ON THE GRAIN OF FILTERS AND THE GROWTH OF BACTERIA THROUGH THEM.

WITH REFERENCE TO THE DOULTON, PASTEUR, BERKEFELD AND SLACK AND BROWNLOW FILTERS.

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Plates I and II.

Plagge in 1886 observed that but few water filters prevented the direct transmission of micro-organisms and that all gave contaminated filtrates after being a few days in operation. This result has been widely confirmed, especially by Woodhead and Wood (1894, 1898), and by Bulloch and Craw (1906). Plagge considered that in the case of those filters which did not permit of the immediate or direct transmission of germs the subsequent failure of the filters was due to the gradual growth of the organisms through the filter mass, a view which has been adopted by v. Esmarch (1902) and is generally accepted.

It did not seem to me, however, to be proven that indirect or delayed transmission was entirely due to growth through the filter, and the following investigation was made to determine whether a mechanical acceleration is caused by the water current sweeping the micro-organisms through the filter mass.

As numerous data had already been collected by Bulloch and Craw (1906) and Bulloch, Craw and Atkin (1908) on the filtration of contaminated water through porcelain and Kieselguhr filters under pressure, it was only necessary to determine the rate of transmission of a highly motile germ when the pressure on both sides of the filter was the same.

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Work having a bearing in this connection has already been carried out by Cambier (1901) who used the Chamberland "*F*" filter for the isolation of *B. typhosus*. He filled the bougie with bouillon and placed it in a tube of bouillon. On inoculating the interior of the filter and incubating at room temperature the bacillus either did not come through or did so only after many days. If, however, he incubated at 37—38° C., the bacillus came through the filter mass "in a short time," *e.g.* 1½ days. Kirsch (1903) found that the shortest period for transmission through the Chamberland filter was 13 hours, but often *B. typhosus* required days to penetrate, and Bienstock (1903) had made similar observations.

In the following experiments the Doulton, the Berkefeld and the Slack and Brownlow filters have been compared with regard to their relative transmission of *B. pyocyaneus* and *B. prodigiosus*.

Method. The filter was placed in a large test tube containing sufficient neutral broth to cover two-thirds of the filter mass. The nozzle which protruded from the test tube and the top of the latter were bound with cotton wool and the nozzle exit similarly plugged. The whole was then heated in the autoclave to 120° C. for one hour and subsequently incubated for four days at 37° C. to prove sterility of the bougie and medium. 1 c.c. of a strong broth culture of *B. pyocyaneus* was then introduced into the interior of the bougie and daily observations of the external medium were made, during incubation at 37° C., for the first visible trace of fluorescence and cloudiness. A more extended series of experiments was made in exactly the same manner using *B. prodigiosus* as the infecting organism. Every few days a loopful of the contents of the bougies was withdrawn and tested by incubation at 37° C. with broth to ascertain whether the interior of the candle contained living bacteria.

Examination for growth of B. pyocyaneus.

Doulton filters, Nos. (1), (2), (3) and (4) were tested and did not show any trace of contamination in the external medium until 14, 18, 17 and 7 days, in the respective cases, had elapsed after the time of inoculation.

Berkefeld filters, Nos. (1) and (2) were highly contaminated after three days and two days respectively. In the case of No. (2) growth probably took place earlier, no observations having been made until the end of three days.

Examination for growth of B. prodigiosus.

A set of *eight Doulton filters* was compared with *four Berkefeld filters* and *four Slack and Brownlow filters* under the same conditions.

Of the *Doultons* one only gave trace of growth after one day, two filters showed contamination after three days, one after four days, one after seven days, one after eight days, one after ten days, and one did not give the slightest sign of growth until 29 days had elapsed. The filter noted as being contaminated after ten days had only a very faint opalescence which remained constant in intensity for 36 days notwithstanding the fact that the interior of the bougie contained a rich culture of living bacteria.

All *four Berkefelds* were highly contaminated after one day and all *four Slack and Brownlow filters* showed a very rich growth after one day. To ascertain whether the Doulton filter maintained its great superiority over the Berkefeld and the Slack and Brownlow filters with uniformity, a further batch of *six Doulton filters* was tested and at the moment of writing this paper only one is slightly contaminated, the period of incubation for penetration having been 14 days—the remaining five filters show no trace of growth. Thus out of 14 Doulton filters only one gave contamination approaching that obtained by the Berkefeld and by the Slack and Brownlow filters.

