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REMARKS ON A

NEW METHOD

(INTERLAMELLAR FILMS)

OF

STUDYING THE DEVELOPMENT OF MICRO-ORGANISMS.

AND

THE MUTABILITY OF THEIR CHARACTERS AND PROPERTIES.

BY

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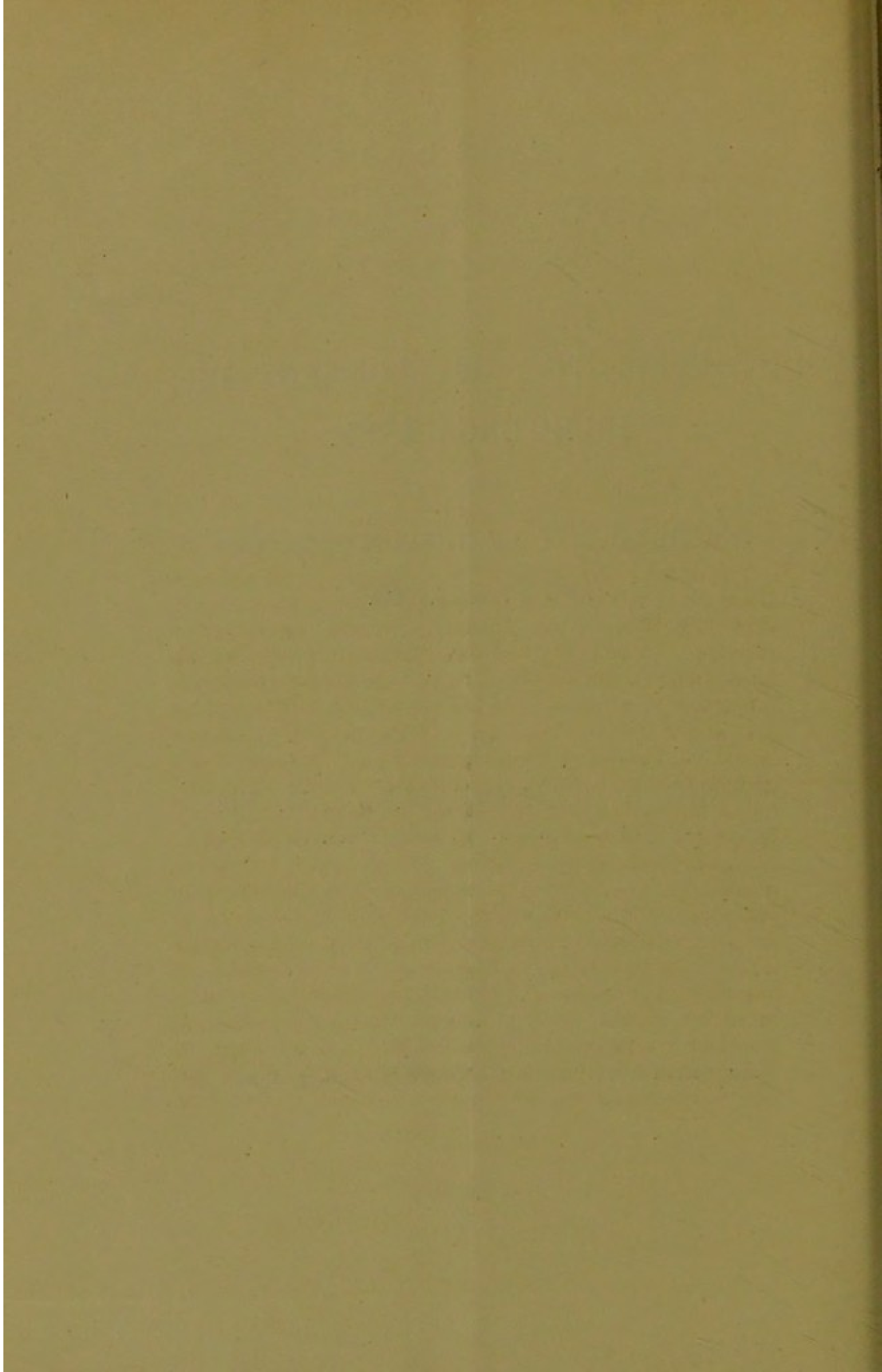


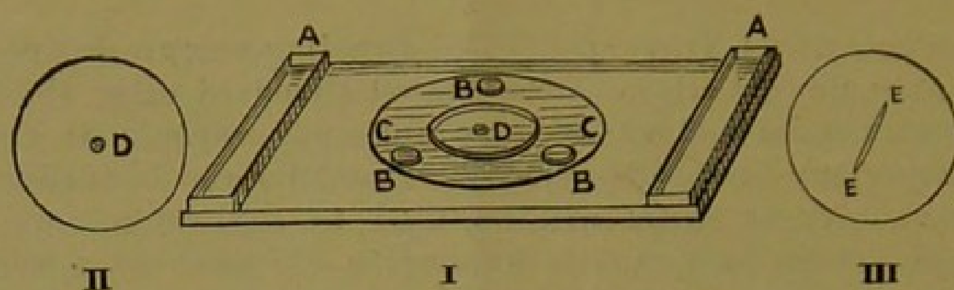
NEW METHOD OF STUDYING THE DEVELOPMENT OF MICRO-ORGANISMS.

