether induced by normal or accidental circumstances, insures live substance, at any rate in the case of the bacteria in which it was observed; of being in a peculiar state permitting a later rebuilding of morphology.

Without being the only process of reproduction of bacteria, the granular forms stand for a phase in the cycle of bacteria which under special conditions is probably the form of resistance of the live non spore-forming element against the causes of destruction which may threaten.

CONCLUSIONS

1°—Bacteria studied are nucleate cells.

2°—The nucleus of these cells is dispersed affecting chromidium form which with the evolution of the bacterium towards reproductive activity mobilises and condenses into granulations.

3°—The localisation at the poles indicates an amitotic process, the details of which cannot be followed up owing to the unsatisfactory nature of the apparatus used for observation.

4°—The irregular distribution of the chromidal substance inside bacterial cells, in the same manner as its regular distribution in cells of the same nature, seems to indicate that cells of the same species are able to divide and multiply by different processes.

5°—The growth of these cells and
their reproduction are closely connected with the growth and reproduction of the chrocidial corpuscles.

6o—The growth and reproduction of these cells takes place through the emission of granules inside the protoplasm, disposing themselves for the interior division of the cell, or else through the emission of granules outside the organism studied, which will give rise to a new reticulum about to build up a new-formed cell.

7o—The growth and reproduction of these cells may take place in the length direction or laterally, providing for transverse planes of division (Cocci, coli, and dysentery bacilli) or longitudinal planes of division like the branching of a tree (diphtheria and tubercle bacilli, sometimes B. coli).

The presence of a granular dust in beginning cultures (coli, diphtheria, tubercle bacilli), and in old cultures or cultures having undergone lysis (tubercle, dysentery bacilli), the variability of size of the granulations, the presence of a cytoplasmic residue enveloping some of them, the possibility of finding, in fresh and in fixed preparations of cultures of different ages, of forms which might be the links in a chain of growth ranging from the granular dust to the rod-shaped forms, are all reasons which make one presume, with every likelihood of truth, that there is a development of the chrocidial granulations, from the primordial phase of a simple granule to that of the bacterial cell.

Observation of unfixed diphtheria and coli bacilli seem to demonstrate this, and the filterability of the granular form giving rise to sub-cultures and to infection give a formal demonstration of it; this is in agreement with the experimental results of the work on tubercle bacilli quoted at the beginning of this paper and of that on dysentery bacilli as was also ascertained by HAUDUROY (16).

GENERAL CONCLUSIONS

1o—The chrocidial granulations which make up the bacterial cell are able individually to reproduce the species.

2o—The form which furnishes the character of the bacterium used in bacteriological systematics is a phase of the evolution of the live substance and corresponds to that of the complex organism.

3o—The granular form represents therefore in the life-cycle of bacteria a phase which insures the perpetuation of the species.

4o—Bacteria have a life cycle which may be summarised in the following phases of their nuclear activity.

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\begin{align*}
\text{A—Germinal phase} & \quad \begin{cases} 
\text{Granular Dust} \\
\text{Free granulations} 
\end{cases} \\
\text{B—Phase of growth} & \quad \begin{cases} 
\text{Granular multiplication} \\
\text{Cellular organisation} 
\end{cases} \\
\text{C—Phase of desintegration} & \quad \text{Cellular desintegration.} \\
\end{align*}
\]

\[
\begin{align*}
\text{Intracellular emission and organisation of granules} & \quad \text{Extracellular emission and organisation of granules} \\
\text{Cellular division and reproduction.} \\
\text{Granular Dust} & \quad \text{Free Granulations.} \\
\text{Granular desintegration Lysis} & \quad \text{Multiplication of granulation.} \\
\text{Cellular organisation.} 
\end{align*}
\]
EXPLANATION OF PLATES 13—15.

Fig. 1 to 8. B. diphtheriae.

Fig. 1—Parkes strain. Fragment of gelose colony (18 hours), placed between cover-glass and slide, stained with methylene blue without fixation.

Granular coco-bacillus, rod-shaped and flexuous form.

Figs. 2-3—Recently isolated strain.

Fragment of an 18 hours gelose colony stained between cover-glass and slide by SALOMONSEN stain.

