Microbiology.— *On differential filtration* [1]). By A. PIJPER.

(Communicated at the meeting of December 17, 1927).

The dimensions of bacteria, as given in textbooks, are unsatisfactory. Such statements are not only too vague, as for instance when a bacillus is said to be 0.5 to 1 micron thick, but the different authors also often contradict one another. Thus it seems to be quite unsettled whether for instance b. typhosus is thicker than b. coli or whether the reverse is true.

This condition of affairs becomes comprehensible when one realizes that these determinations of size are performed by means of eye-piece micrometers, on material of varying origin, and usually including a small number of individuals only, and that therefore the "measurements" obviously are mere approximations.

A more exact knowledge of the relative sizes of bacteria seems to me to be of sufficient importance, theoretically and practically, to merit calling attention to measurements which can be performed by the diffraction-method as indicated by me [2, 3, 4]). The results of these measurements throw new light on certain unexplained phenomena, and point the way towards a new method for isolating bacteria, which method I have designated differential filtration.

The diffraction-method makes use of the fact that many kinds of bacteria in surface-cultures show such a regular arrangement of the individual microbes that the culture can be employed as a diffraction grating. In the case of staphylococci their spherical shape readily explains this. In a staphylococcal surfaceculture consisting of two layers of bacteria, these two layers will naturally fit themselves together in such a way that small apertures will be left open between the cocci, and these small apertures will be situated on straight lines intersecting at angles of 60°. Small variations in the size of individual cocci here and there will cause these lines to wheel about as compared with the lines at other spots. Such a culture may therefore be regarded as a grating with equidistant lines running in all possible directions. The distance between the lines is a function of the thickness of the cocci. Under suitable conditions therefore staphylococcal cultures will produce circular spectrums, the size of which is a function of the thickness of the cocci. A further consideration of the

[1]) Part of the apparatus used in these experiments was procured through the kind assistance of the Research Grant Board of the Union of South Africa.

[2]) The Med. Journal of S. Africa, August, 1918.

[3]) S. African Medical Record, June, 1923.

[4]) S. African Medical Record, June, 1925; October, 1925.

physical aspects of the matter would take up too much space. It is quite feasible to produce, with the aid of staphylococcal cultures, beautiful and bright spectrums with diameters of several centimeters. The diameter of a given coloured circle can be recorded directly by suitable means, or can be registered by means of a colourphotograph. In either case an accuracy of at least one millimeter can be achieved.

Apart from staphylococci I have also found the method applicable to bacteria of the coli-typhoid group. This suggests that in surface cultures of these bacilli, the individuals stand upright, at right angles to the surface of the culture, because otherwise the regular pattern necessary for its proper function as a diffraction-grating, could not be obtained. Considerations of a mechanical nature support this assumption. The thickness of the bacilli then plays the same part as the diameter of the cocci, and determine the dimensions of the spectrums.

This is, briefly, the diffraction-method of measurement. Constant results can only be expected if one scrupulously adheres to an established technique, both as regards the preparation of mediums and the planting out of cultures. The most disturbing factor is desiccation, to which the size of bacteria has proved to be most sensitive, and surface-cultures naturally are much subject to desiccation. Old and thick cultures therefore are unsuitable.

Working on these lines I have succeeded in establishing the sizes of the spectrums which belong to given kinds of bacteria, and in calculating values therefrom for the mean relative thickness of these bacteria. The relation between the size of the elements of the grating and the thickness of the bacteria may be regarded as constant. The thickness of the bacteria however here includes the ektoplasm, of which little is known. It therefore at this time seems better not to attempt to give absolute values for the thickness of the bacteria, but to use the thickness of b. typhosus as the unit measurement. The b. typhosus was chosen because this bacterium exhibits such a remarkably small range of variation in its other characters. It may be added that the results of the diffraction-measurements otherwise compare very well with the accepted dimensions of bacteria, as all calculated values lie in the neighbourhood of 1 micron.

In this way I found, that, expressed in b. typhosus units, b. coli Escherich had a thickness of 1.11, b. cloacae of 1.08, b. neapolitanus of 1.15, b. lactis aerogenes of 1.38, and various not further classifiable representatives of the group of about 1.3. In every instance more than one strain of bacilli was examined, and of typhoid bacilli more than twelve strains were measured. A single strain of paratyphoid-B bacilli measured 0.98 and a single strain of b. pyocyaneus 0.88.