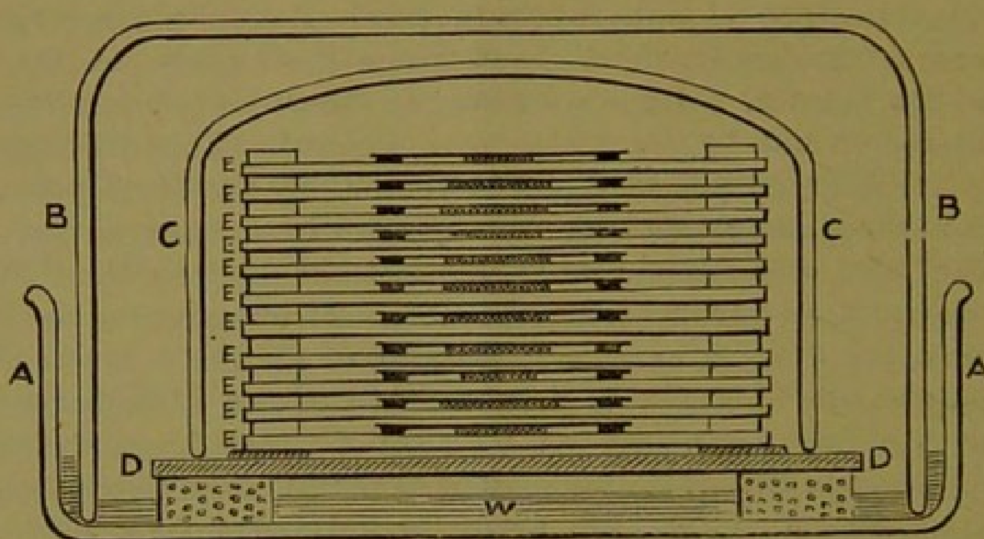
THOSE who have followed the discussions which have taken place between the partisans of the constancy (Koch, Zopf) and those of the mutability of pathogenic bacteria (Davaine, Naegeli, Pasteur, Buchner) know what stress has been placed on the impurity of cultivations the pathogenic properties of which seemed to have altered. It is evident that one of the simplest ways to solve this vexed question would be, instead of studying the mixed products of the germination of a number of sores, to isolate one spore, and follow its development through all its stages, and the development of successive generations of organisms all derived from the same original spore, and cultivated in various media. If it were potsible to follow thus the history of one spore and its progeny, it would only be necessary, in order to obtain definite results, first to study the complete series of morphological changes which occur, when the descendants of the same individual are cultivated severally in various media, then to connect certain physical and chemical alteration of the various media with stages of development, modified and unmodified, and finally to find how the properties of the organism at each developmental

stage are or are not modified by external circumstances. I had already attempted to carry out this plan by means of a dilution method, such as that used by Brefeld and others since, early in 1881, when working at the organisms of suppurating mucous membranes, a work which I gave up owing to the failure of the methods I was using then, and the special difficulties connected with the subject. I was, however, already then able to satisfy myself that phenomena analogous to those of karyokinesis were of constant occurrence in multiplying bacteria, and gave rise to many appearances which have been observed by others, though not explained fully yet.

About the middle of last year, whilst studying the development of certain pathogenic moulds and other parasites, I felt again the need of following closely the development of single organisms. I failed by plate and drop cultivations to obtain the results I wanted, partly owing to the effects of liquefaction of certain media or of the mobility of others, partly also owing to the form assumed by drops. I was then led to adopt a new mode of cultivation which, although not perfect in many of its details, has yielded results which so far have been satisfactory, and some of which have been exhibited this year at a meeting of the Pathological Society (May 5th). The principle of the method is to enclose a thin layer of the nutrient medium between two parallel plates, so as to force the organism to grow in definite directions. Owing to the effects of capillarity the most fluid nutrient media become, so to speak, fixed, provided evaporation be prevented, and they become as available as the solidified ones. The method can, of course, be varied in many ways ; but one of the simplest plans, and one of those which I have used with satisfactory results, is the following. At each end of a glass slide (1½ in. by 3 in) a narrow slip of glass is fixed (A A). This, as will be seen later on, is to act as a support. (The surface of the slide on which these slips are fixed will be called the upper