Short forms showing the reticular condensation of the chromatic substance; others showing continuity of extremities by means of granules, others showing free granulations laterally appended.

Fig. 4—Same preparation.

Granular cluster connected by very delicate granular threads.

Fig. 5—Serum culture of 48 hours.

Recently isolated strain.

Sublimate-alcohol fixation and staining with DELAFIELD’s iron haematoxylin.

Short and long rod-shaped forms showing a chromatic condensation in granules connected by a fine thread in the long forms; the thread is not visible in the short forms.

Fig. 6—PARKES strain. Gelose culture (18 hours).

Preparation fixed in sublimate alcohol and stained by DELAFIELD’s iron haematoxylin. Granular forms from the shape of a dot to that of cocci.

Cocco-bacilli with a differentiation which allows one to see inside them a fine reticulum with granulations. Cocco-bacilli with a condensation of chromatic substance at the poles.

Forms showing one and two granulations appended at one of the poles.

More or less short coco-bacillus forms without any marked differentiation.

Long flexuous form showing a finely granular reticular structure.

Fig. 7—Same culture. Sublimate-alcohol fixation, HEIDENHAIN’s iron haematoxylin stain. Granular dust in a finely granular stroma.

Coccus forms without differentiation.

Rod-shaped forms with a condensation of the chromatic substance, forming polar and central granulations.

Cocco-bacillus forms with clear chromatic differentiation, showing a central narrowing which indicates a future plane of division.

Cocco-bacillus forms with appended polar granulation.

Long flexuous forms, finely granular and with clear and large granules connected by a fine reticulum.

Fig. 8—Recently isolated strain.

Serum culture (48 hours). Sublimate alcohol fixation, HEIDENHAIN’s iron haematoxylin stains.

Forms ranging from a simple isolated granule to cocci, coco-bacilli, bacilli with polar granulations and finely granular threads.

Figs. 9 to 21—Bacillus coli.

Fig. 9—Strain recently isolated from the faces of a case of enteritis.

Colonies of six hours’ growth on gelose extended over a slide, fixed and stained by May Grunwald fluid and stained over with Giemsa fluid.

Bacillus forms showing clearly the chromatic substance condensed at poles and centre in the shape of large granules, allowing the cytoplasm of the cell to be seen. These forms seen in small groups reveal a certain parallelism between the granulations. The background of the culture is pale with a pink hue from the culture medium.

Figs. 10-11—Other field of the preparation, showing different bacterial forms, from simple granular dust, isolated granules, cocci to the form of rods with the chromidial substance clearly granular or without any evident differentiation.

Figs. 12-13-14—Culture on gelose spread over slide. Observation of the fresh material 4 hours after inoculation of medium. Incubator at 37°C.

Bacilli with refractive polar and central points. In some forms noticeable differentiation is seen. In other forms a close interrelation is seen between the polar refractive dots, of one bacillus another. One or two forms show an apparently appended refractive dot.

Fig. 14—Imprint preparation. Sublimate-alcohol fixation. HEIDENHAIN’s iron-haematoxylin stain. Same culture. Rod-shaped forms without any noticeable differentiation.

Figs. 16-17—Same preparation.

Forms, some of which clearly show the condensation of chromatic substance as granules inside bacilli and others showing granulations outside the bacterial body and seeming to have arisen from it. One or another free granulation.

Figs. 18-19—Same preparation.

Granular dust and bacilli with appended granulations.

Fig. 20—Same culture. Methylene blue stain without fixation.

Disposition of bacilli in a colony.

Fig. 21—Fragment of a colony stained with methylene blue without fixation and seen in suspension in the staining solution.

Forms showing close interrelation between the granulations of the body of one and another bacillus.

Fig. 22—B. diphtheriae. NEISSER stain.

Fig. 23—Dysentery bacillus of SHIGA type.

Culture on gelose spread over a slide, fixed and stained with the MAYGRUNWALD stain and stained over with GIEMSA. Colony of 12 hours growth.

Disposition of the bacilli in a colony.

N. B. The figures illustrating the other preparations of dysentery bacilli are not given as they show nothing of special interest that is different to that which has been depicted for B. coli.