Conclusion. As no porcelain or other filter which I have tested under pressure has given sterile filtrates for more than a week, and as many of the Doulton filters showed sterility of the test medium, when growth through the filter mass took place in the absence of pressure, for much longer periods, I conclude that the so-called "indirect contamination" is not due to growth through the filter alone, but is highly dependent upon the current of fluid passing through. The Doulton filter further proved itself superior to the Berkefeld and the Slack and Brownlow filters in preventing the growth of micro-organisms through the filter mass.

On the grain of the filters examined.

The above investigation seemed to indicate that there is no essential difference between direct contamination and the so-called "indirect contamination" so far as the filters tested are concerned, especially when the filtration takes place under pressure. The differences in time of contamination of the filtrate may depend upon two factors at least:

(1) The chemical natures of the filter mass and the material subjected to filtration,

(2) The physical configuration or micro-structure of the filter mass and the physical magnitude of the contaminating matter.

It seems to me to be highly probable that in the filtration of fluids containing colloidal substances, suspended matter, or micro-organisms, the chemical nature of the filter mass will be eliminated very rapidly as a factor in the efficiency of the filter, owing to the formation of a coating of foreign material, derived from the fluid, over the chemically active surface. On the other hand the size of the constituent grains of the filtering material must condition the porosity and consequently the permeability to micro-organisms in a high degree so far as direct transmission is concerned, and probably also, in the above experiments, conditions the rate of growth through the filter, as larger pores will permit of a quicker diffusion and convection of nutrient medium. That the physical nature is the chief factor governing the efficiency of the filter seems to be admirably confirmed on comparing the above results for growth through filters with the micro-photographs (Plate II, Figs. 1 to 4) of thin translucent sections of the filter masses.

The size of pore—the lighter portions in the photographs—is very small in the Doulton filter, relatively greater in the Pasteur-Chamberland, very much greater in the Berkefeld, and in the case of the Slack and Brownlow filter the pores are of striking magnitude. Each of the photographs represents magnification of 100.

The order in which the filters arrange themselves as regards size of pore, or grain, is exactly the same as the order of arrangement for growth of bacteria through them, *e.g.* the Doulton filter shows the smallest grain and gives the least growth. I conclude therefore that the grain of a filter is a very important if not the most important factor in governing the growth of bacteria through the filter mass.

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EXPLANATION OF PLATES.

PLATE I.

PHOTOGRAPHS OF GROWTH.

Fig. 1 is a comparison of two Doulton tubes, one Berkefeld and one Slack and Brownlow after inoculation with *B. prodigiosus* and incubation for two days at 37° C. The Slack and Brownlow filter (S) was highly contaminated, the Berkefeld (B) markedly, and the two Doulton filters (D, D) not at all.

Fig. 2, two Doulton filters (D) inoculated with *B. prodigiosus* and incubated for 11 days, one Doulton (D) incubated for 18 days, in the centre of the figure, and two Berkefeld filters (B), at the extreme sides of the figure, incubated for three days only at 37° C. indicates contamination (opalescence) in the cases of the Berkefeld and sterility (transparency) in all the Doulton filters.

Fig. 3 represents two Doulton tubes A and B of Fig. 2 after incubation for 32 and 25 days respectively with *B. prodigiosus*. A faint opalesence was present in the 32 day tube which was certainly not due to micro-organisms, for it remained constant from the 10th till the 40th day.

Fig. 4 shows two Doulton and one Berkefeld filters inoculated with *B. pyocyaneus* and incubated for 11 days at 37° C. The lower end of the Berkefeld bougie is nearly on a level with the lower ends of the Doulton filters, but owing to the luxuriant growth of micro-organisms through the former (B) its outline is obscured. The Doultons (D, D) on either side are quite transparent and showed no trace of growth.

PLATE II.

MICRO-PHOTOGRAPHS OF GRAIN.

Thin Translucent section magnified to 100 diameters.

Fig. 1, Doulton White filter tube.

Fig. 2, Pasteur-Chamberland filter tube.

Fig. 3, Berkefeld filter tube.

Fig. 4, Slack and Brownlow filter tube.

