THE METHOD OF DIVISION OF THE ROUGH AND SMOOTH TYPE OF COLONIES AMONG BACILLI OF THE SALMONELLA GROUP.

By MURIEL M. NUTT.

(*From the Department of Bacteriology and Preventive Medicine, University of Manchester.*)

(With 8 figures.)

In 1921 Arkwright described two distinct types of colonies which appeared when cultures of *Bacillus dysenteriae* Shiga were plated on agar. One which he called the "rough" type had a coarsely granular surface rather like morocco leather, and an irregular margin. The other which he called the "smooth" type had a regular edge and a smooth surface. He found the same differentiation into rough and smooth colonies occurred among other members of the group, in *B. typhosus*, in *B. paratyphosus* B, and in *B. enteritidis* Gaertner. Since then these two types of colonies have been described in many other organisms; in streptococci by Cowan, in pneumococci by Griffith and by Reimann, in pasteurella by de Kruif, in meningococcus and gonococcus by Atkin, in the cholera vibrio by Shousha.

The authors above cited have directed their attention to the cultural characters, growth in broth, agglutinability, etc. It seemed possible, however, that there would be some differences in the method of growth of the individual bacteria comprising the rough and smooth colonies. To determine this the growth and division of living cells, from rough and smooth strains, was observed by dark ground illumination. Starting in each case from a single cell, or one in the first stage of division, and using a solid medium so that the daughter cells remained in contact, their growth into small clusters or tiny colonies was watched.

Technique.

Young actively growing organisms are obtained by plating on agar from a casein broth culture grown for 16 hours at 22° C. and then incubating the plate at about 30° C. for 16 hours. In *B. aertrycke* (mutton) and other members of the group the colonies thus obtained are about 1 mm. in diameter and contain actively dividing organisms.

A drop of melted agar (1·5 per cent.) is placed on a coverslip and an emulsion from a young colony is quickly made in the agar. The slide is then carefully lowered over the drop and pressed flat. This must be done rapidly otherwise the agar sets, and the preparation is both uneven and too thick. The coverslip is then ringed round with paraffin wax to prevent drying.

Gelatin of 6–10 per cent. may be used instead of agar, but in this case the slide cannot be incubated above 25° C. and the growth is therefore much slower than when agar is used.

(1) *Rough culture of* B. aertrycke (*mutton*).

Preparations of a rough culture of *B. aertrycke* (mutton) were made and observed as described, isolated normal looking organisms being singled out for study. Such organisms contained few refractile granules and had sharply defined contours. Preferably a single organism was chosen, or one in which there were signs of division, evident as slight lateral constrictions. Drawings were made at stated times with a camera lucida and the exact position of the organisms recorded by means of the Vernier scale before each slide was placed at 37° C. After about half to three-quarter hour the preparations were re-examined and the organisms again drawn. There was found to be a definite lag period varying somewhat in length during which the cells increased in size without division. Throughout the day the preparations were examined at short intervals, drawings made of the different stages, and the slide replaced at 37° C. after each examination. Division once started usually proceeded rapidly and by the end of the day (6–8 hours) the single organism had divided into a little cluster of cells in which it was difficult to make out the actual numbers.