Similarly it was found that staphylococci from a carbuncle had a thickness of 1.08 b. typhosus units, and various cocci from furuncles of 1.11, whilst white staphylococci were 1.3 thick, and staphylococci from air reached 1.6.

Very young cultures often seemed to contain thicker bacteria than older cultures. This initial stage was followed by a period of several hours during which constant values were recorded, and after that the bacteria steadily became thinner. For this terminal change the unavoidable desiccation must be held responsible. The initial, and perhaps only apparent, increased size may perhaps be due to the scantiness of growth in the early stage, when the grating may still consist of one layer of bacteria only, which produces an entirely different type of grating. Perhaps also we are here dealing with the phenomenon described by others on the strength of microscope-measurements, viz., that young individuals in fresh cultures show enlarged sizes [1]).

The diffraction-method thus demonstrated that certain bacteria under given conditions, amongst which a sufficient degree of humidity appears supreme, show constant differences in thickness, and that generally speaking the thickness stands in inverse relation to the pathogenicity.

The idea now suggested itself that the discovered differences in thickness might throw some light on well-known but unexplained phenomena, and that perhaps some practical application might be found.

FRIEDBERGER's experiments on the ascent of bacteria have remained unexplained. FRIEDBERGER dipped strips of filterpaper into fluids containing mixed suspensions of bacteria, and noticed, by placing these strips after a few minutes on solid media, that some kinds of bacteria ascended higher into the filterpaper than others, or as I would prefer to put it, that some kinds of bacteria were carried to greater heights on the ascending current of fluid than others. Attempts at explanation have failed, motility was proved to play no part, and the conception of adsorbability brought no light. The matter becomes very simple when one remembers that it is extremely probable that the thinner bacteria find it easier to pick their way between the obstructing fibres of the filterpaper when carried upwards by the current. As a matter of fact it appears from FRIEDBERGER's reports that those bacteria which ascend highest, prove to be the thinnest when measured by the diffraction-method. The order in which FRIEDBERGER places bacteria according to their powers of ascent, corresponds with the order in which they have to be placed according to their thickness. FRIEDBERGER is inclined to accept this explanation [2]), although he omits to mention this new principle in his recent communication on the subject, in which he describes an application of the filterpaper-method to the purification of vaccine lymph from contaminating staphylococci [3]). Miss SLUITER, who performed experiments on the ascent of red blood cells, admits the possibility that the size of the cells plays a part [4]).

From this perfectly natural explanation of the ascent of bacteria in

---

[1]) HENRICI, Science, Vol. **61**, 1925.

[2]) Private communication.

[3]) Deutsche Med. Woch, 1927.

[4]) These Proceedings, Vol. **28**, 1925.

filterpaper, it is only a small step to the explanation of certain experiments on filtration, which also stand in need of elucidation. CARNOT and GARNIER [1]), and later CARNOT and WEIL—HALLE [2]), have claimed, and this has later been partly confirmed by PLANTENGA [3]), that when mixed suspensions of bacteria, containing typhoid bacilli, are brought into one limb of a *U*-tube, which is filled with broth, and contains sand at the bend, the typhoid bacilli will be the first to appear in the other limb, leaving behind the other bacteria. The explanation was sought in the greater motility of the typhoid bacilli, and the French authors speak of "auto-sélection par motilité". FETSCHER's method which pursues the same object and according to FETSCHER is based on the same principle, replaces the sand by a woollen thread [4]).

Both methods were extensively tried by me. The results up to a point agree with the inventors claims, but they at the same time have confirmed my contention that the motility does not play the part ascribed to it, and that here also differences in thickness afford a better basis for explanation. When non-motile bacteria are used, such as staphylococci, the experiments succeed just as well, and it is always that bacterium which comes through first which by the diffraction-method was found to be the thinnest.

As all these methods for the isolation of bacteria are obviously dependent on an identical principle, viz., that thinner bacteria more easily find their way through narrow paths, it seems justified to speak in all these cases of differential filtration. Whether the driving force has to be looked for in active growth, diffusion, currents generated by heat, or motility, to my mind seems to be without importance for the explanation of the phenomena.

