THE PLEOMORPHISM OF THE COMMON COLON BACILLUS.¹

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THE pleomorphism of the common colon bacillus has been noted by most observers. The forms to be seen on one cover-glass are often many and various, ranging from small coccus-like forms to long thin rods; and in the same culture at different times different shapes have been noted; sometimes short coccus-like forms are found to be in the majority, sometimes longer rod-like forms; and in some cases threads $6\ \mu$ in length, and even spirals have been noted. Beyond this it has been observed that bacilli from different sources, grown on similar media, differ from one another. Vincent Harris² isolated colon bacilli from man, the cat, rat, rabbit, and guinea-pig, and found that bacilli giving, in general, similar chemical reactions often differed morphologically. One kind is described as "small stout bacilli almost of coccus form," another as "large stout bacilli with no tendency to the coccus form." Gordon,³ on the other hand, working with some twenty kinds from different sources, found that although differences did exist, they were ill-defined and inconstant. Be this how it may, sufficient has been said to show that the colon bacillus may vary in shape and size somewhat considerably. The object of the present inquiry is to determine what changes in shape, if any, result from changes in the nutrient medium. To this end I took one bacillus and grew it in different media of known constitution, and then compared the results. The bacillus used was from the guinea-pig; it did not liquefy gelatine; in peptonised broth it grew rapidly with a scum on the surface and deposit at the bottom, and gave the indol reaction in a few days; in gelatine shake cultures it produced gas; it curdled milk, rendering it acid. It was motile, and in broth was pleomorphic, its length varying from $2\cdot5\ \mu$ to $\cdot5\ \mu$.

The method adopted was to remove a single colony from a gelatine

¹ Read before the Pathological Society, 7th December 1897.

² *Journ. Path. and Bacteriol.*, Edin. and London, 1896, vol. iii. p. 314.

³ *Ibid.*, 1897, vol. iv. p. 439.

plate culture, and place it in peptonised broth. After from two to six hours the test solutions were inoculated from this broth tube, so that in each solution were placed like bacilli with a like history; comparative results were thus ensured. The substances on which the bacillus was grown in this way were peptone, albumin, glucose, leucine, and ammonium tartrate. Numerous experiments were made, and, with the exception of the forms grown on leucine, the morphological characters of the organisms grown in each solution were fairly constant. At the end of the experiments plate cultures were made; and when impurities were detected, either by plate or direct examination, the results were discarded.

It may be well to describe the bacillus as it occurs in the different media.

In a 5 per cent. solution of Witte's peptone (a substance which, I must add, consists chiefly of albumoses) the bacillus grows rapidly. In twenty-four hours it presents the following characters:—In shape it is rod-like, with ends more or less rounded, in a few cases rather tapering; the sides are parallel. It is pleomorphic, in size varying from tiny coccus-like forms to long rods; but the majority of forms are from $2.4\ \mu$ to $1\ \mu$ in length, and from $0.6\ \mu$ to $0.3\ \mu$ in thickness. Less commonly forms from $1\ \mu$ to $0.6\ \mu$ by $0.5\ \mu$ to $0.3\ \mu$ are seen; still fewer are coccus-like forms $0.6\ \mu$ to $0.5\ \mu$ in diameter; while rarest of all are forms 3 to $5\ \mu$ in length. Bacilli are frequently seen end to end in pairs, and occasionally in chains of three or four. Many, especially the smaller, are stained uniformly, and appear homogeneous; but large numbers, usually the majority, are seen to be more deeply stained at the ends than in the middle. The portions at the ends which stain more deeply are of varying size, and are generally less than one-third of the length; they are clearly marked off from the central portion, which stains to a variable degree, sometimes scarcely at all, sometimes almost as deeply as the ends. In a solution examined from day to day signs of a progressive degeneration are observed: irregularities of shape and staining capacity appear, and there is an increasing quantity of debris. The bacilli that remain regular in outline decrease in size, or rather there is an increase of smaller forms, so that from the tenth to the thirtieth day the majority are from $1.4\ \mu$ to $0.5\ \mu$ in length, and tiny forms $0.6\ \mu$ by $0.3\ \mu$ are much commoner. Nearly all stain deeply at the ends, while the body is stained lightly or not at all. There are always some forms, however, that stain well and uniformly, and are of the original size; in the later stages it is only these forms which are seen to be in pairs. These forms, which get fewer and fewer, are well shown in preparations stained with methyl-blue, since the great majority after about eight days do not stain at all in this way, so that these few normal forms are readily distinguished. This has been noted in the case of other microbes; and Washbourn,¹ remarking the fact in

¹ *Journ. Path. and Bacteriol.*, Edin. and London, 1896, vol. iii. p. 214.

the case of the pneumococcus, suggests that the well-stained forms, few in number, act as spores; and the fact that in the case of the colon bacillus these are the only forms to be seen in pairs supports that view. I may add that at no time has staining for spores yielded any positive result.

