NOTES.

THE FORMATION OF BACTERIAL COLONIES[1].—During the detailed examination of a large number of forms or species of Bacteria from the Thames, I have been struck, as have other investigators, with the extreme difficulty—not to say impossibility—of successfully employing the diagnoses given in authoritative works, such as Eisenberg's Bakteriologische Diagnostik. Over and over again prolonged cultures of a given form showed departures in sometimes one and sometimes another direction; and although the generality of the characters sufficed to diagnose a type nearer to one or another of the accepted 'species,' the variations were so numerous that it was difficult to do anything with them beyond describing all the 'varieties.'

This was not due to differences in the cultures in the ordinarily accepted meaning of the word, because half a dozen forms of the same type varied when cultivated side by side in the same media, and consequently it was impossible to admit that differences in the conditions of culture (in the ordinarily accepted meaning of the term) were responsible.

The suspicion therefore arose that one and the same 'species' of *Bacterium* will differ in its behaviour according to the vicissitudes it has been subjected to in the river previously to its capture: in other words, that 'varietal' forms occur stamped for the time being with acquired characters.

On the other hand, the possibility existed that in cultures side by side, assumed to be identical, and still more in the case of cultures in media prepared according to the same formula but at different times—e.g. two brews of peptone-gelatine, or two potato-tubes prepared from different potatoes—causes of variation might exist far too subtle for detection, but which nevertheless have their effect on the sensitive organization of the Schizomycetes.

Consequently I attempted the task of cultivating gelatine-plate-colonies under such circumstances that the very earliest stages of development could be observed directly under the highest powers of the microscope, so as to see how colonies are built up from the first division of a Bacillus until the usual macroscopic characters of the colony are recognizable, as in ordinary plate-cultures.

This was done by isolating simple Bacteria in a hanging drop of

[1] Read before the Botanical Section of the British Association at Ipswich.

gelatine by the method described in my Fourth Report to the Royal Society Water-research Committee [1], and so arranging matters that the gelatine-drop, &c. forms a plate-culture, the floor of which is the thin cover-slip. In fact, if we suppose a modified Petri-dish with the glass floor so thin that the whole thickness of the gelatine film can be optically pierced by a one-twelfth immersion, the essential conditions are obtained.

When the colonies become visible to the unaided eye, it is easy to inoculate a tube and test the purity of the culture, &c., by the ordinary methods.

In other cases, I utilized the method of preparing *Klatschpräparate.* A sterile cover-slip is held by sterile forceps and laid flat on a growing colony, a thin film of nutrient gelatine having been distributed over the contact side of the cover-slip: this is then lifted and used as if it were a hanging-drop preparation. The adherent Bacilli, fixed *in situ* on the film of gelatine, can then carry on their growth, &c., under observation.

It usually happens that when a rodlet, fixed in solid gelatine, grows to twice its length and then divides, the two daughter-cells slip one over the other *as if the elasticity of the gelatine* made itself effective on the free distal ends, and this phenomenon is repeated, so that when say a dozen or twenty divisions have occurred, we have a group of rods irregularly side by side in a spindle-shaped micro-colony. It is in this way that the frequent occurrence of 'whetstone-shaped' and 'oval' submerged colonies is to be explained. Subsequently, as the gelatine is softened around, these may gradually round off to a spherical shape.

Another proof that the elasticity of the gelatine comes into play is afforded by such observations as the following. A *Micrococcus* or *Bacillus* having divided many hundreds of times, forms a perfectly spherical colony. The gelatine then softens at one point on the periphery of the submerged sphere, whether by local increase of enzyme or otherwise does not matter, and a rounded protuberance of the colony at once forces its way into the softer gelatine, converting the whole colony into a pyriform shape. As this occurs, all the diameters of the rounded part of the pear—i.e. the previous sphere—perceptibly diminish. In other words, the elastic pressure of the gelatine forces the colony to bulge into the yielding gelatine. Later on other local protuberances may occur in the same way, and the rapidly enlarging colony then assumes moruloid or lobed shapes, such as are common in submerged colonies.

[1] Proc. R. Soc., vol. lviii. pp. 12 and 13.

If the direction of least pressure on a submerged spherical colony of the above description happens to be at right angles to the free surface of the gelatine-film, then the colony bursts to the exterior and emerges as it were like a fountain, oozing out its Bacilli or Cocci, &c., in the form of a button-like drop, or a flat cake, film, &c. according to circumstances. In these cases the dark submerged 'manubrium' of the colony shows long after emergence the point in gelatine where the Bacilli burst through.