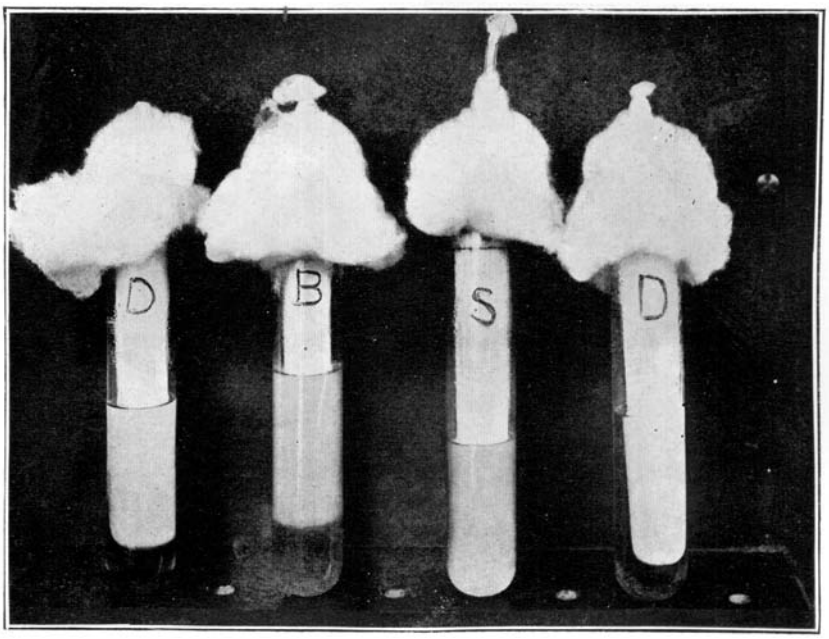

Fig. 1.

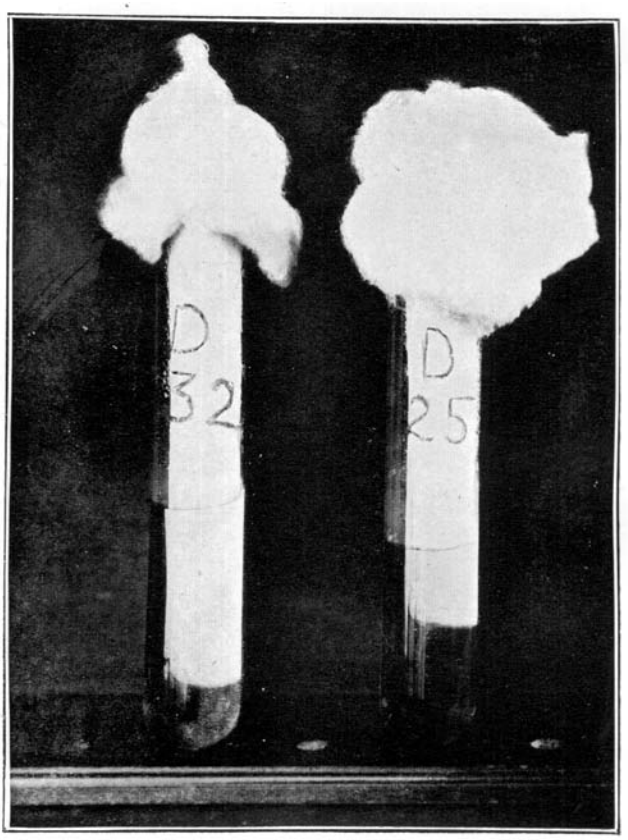

Fig. 3.

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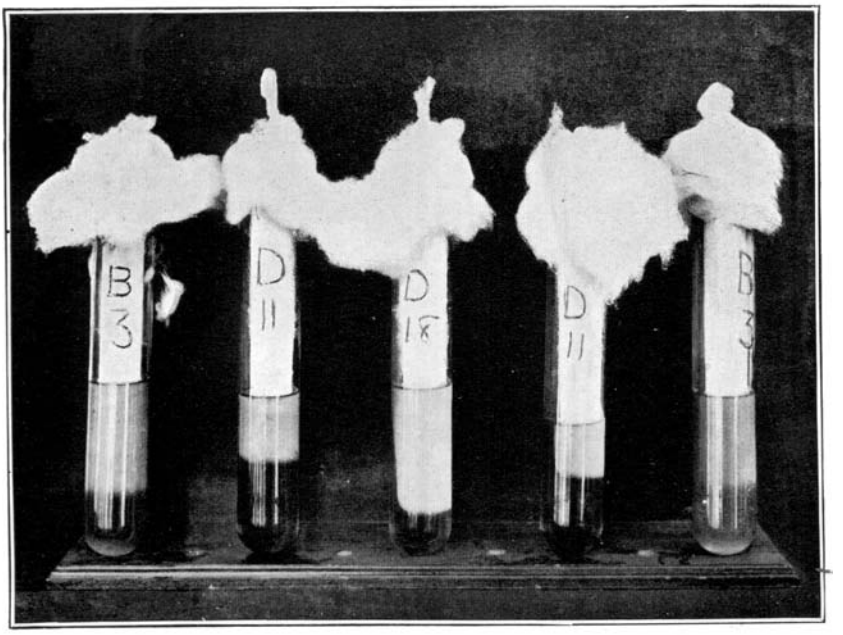