This staining of bacilli at the ends and not in the middle is of interest, since it occurs in the majority of forms, and since it appears in quite young cultures—I have seen it as early as five hours after inoculation. If such bacilli are examined while alive, the ends appear darker and more granular than the bodies, which are translucent and homogeneous. These darker portions are rounded in shape, and are distinctly outlined from the central clearer portion; the dots at the two ends are usually equal in size, but in older cultures one may be larger than the other. These bacilli are motile, and are seen in pairs. Those, on the other hand, which show no such differentiation of parts, are dark and faintly granular throughout. The appearance suggests that there is an aggregation of certain parts of the protoplasm, parts which possess a stronger staining capacity, at either end. In specimens stained with Ziehl's carbol-fuchsin it is seen that the bodies of the bacilli colour to a varying degree, from being almost as deeply coloured at the ends to being quite unstained; in lightly stained specimens the contrast between ends and body is greater than in those more deeply stained.

The phenomenon has been described by Emmerich and by Buchner,¹ who showed that it could always be produced by the addition of 3 per cent. of soda to the broth in which the organism was grown. Moreover, there is further evidence to show that it is due to the alkalinity of the medium. A peptone tube seven days old, markedly alkaline, in which the majority of forms showed this differentiation, was rendered acid by the addition of decinormal hydrochloric acid; after twenty-four hours there were very few bacilli that did not stain uniformly. Again, if to a series of tubes sufficient acid is added from time to time to keep them about neutral, the staining at the ends is much less marked than in control tubes not so neutralised. When the bacillus is grown on a mixture of peptone and glucose, so that the solution is always acid, very little staining at the ends appears; the forms nearly all stain uniformly. If bacilli showing this appearance, from a peptone tube, are placed in a glucose solution, in three to six hours all trace of it has disappeared; while if from this glucose solution a fresh peptone tube is inoculated the change of structure is again produced. It may be regarded, then, as a change in the constitution of the protoplasm of the bacillus, resulting from a change in the medium, the change being from neutral to alkaline.

Grown on a 1 per cent. peptone solution the bacillus presents much the same features. It is, however, rather shorter and thinner,

¹ *Arch. f. Hyg.*, München u. Leipzig, 1885, Bd. iii. S. 291 und 361.

the majority of forms being $2.2\ \mu$ to $0.8\ \mu$ long, and $0.4\ \mu$ to $0.3\ \mu$ broad. The aggregation of staining material at the ends is much less frequently met with. The first difference may be explained by the dilution of the nutriment; the second by the solution being less alkaline; for when, by the production of ammonia the solution becomes more alkaline, the appearance is quite common.

Grown on peptone, anaerobically, in an atmosphere of CO_2 , the appearances are much the same; nor does the addition of a reducing agent such as sodium formate affect the size, shape, or constitution. In a solution consisting of one part of egg albumin, and four parts of a 1 per cent. saline solution, the bacillus does not grow at all well, only a few forms being seen. In shape these resemble the peptone forms; they are rather smaller, however, the majority varying from $1.8\ \mu$ to $0.8\ \mu$ in length, by $0.5\ \mu$ to $0.3\ \mu$ in thickness, though larger and smaller forms may be seen. This decrease in size may be due to the fact that albumin is not such a favourable medium for the growth of this bacillus as is peptone. If a broth is added to the albuminous solution a better growth is obtained, and the size of the bacillus is equal to that produced in peptone. The aggregations at the ends are not seen, except in a few cases in old tubes to which broth has been added; but the solution never becomes more than faintly alkaline.

A sugar solution of the following composition was used. A quantity of 5 or 6 grms. of glucose was dissolved in 100 c.c. water; to this 2 grms. of prepared chalk and 1 c.c. of ordinary peptonised broth were added. In this medium, then, we have a very small proportion of nitrogenous matter present. The bacillus grows fairly well in this solution, and after eighteen hours large numbers are always to be found. The forms are pleomorphic, but not to such an extent as in peptone. The majority are coccus-like forms; some are square with sharply cut sides, others are irregularly rounded, and some are almost as round as true cocci. Besides these, there are many short rods or ovals, and a few longer rod-like forms as in peptone.

The majority are under $1\ \mu$ in length, varying from $1\ \mu$ to $0.4\ \mu$, while the thickness varies from $0.7\ \mu$ to $0.4\ \mu$. There are always present forms longer than $1\ \mu$, ranging from $1\ \mu$ to $1.8\ \mu$, but it is rare to see longer forms than this. The coccus-like forms vary from $0.7\ \mu$ to $0.5\ \mu$ in diameter, the extremes being from $1\ \mu$ to $0.4\ \mu$.