one hereafter.) Three small drops of sealing-wax are dropped on the upper surface of the heated slide (any other thick cement, solid and emitting no antiseptic vapours, at the temperature of the body, may be used instead of sealing-wax). These drops will be used to support a cover-glass (C) an inch and a quarter in diameter at a certain distance above the slide, and therefore must form the apices of a triangle capable of being inscribed in the circumference of such a cover-glass. Before placing the drop of fluid on it, the slide must be thoroughly sterilised in the flame of a Bunsen burner, or otherwise (the sealing-wax does not interfere with this process). Then the slide is inverted or placed under a thoroughly sterilised plate. A cover-glass (C) one inch and a quarter in diameter is sterilised also, and the surface which is to be next to the slide is carefully protected from the access of any germ or dust. On this surface a very small drop (D) of sterilised material may be placed, and this drop touched with a wire charged with a few organisms. A number of cover-glasses being prepared in this way, they may be examined over a sterilised plate with a pretty high power, inoculated surface downwards, and not in contact with the supporting slide, which must also be thoroughly sterilised, until a drop is found to contain the number of organisms wanted. Instead of a drop a streak (E E) can be used, according to the nature of the organism investigated. On the upper surface of the sterilised slide a drop of sterilised nutrient or other medium is deposited by means of a perfectly sterilised pipette. The size of the drop depends on the thickness one desires to give to the preparation or the surface one intends to cover. The diameter of the enclosed film should, in order to prevent contamination, never be more than three quarters of an inch when the cover is not more than an inch and a quarter in diameter. (I often use larger covers and slides, but this in most cases has no advantage.) The centre or the side of such a drop may now be inoculated (in case the cover has not been pre-





I.—A slide with interlamellar film, ready for incubation. A A, Side rests. B B, Drops of sealing-wax supporting the cover. C C, Cover-glass compressing a drop of nutrient material. D, Very small drop of gelatine or other material containing a few spores. II.—A cover-glass with very small central drop inoculated (D). III.—A cover-glass with a small central streak (E E) containing micro-organisms. IV.—Diagrammatic representation of eleven interlamellar cultivations in a moist chamber, showing the disposition which I have adopted both for this method and for other forms of film or plate cultivations. A, Outer basin containing a thin layer of water (W) at the bottom. B, Covering basin with a flat bottom, allowing a series of moist chambers to be piled one above the other in the incubator. C, Inner bell preventing condensed water falling upon the slide. D, Plate supported by pieces of cork. E's, Slides.

viously inoculated). Then the cover is placed over the drop. It should be well supported by the three drops of sealing-wax, and should not at this stage flatten much the drop underneath. A heated rod is then applied successively over the three drops of sealing-wax until the inoculated fluid has spread evenly over a certain surface under the central parts of the cover; the preparation is then ready for the incubator. It has, however, to be kept in mind that, owing to the free access air has to the surface of the inoculated fluid, it is necessary to keep the preparation in a moist chamber. The extremely small size of these inoculated slides allows of a large number being packed in an extremely small space. Before incubating the preparation it is necessary to select out of the micro-organisms which have been sown into the fluid one or several, the position and relation of which are carefully noted. For this purpose divided cover-glasses or slides, or, as I prefer, a finder, can be used. I have in this way followed the development of bacteria and spores of pathogenic Pyrenomycetes for days and weeks. I have devised many improvements for regulating the thickness of the enclosed film and making its thickness perfectly even, but these are not necessary to the success of the method, and their description would obscure its main object.

Now it will appear to many that this is nothing else than a drop cultivation, and to others that it is a film or a plate cultivation. It is all this, but I claim that it is something more; for by using an "*interlamellar film*," as I feel inclined to call it, the free surface of the medium is limited to the space existing between the two glass lamellæ used, whilst in ordinary plate, film, or drop cultivations the surface in contact with the air is very extensive; by the interlamellar method a side view, so to speak, of the cultivation is obtained; by the drop method a surface view. By the interlamellar method organisms placed at various distances from the free surface of the medium can be

followed in their development step by step,[1] a thing utterly impossible in drop cultivation. By the interlamellar method it is possible to follow certain chemical changes occurring along a growing filament or colony extending in a direction which can always be determined ;[2] this is impossible to the same extent in drop cultivations. By the interlamellar method it is possible to follow for weeks the development of the same individual, or group of individuals, even in the midst of a fluid material; this cannot be done for any considerable length of time in drop cultivations. I feel, therefore, justified in claiming for the method some advantages. I wish, however, to state clearly that it has disadvantages of a serious kind when the objects in view are not those which I have tried to explain, and therefore I do not offer this new method as anything more than a help to those who may try to solve some of the questions to which I have referred. On some future occasion I hope to be able to give further details regarding the modifications which have been suggested to me by circumstances and the general nature of the results I have obtained.

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[1] In this way the branching of many bacilli can be demonstrated. This branching, which is supposed by most authorities not to exist among the schizomycetes except in a spurious form, can by this method be easily demonstrated. I had, it is true, been led to believe in its existence from the study of organisms grown differently, but by this method branches can be seen to arise from definite filaments. Dr. Slater, who has kindly made many observations with me for the study of this point, has obtained results confirming entirely my views.

[2] By using media containing substances capable of forming insoluble compounds with the products of the metabolism of micro-organisms, the gradual formation of these metabolic products can be followed and demonstrated. Thus I have lately been able to demonstrate the formation of oxalic acid out of various substances, such as gelatine, starch, gum Arabic, and possibly cellulose. I have been able to show that the formation of this acid begins only when growing filaments have free access to air—a fact cf gieat significance in connexion with Pasteur's teachings.