A B

Fig. 2.

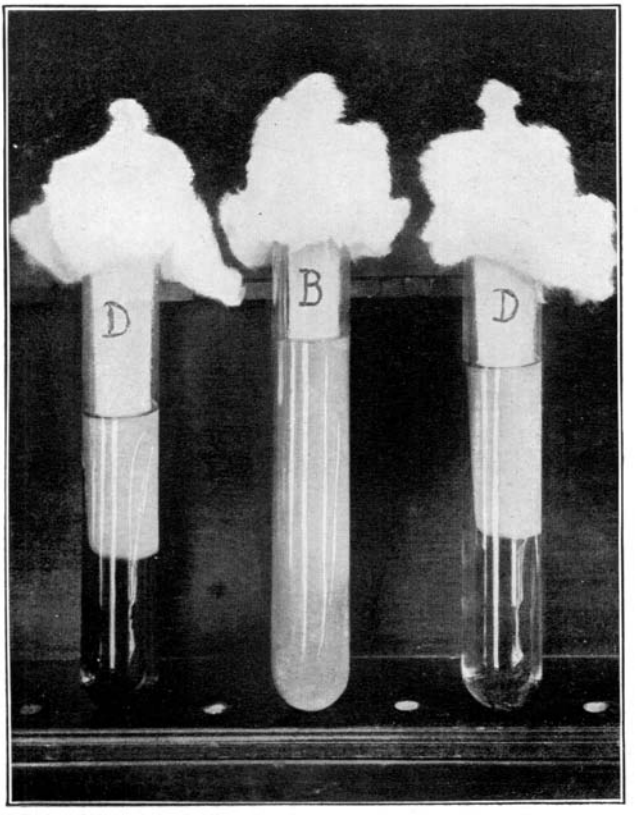

Fig. 4.

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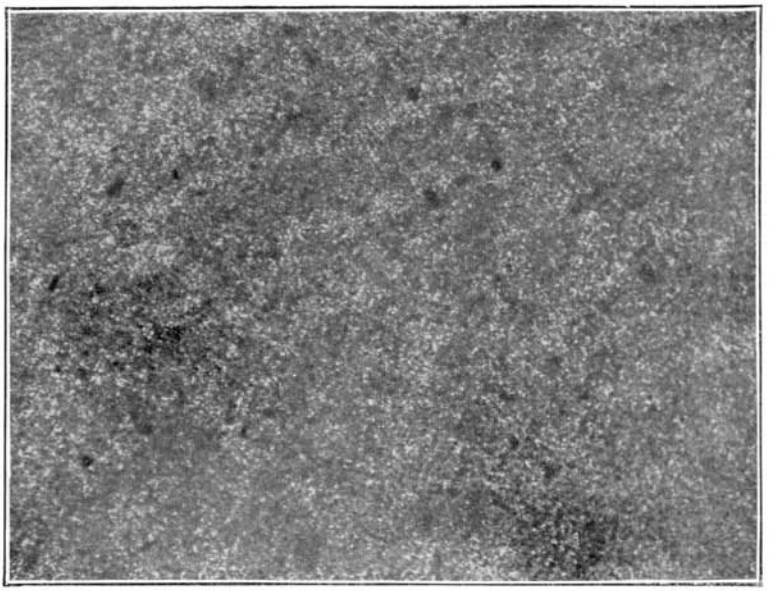

