DIFFRACTION PATTERNS PRODUCED BY BACTERIA

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(With Two Text-figures.)

I.

When Pijper in 1918 described his diffraction method of measuring the dimensions of small objects, the first application he made of it was to measure the dimensions of bacteria. From cultures of members of the coli-typhoid group on agar slants he obtained excellent spectra, which appeared as a series of circular rings (Pijper, 1919 *a*, *b*). These he regarded as being produced in the same way as are the patterns obtained from a grating whose slits are distance D apart, and, applying the formula

$$D=\lambda\sqrt{f^2+r^2}/r \qquad \ldots\ldots(1),$$

where λ is the wave-length of the first coloured ring whose radius r is measured, and where f is the focal length of the lens used to focus the patterns, he arrived at the conclusion that the "slits" in the particular kind of "grating" formed by the bacilli are about $0{\cdot}9\,\mu$ apart. He was thus led to regard the "grating" as being made up of bacilli standing on end perpendicularly to the surface of the nutrient medium, for $0{\cdot}9\,\mu$ is about the diameter of the cross section of the bacteria with which he worked, and he thought that the patterns were produced by the diffraction of light through the roughly triangular spaces which occur between circular objects which are closely packed together. If all the triangular spaces were similarly oriented, triangular patterns would result, and Pijper attributed the circular spectra which he obtained to the roughly triangular spaces being oriented in all directions.

This idea of an orderly arrangement of bacilli at surfaces is defended by Pijper with considerable ingenuity, but the theory underlying his conclusions is faulty. The error lies in treating the patterns as being produced by a regularly arranged grating, instead of as the result of the diffraction of light by rod-like objects arranged at random. Exactly the same mistake was made by Berganzius (1921) (and also by Pijper himself) in dealing with the case of the diffraction patterns produced by films of red cells; these at first were looked upon as being produced by diffraction through the spaces between contiguous cells, instead of by diffraction by the circular discs themselves, and Berganzius even believed that only when the distance between the cells is the same as their diameter do the spectra provide a value for the latter. Millar (1926) corrected this mistaken idea for the case of red cells, and in this paper I propose to do the same thing for the case of rod-shaped bacteria.

II.

Consider the case of a rod-shaped bacillus of length a and of thickness b lying in a plane upon which parallel light of wave-length λ is incident. The diffraction pattern which results is the same as that which would result from a rectangular aperture of the same dimensions, and there will be a series of minima arranged parallel to the two sides of the rectangle. Those parallel to the side a will make diffraction angles θ_1, and those parallel to the side b diffraction angles θ_2, such that

$$\operatorname{cosec}\theta_1 = a/m\lambda \qquad \ldots\ldots(2\,a),$$

and

$$\operatorname{cosec}\theta_2 = b/m\lambda \qquad \ldots\ldots(2\,b),$$

where m takes on the successive values 1, 2, 3, etc., thus giving a whole series of minima in either direction. The crossing of these bands of minimum illumination will give rise to a series of bright rectangular patterns, in the approximate centre of each of which the illumination will rise to a maximum; the illumination in the rectangular patterns situated along the axes of the rectangle will be much greater, however, than that in the remainder. If there are N such rod-shaped objects oriented identically, the patterns will be in the same position, but increased N times in intensity.

If the rod-shaped objects are scattered at random, so that they are oriented in all kinds of ways, the problem becomes a good deal more complicated, but fortunately it is possible to arrive at an approximate solution if the length of the rods is much greater than their breadths, for under these circumstances the two expressions (2 a) and (2 b) can be treated separately, and we can imagine two sets of patterns, one produced by the lengths of the rods, and one by their breadths. Further, because of their low intensity, we can disregard all the maxima except those which occur along the axes of the respective rods, for the former have intensity

$$I = 16/\pi^4(2m+1)^2\,(2m_1+1)^2 \qquad \ldots\ldots(3),$$

in which m and m_1 might have different numerical values, as we might be dealing with, say, the maximum lying just beyond the second minimum parallel to the length but the third minimum parallel to the breadth. The maxima situated along the axes, however, have the very much greater intensity

$$I = 4/\pi^2(2m+1)^2 \qquad \ldots\ldots(4).$$

Now if the rods are scattered at random, the two series of linear minima will become two series of circular minima, and the maxima will appear between them as bright circular bands.

The next step is to show that the series of maxima and minima corresponding to the lengths a and the series corresponding to the breadths b occur at such different diffraction angles when the rod is, say, five times as long as it is broad, that the two series do not affect each other in any important way. This is best illustrated by the graph of the two illumination functions shown in Fig. 1, which was made by calculating the position of the minima and maxima, from expression (2 a), for bacilli

10μ long, the position for the minima and maxima, from expression (2 b), for bacilli 2μ broad, and the respective intensities from expression (4), λ being taken as 0·5461μ throughout. It will be observed that the illumination function for the lengths gives quite small diffraction angles, and that by the time $\theta = 15°$ the intensity is virtually zero: the first minimum for the series of patterns corresponding to the breadths, on the other hand, occurs at 16·6° and while the first maximum occurs at about 25°, and so the illumination in the bright ring seen in that region is virtually entirely due to the breadths of the bacilli.