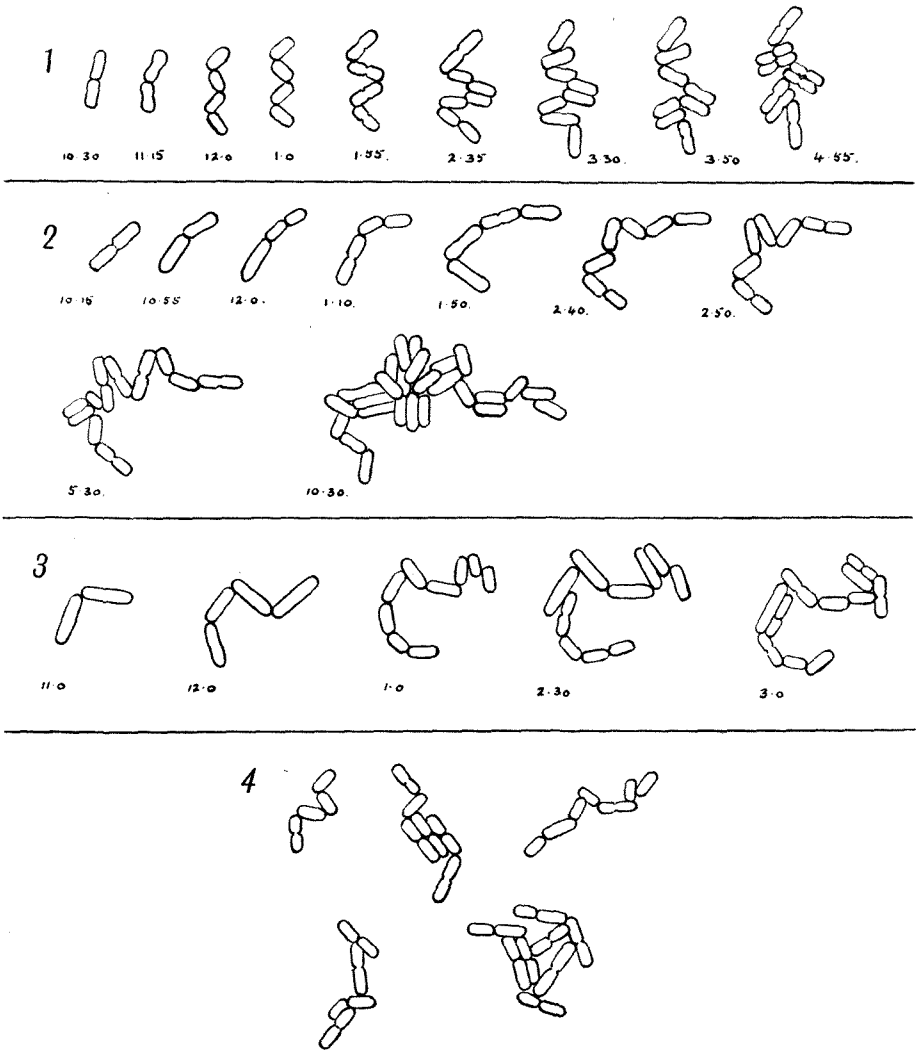
In rough strains of *B. aertrycke* (mutton) a characteristic mode of division can be observed. After the organism has divided once or more, two contiguous cells bend sharply at an angle. The bend may occur at the first division (Fig. 3), but a very characteristic form is a chain of four which then bends sharply at the middle (Figs. 1 and 2) so that the central pair of cells come to lie at an acute angle to one another, while the angles between these and the two terminal cells may be either acute or obtuse. In the later divisions the same sharp bending between adjacent cells is seen repeatedly, so that the final cluster in a typical rough culture looks "rough" owing to the projections or angles which stick out from the side.

(2) *Smooth cultures of* B. aertrycke (*mutton*).

Preparations of smooth cultures were made and observed the same way as described for the rough type. In this case instead of the organisms bending at angles after division, they are seen to slip past each other and lie side by side. The slipping may occur at the first division, or there may be a chain of four produced as in the rough type. This, however, is relatively uncommon, and long chains, such as are frequently observed in rough cultures never occur. Even when short chains are formed, the next stage consists in a slipping movement at some intercellular division, never in a bending movement as in the rough forms. The final cluster in a smooth culture is even in appearance owing to the cells sliding past each other and forming smooth masses.

Therefore the appearances observed during the multiplication of the organisms suggest that the difference between the rough and smooth colonies

depends essentially upon the degree to which contiguous cells adhere to each other after undergoing division. In the rough type the bacilli are more adherent than in the smooth, with the result that the chains of bacilli bend and thereby give rise to the rough granular growth.



Figs. 1, 2. Rough strain of *B. aertrycke* (mutton).
Fig. 3. Rough strain of *B. enteritidis* Gaertner.
Fig. 4. Typical rough groups of *B. aertrycke* (mutton) after 7 hours at 35° C.

In certain strains of *B. aertrycke* (mutton) this character is very well marked. It is not however always so. Some of the organisms in a rough culture may show a tendency to slide and form smooth masses, or occasionally an organism which starts by dividing in the rough manner may show in a few

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divisions the sliding type. It must always be borne in mind however that an apparently rough culture may in reality be a mixture of rough and smooth.

Cultures of both rough and smooth types were therefore obtained from single cells, using the method described by Topley, Barnard and Wilson. In such cultures although sliding stages were sometimes noticed interspersed between typical bending stages, an organism in a single cell rough culture has