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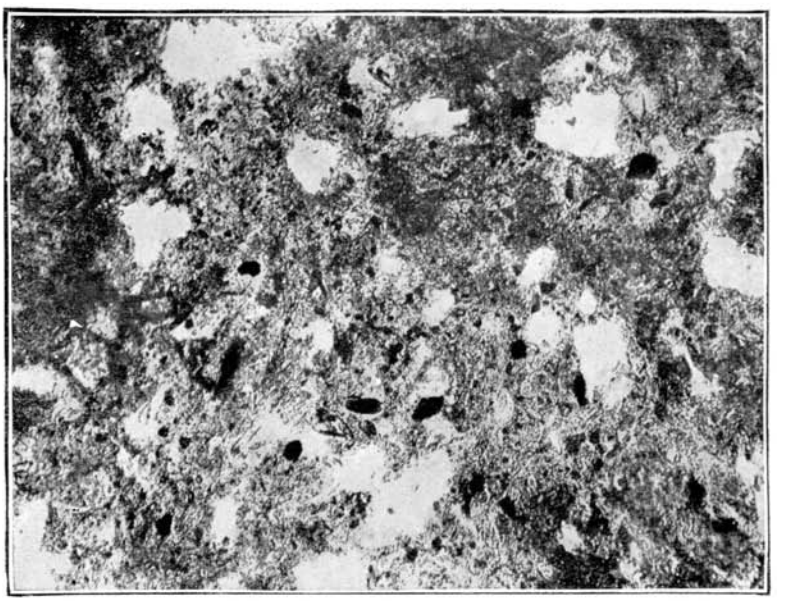

Fig. 3.

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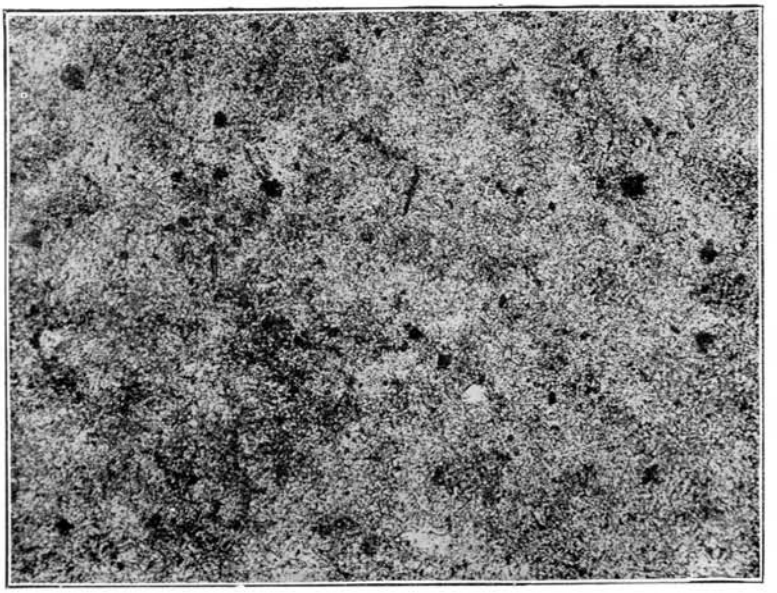

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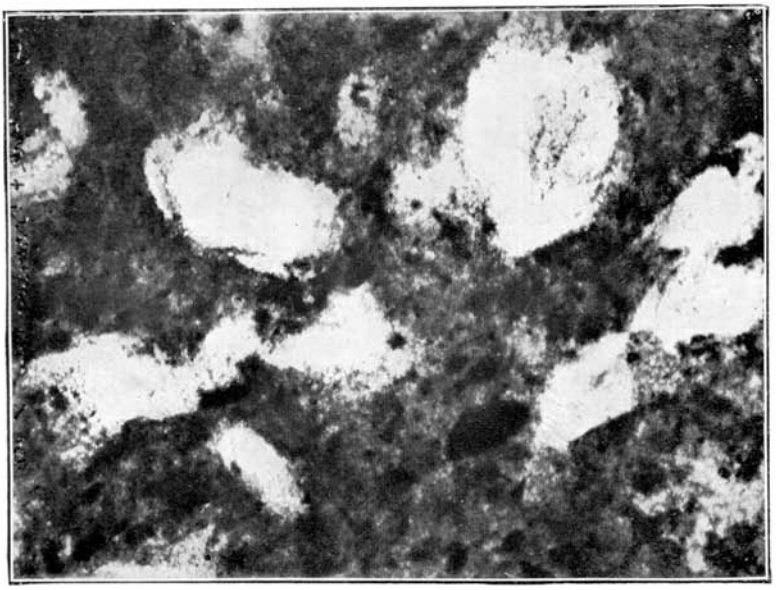

Fig. 4.

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