It proved very difficult to procure sterile sand in sufficient quantity. FETSCHER's apparatus is expensive and fragile. The experiments therefore were continued with cotton wool as filter-material. In a test tube a glass tube was placed, about half as wide as the test tube, and three quarters of its length. This inner tube was bevelled at its lower end, and a rim at its upper end prevented too close contact with the outer(-test)tube. A fairly tight plug of cotton wool, about 2.5 centimeters long, was pushed into the inner tube up to a distance of about 2.5 centimeters from its lower end. Fluid, usually broth, was then poured in, to a level a couple of centimeters over the upper end of the cotton wool plug, and the whole apparatus was sterilized in the usual way. If now bacteria were brought into the upper end of the inner tube, over the cotton wool plug, then these bacteria after a time found their way through the cotton wool and appeared in the outer tube. With this kind of apparatus a large number of filtration experiments were performed. Mixtures of bacteria in varying concentrations

---

1) C. R. Soc. Biol., 21 juin et 5 juillet 1902.
2) C. R. Acad. Sc., T. **160**, N°. 4, 25 janvier 1915.
3) Ned. T. v. Geneesk., 1925.
4) Mediz. Klinik, 1926, N°. 35.

and quantities were inoculated into the inner tube, and with intervals of 12 hours the contents of inner and outer tubes were plated and examined. In seven instances a few platinumloopfuls of a mixture of equal parts pathogenic and non-pathogenic staphylococci were brought into the inner tube. The fluid used was ordinary broth, and control tests showed that these bacteria endured one another's presence quite well for many days. The thickness of the staphylococci used in the different experiments varied from 1.08 to 1.2 b. typhosus units for the pathogenic cocci, and from 1.3 to 1.6 units for the non-pathogenic ones. In six out of the seven instances a pure culture of the pathogenic staphylococci was found in the outer tube after due waiting, the seventh time a few non-pathogenic cocci had also passed through, but they were a small minority.

Similar experiments with typhoid bacilli combined with staphylococci yielded four times out of four experiments the result that a pure culture of typhoid bacilli made its appearance in the outer tube, and it was immaterial whether pathogenic or non-pathogenic cocci were used.

Inoculations of a mixture of micrococcus abortus and a pathogenic staphylococcus did not produce the expected result as long as the fluid employed was broth. It was expected that the micrococcus abortus, which is very much smaller than any staphylococcus, would filter through first. From control platings from the inner tube however it became clear that the antagonism between micrococcus abortus and staphylococci was so pronounced that after a very few hours no living micrococcus could be detected in the inner tube. When now the broth was replaced by saline, then the results were quite different, and twice out of two experiments the micrococcus got through first in pure culture. Of course there was no multiplication of the germs in the saline, and this rendered it comprehensible that the results were delayed several days.

It may justly be stated that these experiments with non-motile bacteria, fully confirmed the assumption that the thickness of the bacteria is the decisive factor.

A mixture of typhoid bacilli and (non-motile) b. lactis aerogenes inoculated into the inner tube, gave 30 times out of 33 attempts the result that in the outer tube a pure culture of typhoid bacilli appeared. In the remaining three instances the typhoid bacilli were in the majority in the outer tube, and it seems likely that if only the plating had been done somewhat earlier, the success would have been complete in these instances as well. Now the difference in thickness between typhoid bacilli and b. lactis aerogenes is very marked, viz. 1 as against 1.38. That this difference in size is the decisive factor and not, as might be suggested, the difference in motility, was demonstrated by further experiments in which so much typhoid serum was added to the tubes that the typhoid bacilli quite lost their motiliy. The results of the filtration were unchanged, both in broth and in saline.