Comparing these shapes and sizes with those of the peptone albumin forms—in proteid the majority are over $1\ \mu$ in length, in sugar the majority are under. In proteid the forms are two to four times as long as they are broad, in glucose they vary from being twice as long as they are broad to having equal length and breadth. In glucose the average form is shorter but broader than in proteid; and while in proteid coccus-like forms are infrequent, in glucose they form the majority.

To make sure that this change of shape was due to the presence of glucose, and not to the dilution of the nitrogenous material, control tubes of peptonised broth to the same dilution (1 in 100) were inoculated. Forms were obtained rather smaller than those in peptone, but alike in shape.

The glucose forms stain well and uniformly, and from day to day show very little change; examined alive, the bodies seem homogeneous throughout, and slightly more translucent than proteid forms; no aggregations of protoplasm towards the ends are ever seen. Examined after four or five months the forms show much the same appearance, presenting in this way a great contrast to the proteid forms, and stain well and uniformly, as do they also in dead cultures, where in only a few forms is any irregularity seen. If, in this glucose solution, the amount of nitrogenous material is increased, the number of longer forms increases, and forms from 2 to 1 μ in length are much commoner, while the thickness remains the same. Thus, in one tube of this description, the average length was from 1.4 μ to 0.8 μ , and the thickness 0.8 μ to 0.5 μ . The addition of nitrogenous matter, therefore, increases the average length, while the thickness remains the same.

In a 5 per cent. solution of ammonium tartrate, with a small percentage of sodium chloride and potassium sulphate added, the bacillus grows very slowly; its growth is increased and hastened by the addition of a small amount of peptonised broth (1 c.c. to 100 c.c.). The large majority of forms in this case vary in length from 2.5 μ to 1 μ , it being rare to find forms under 1 μ in length; the thickness varies from 0.6 μ to 0.3 μ . Long forms up to 3.5 μ may be seen, and in old tubes there are filaments. They stain well and uniformly, and in old tubes the ends are sometimes more deeply stained than the bodies.

It is in this case that the greatest contrast to the sugar forms is observed. In proteid solutions there are always a number of short, stout forms; here such forms are scarce. And it may be noted that ammonium tartrate contains a larger proportion of nitrogen than proteid.

In a leucine solution, consisting of 2 per cent. leucine, 0.5 per cent. of sodium chloride and potassium sulphate, the bacillus grows well, producing a turbidity in twenty-four hours, and the forms are seen to be numerous. The results in this case were rather divergent, and only a wide limit of size can be given. The length may vary from 1.8 μ to 0.5 μ , the thickness from 0.6 μ to 0.3 μ , and perhaps those under 1 μ in length are rather commoner than those over.

Besides the above-mentioned method of inoculating a series of these different solutions from the same source, one solution was frequently inoculated directly from another. Thus glucose solutions were inoculated from a peptone, two to six days old; in four to six hours all "end staining" disappeared, and the bacilli had increased in

thickness, but had not diminished in length; in twenty-four hours they had diminished in length, and typical glucose forms were seen; a peptone solution was then inoculated from this, and in twenty-four hours typical peptone forms were seen. In this way one solution was inoculated from another, and generally the character of the bacillus was changed in twenty-four hours. In the case of some bacilli grown on sugar for four to five months, the alteration on introduction into other media took a little longer; but in no case have I succeeded in producing any permanent alteration in shape or structure.

SUMMARY AND CONCLUSIONS.

These results may be summed up by saying that in such nitrogenous media as proteid and ammonium tartrate, the average length of the bacillus is greatest in proportion to its breadth; while in glucose, to which a minimum amount of nitrogenous material has been added, it is shortest; and that in leucine, which contains an amount of nitrogen intermediate between the first two groups, the bacillus is intermediate in size; while if in the glucose solutions the nitrogenous material is increased, there is an increase of longer forms.

From these results it may be concluded that the composition of its food stuff plays a considerable part in determining the shape, size, and structure of the bacillus; that it is not the only cause of its variations in shape and size is shown by the fact that large differences in these respects are met with in any one solution; the substances used varying, however, in this respect, the bacillus being more pleomorphic in proteid and leucine than in sugar or ammonium tartrate. Although in this series of experiments the average length of the bacillus was greatest in an ammonium tartrate solution which contains a high proportion of nitrogen, and least in a glucose solution which contained only a minute proportion of nitrogenous matter, and although in other solutions there was a certain correspondence between the average length of and the amount of nitrogen present, yet I do not think the facts sufficient to warrant any generalisation on the subject. I merely wish to draw attention to an interesting and significant point.

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