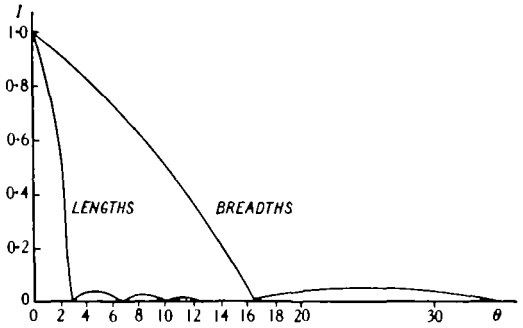

Fig. 1. Graph of the illumination functions for bacilli of length 10μ and breadth 2μ, the patterns produced by the lengths being treated separately from those produced by the breadths. Ordinate: illumination in arbitrary units; abscissa: diffraction angle in degrees.

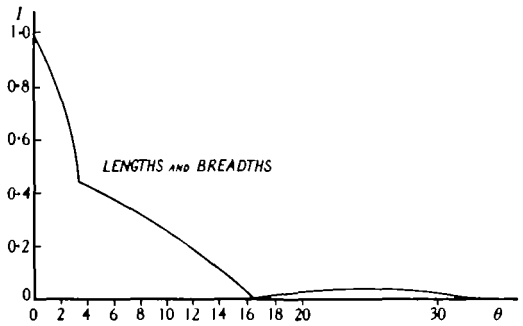

Fig. 2. The illumination function as it would appear; this being a composite of the two curves in Fig. 1. The first minimum and the maximum beyond it correspond to the breadth component only; the effect of the length component is merely to change the way in which the illumination falls to the first maximum. Ordinate: illumination in arbitrary units; abscissa: diffraction angle in degrees.

The illumination function which arises when the light is diffracted by the rods lying at random is accordingly that shown in Fig. 2. This shows a first minimum in the neighbourhood of 16·6°, and a bright band between this minimum and the second minimum at 33·2°; the minima and the bright band, however, correspond entirely to the breadths of the rods, for the illumination produced by the diffraction of light by their lengths is virtually zero at such great angles. The only effect produced by light diffracted by the lengths is to bring about a distortion of the usual illumination function in the region of 3°, and this, of course, is not observed by the eye. The eye observes, however, the minimum at 16·6° and the bright ring lying outside it, but these are not the result of the rods being arranged at right angles to

the surface, as Pijper believes, for they occur in just the same position if the bacilli are oriented at random. If experimental verification of this is required, it is a simple matter to show that diffraction patterns of considerable brilliance arise from dried or stained films of bacilli, made in the usual way, in which microscopic examination shows the rods to be lying at random, and for which the first bright maximum of the patterns occurs at an angle corresponding to the observed breadth of the organisms.

One further point requires consideration. Pijper says that at first the bacilli lie in a haphazard way parallel to the surface of the culture, and that as the growth becomes older an orderly orientation takes place with the rods lying at right angles to the surface. It is at this stage that good diffraction patterns are obtained. Still later, as the number of bacteria increases by multiplication, the patterns become feebler, and Pijper explains this by pointing out that all the apertures of the supposed grating become blocked up when more than a few layers of micro-organisms are formed.

If we do not accept the idea of a grating and of an orderly orientation, it is necessary to explain these observations in some other way, and it is not difficult to do so. In very young cultures there are insufficient organisms present to give brilliant spectra, but as the numbers increase the brilliance of the patterns increases, for brilliance is proportional to numbers, other things being equal. It is also dependent, however, on the scatter of the lengths and breadths of the rods about their mean values, just as in the case of discs it is dependent on the scatter of the diameters of the discs. (See Ponder, 1929.) The scatter is known to increase as a culture becomes older, for which reason old cultures, although containing more bacteria, show feebler diffraction patterns.

In conclusion, I may remark that the usual theory applicable to diffraction patterns does not hold more than very approximately when the diffraction angles are as great as those corresponding to the bright rings observed with cultures of bacilli, for these may be as great as 45° if the breadth of the bacillus is 1 μ or less. For this reason I doubt if absolute values can be found for the breadth of bacilli by the diffraction method, although relative values are readily obtainable. The same remark applies to the diffraction patterns obtained with cultures of small cocci, for which the theory is exactly the same as that for red cells scattered at random.

SUMMARY.

By treating the diffraction patterns produced by cultures of bacilli as the result of diffraction through a grating, Pijper has been led to the conclusion that the bacilli are oriented at right angles to the surface of the culture medium. It is shown in this paper that his results are better accounted for by supposing the organisms to be lying at random, under which circumstances only those patterns resulting from the diffraction of light by their breadths are perceptible to the eye.

REFERENCES.

BERGANZIUS, F. L. (1921). *Pflügers Arch.* **192**, 118.
MILLAR, W. G. (1926). *Proc. Roy. Soc.* B, **99**, 264.
PIJPER, A. (1919 *a*). *Med. Journ. of South Africa*, June-July.
—— (1919 *b*). *South African Med. Record*, Sept. 27th, p. 8.
PONDER, E. (1929). *Journ. Exp. Biol.* **6**, 427.