Typhoid bacilli and b. lactis aerogenes exhibit no antagonism and this

facilitated the experiments. Between b. typhosus and b. cloacae the antagonism was found to be so marked that at first it appeared impossible to keep typhoid bacilli alive at all in a mixture of these two bacteria. Their difference in thickness is but small, viz. 1 as against 1.08, which initially threatened to endanger successful differential filtration of b. typhosus. Replacing all sugar in the broth by phosphates did not improve the conditions for b. typhosus. Addition of malachitegreen, caffein, crystal-violet, did not have the desired result. Similarly the mediums of SACQUEPÉE [1]), of NISZLE [2]), of WADE, KELLY and GIBLIN [3]), of FETSCHER [4]), all devised to favour the growth of b. typhosus and keep other bacteria in check, did not succeed in keeping b. cloacae back so that b. typhosus could be kept alive. In the end it was found that only the medium described by WILSON and BLAIR [5]) (on 100 cc. broth 5 cc. of a 20 % glucose solution, 2.5 cc. of a 20 % sodiumsulphite solution, and 2 cc. liquor bismuthi B. P.) was able to show live typhoid bacilli 12 hours after inoculation with equal quantities of b. typhosus and b. cloacae. If ten times more b. cloacae than b. typhosus were introduced, then after 12 hours no living typhoid bacilli could be found.

Filtration experiments with this medium succeeded quite well. In 18 out of 21 attempts the outer tube showed a pure culture of typhoid bacilli after 12 hours. In some instances markedly fewer typhoid bacilli than b. cloacae had been inoculated into the inner tube, sometimes the proportion was 1 : 1000, or 1 : 10.000, in one case even 1 : 1.000.000. Even then pure cultures of typhoid bacilli were found in the outer tube. In one experiment where the proportion was 1 : 100.000, chiefly typhoid bacilli were found in the outer tube with a slight admixture of b. cloacae. These results of differential filtration are, apart from the slight difference in thickness of the bacilli, all the more remarkable because this strain of b. cloacae was at least as motile as b. typhosus.

Good results were also achieved in the differential filtration of typhoid bacilli versus b. coli ESCHERICH. The antagonism which in this case appeared in broth, could be kept in check by making the filtration take place in brilliantgreenpeptone water. In seven out of eight attempts a pure culture of typhoid bacilli was found in the outer tube, and the eighth time a few colonies of b. coli appeared, together with numerous colonies of b. typhosus. It made no difference whether equal parts of b. coli and b. typhosus or one part b. typhosus against one hundred parts b. coli were placed in the inner tube.

Still further differential filtration experiments were performed with 24 representatives of the coli-typhoid group, which all had been found

---

[1]) Bulletin Inst. Pasteur, 30 Sept. 1926, N^0. 18.

[2]) Centralbl. f. Bakt., July 21, 1927.

[3]) Centralbl. f. Bakt., Aug. 10, 1926.

[4]) l. c.

[5]) Journal of Pathol. and Bact., July 1927, Vol. **29**, N^0. 3.

thicker than b. typhosus by the diffraction-method. As a rule the bacilli varied so much from known forms in their biochemical reactions that no names could be given to them. In half of the cases it appeared that their antagonism to b. typhosus was so strong that typhoid bacilli could not be kept alive together with them in any medium, even for a few hours. With the remaining twelve, differential filtration against typhoid bacilli was attempted, sometimes in brillantgreenpeptonewater, sometimes in WILSON and BLAIR's medium, and in eleven out of the twelve instances a pure culture of typhoid bacilli appeared in the outer tube.

SUMMARY.

The relative thickness of staphylococci and of bacilli of the coli-typhoid group can be determined by the diffraction-method of measurement described.

The thickness of these bacteria remains constant under suitable conditions.

Generally speaking, pathogenic representatives of the two groups are thinner than non-pathogenic ones.

The explanation of FRIEDBERGER's filterpaper-ascent experiments and of so-called sandfiltration of b. typhosus must be sought in differences in thickness of the bacteria concerned, and not in differences in motility.

A technique is described which, by filtration through cotton wool, allows the separation of thinner microbes from thicker ones, and this is called differential filtration.

As b. typhosus is a particularly thin bacillus, differential filtration often succeeds in separating b. typhosus from other bacteria. Where it fails to do so, the failure is usually due to the antagonism of the bacteria concerned, which has the effect of killing the typhoid bacilli before differential filtration can take place. In some instances a suitable filtration medium can counteract this antagonism, but a medium which suits all cases has not been found.

Pretoria, October 1927.