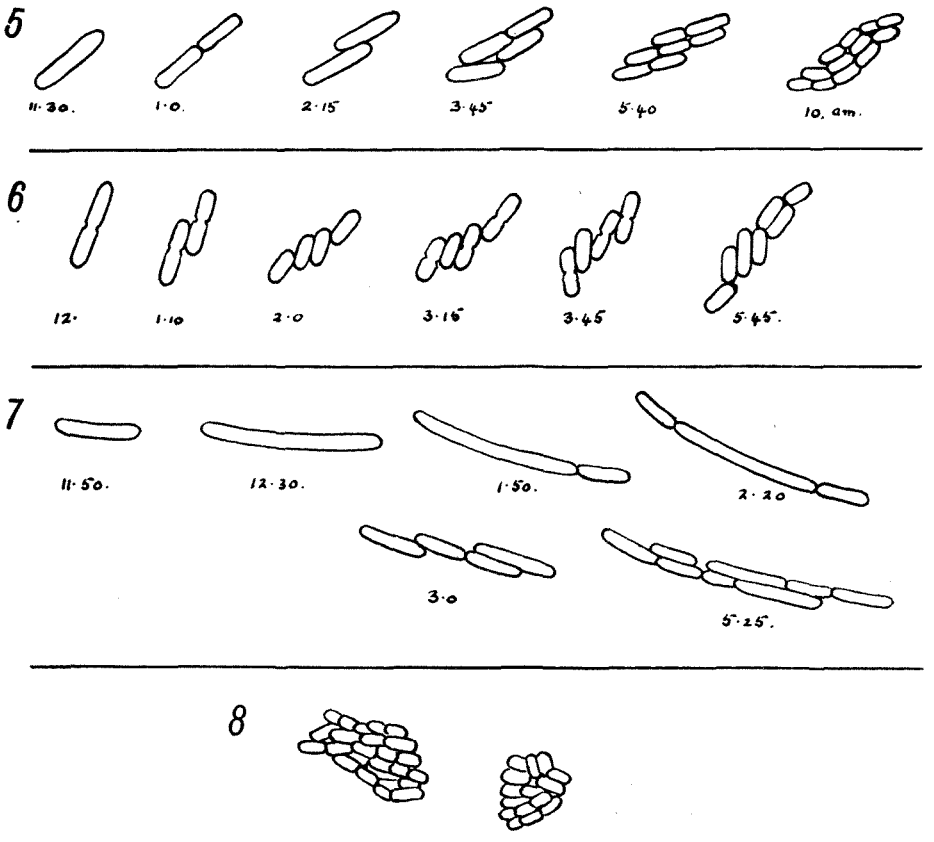

Fig. 5. Smooth strain of *B. paratyphosus* B.
Figs. 6, 7. Smooth strain of *B. aertrycke* (mutton).
Fig. 8. Typical smooth groups of *B. aertrycke* (mutton) after 7 hours at 37° C.
Drawings of Figs. 1–8 made with the camera lucida, using dark ground illumination.

never been observed to divide *throughout* in a typical smooth way. Provided therefore that one is dealing with pure cultures derived from single cells, it is quite easy with practice (even if occasional bacilli show the slipping form of growth) to distinguish the rough from the smooth; the difference is obvious at about the 16-cell stage when the jagged uneven appearance of the rough clusters is sharply distinguishable from that of the regular smooth clusters.

Bending divisions in a smooth culture have never been observed.

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B. paratyphosus B and *B. enteritidis* Gaertner.

Rough and smooth cultures were obtained from *B. paratyphosus* B and *B. enteritidis* Gaertner. In both cases rough cultures showed the typical bending type of division, producing at the end of the day irregular masses with projecting angles. In the smooth cultures the sliding divisions gave rise to the smooth even masses typical of the smooth colony.

Sections of both rough and smooth colonies gave negative results, in that no differences were observable. On the other hand, colonies fixed and stained on coverslips did show a different arrangement of the bacilli at the edge of the colony. In the smooth colony the organisms showed a regular arrangement in rows, which would be expected if the colony were built up by organisms having the sliding type of division. At the edge of a rough colony the organisms appeared to be arranged in a very irregular, haphazard manner, quite different from that in a smooth colony.

The causes that lead to the formation of rough colonies on solid media operate equally in broth. Rough strains of streptococci form chains; rough strains of *B. aertrycke* (mutton) form bent chains leading to the development of irregular clusters. These chains or clusters either sediment to the bottom of the tube, or remain suspended as granules in the clear fluid. The uniform turbidity of a smooth broth culture on the other hand will be due to the even suspension of the organisms which separate or slide past each other, and do not cohere after division.

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REFERENCES.

ARKWRIGHT (1921). *Journ. Path. and Bact.* XXIV. 36.
ARKWRIGHT and GOYLE (1924). *Brit. Journ. Exper. Path.* V. 104.
ATKIN (1923). *Ibid.* IV. 325.
—— (1925). *Ibid.* VI. 235.
COWAN (1922). *Ibid.* III. 187.
—— (1924). *Ibid.* V. 226.
GRIFFITH (1923). *Rep. Publ. Health and Med. Subj. Ministry of Health*, No. 18.
DE KRUIF, P. (1921). *Journ. Exper. Med.* XXXIII. 773.
—— (1922). *Ibid.* XXXV. 561.
—— (1922). *Ibid.* XXXVI. 309.
REIMANN (1925). *Journ. Exper. Med.* XLI. 587.
SHOUSHA (1923). *Journ. Hygiene*, XXII. 156.
TOPLEY, BARNARD and WILSON (1926). *Ibid.* XX. 221.

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