If, on the other hand, the still submerged spherical colony begins to liquefy the gelatine equally all round its periphery, a beautiful play of chemotaxis comes in: the superficial Bacilli arrange themselves at right angles to the periphery, in the zone of liquefied gelatine, and form a radiating fringe. In some cases this may be brought about by softening of the gelatine by a slight rise of temperature, and possibly by other agencies than the direct liquefying power of the Bacilli.

Turning now to emerged colonies. It is pretty clear that the flow over the free surface of the gelatine may be affected by several factors, and especially by the degree of moisture. If a damp film exists, or if the *Bacillus* can slightly liquefy, the free spreading of the colony is favoured much more than when the gelatine is relatively dryer or the *Bacillus* weak, and it is obvious how this may be profoundly affected by tardy emergence, differences in hygroscopic properties of the gelatine-film, dry or moist atmosphere, temperature, &c. Several cases are given where colonies of one and the same form behave in this respect so differently on emergence, that they might be taken for different species, and would probably be so described by bacteriologists unacquainted with these phenomena.

That the liquefying power of Bacteria varies is well known, and I have been able to show that with comparative plate-cultures of one and the same form the rapidity of liquefaction, like the rate of growth, can be affected by exposure to light and other agents, and in these cases the appearance of the resulting colonies may be so different that no one would suppose them to belong to the same species.

In pursuance of the subject, I have made a large number of cultures to see if exposure to light alone will so affect the organism that its after-behaviour is modified. Several species have been exposed in tubes of broth or of water to sunshine, with control tubes shaded from the light. The usual effects—death of Bacilli in the light, and retarded growth of those which survive—were confirmed, but it has not

as yet been possible to obtain a distinct varietal form definitely due to the modifying action of the light alone from these tubes. On growing colonies in the dark, in normal gelatine, the Bacilli retarded by the light-action slowly recover their usual characters after a time. Even after numerous transferences from light-tube to light-tube, and dark-tube to dark-tube, day after day, the same recovery occurs, and the colonies are not affected in any permanent way.

While actually growing in gelatine, on the other hand, very slight exposures affect the appearance of the colonies, and if the effects of varying temperatures (within limits in no way fatal to the plants) are superposed on those of light, some marked changes in shape, rate of growth, and even pigmentation may be induced.

Much research will be necessary, however, before the problems here raised are settled.

Growth is very easily affected by much slighter changes of condition than is usually accepted, and my experiments convince me that plate-cultures 'at ordinary temperatures'—or 'at the temperature of the room' (*Zimmertemperatur*)—so often employed in diagnoses, are useless. The exact temperatures must be quoted, and they must not be allowed to vary.

In many cases, the emerging colonies form extremely thin films on the gelatine-surface, the play of light on which gives the iridescence so often quoted as a character. These films may consist of contorted or coiled tresses of filaments, lying parallel on the flat surface, like coils of rope: if growth is so vigorous that these films become more than one filament thick, the iridescence may not appear: if local liquefaction occurs, the filaments may break up into isolated Bacilli in patches, and curious mottled or mosaic-like, or tortoise-scale-like patterns may occur. If the liquefaction is more vigorous, the whole film may be set in curious amoeboid movements, and rapidly extend over the surface of the gelatine. I have a whole series of forms which seem to connect extremes where liquefaction of ten per cent. peptone-gelatine is rapidly brought about (in forty-eight hours or less) by means of these creeping and almost invisible surface-films, with others where the hyaline film shows no movements and the segments remain connected in coiled filaments.

The size—both length and thickness—of Bacilli may differ in different parts of the same colony; and forms, usually described as non-motile, may often be shown to be motile under given conditions.

Colour-variations have long been known. I have confirmed the occurrence of white varieties of crimson forms, and find considerable variations in yellow pigmented forms. An interesting case is that of white varieties of a violet *Bacillus*, so permanent that I have cultivated it for weeks and even months as a white form, and can only get it to produce its pigment in broth, though otherwise it seems vigorous enough.

In view of these and other results, which I hope to publish later, it seems extremely probable that the following three propositions are true.

(1) That variations in the form, rate of growth, size and colour, and other characters of plate-colonies result from much slighter variations in the gelatine and other environment than has hitherto been recognized.

(2) That, regarding the water of a river as the food-medium, the vicissitudes which a *Bacillus* has been exposed to in this medium previous to its capture and isolation in the laboratory, may have stamped on it such differences that its plate-colonies differ considerably at different times of the year, or even in the same season according to the length of time the individual germ isolated has been in the river.

(3) It is in great part owing to the coincidence of these causes of variation that it is often so difficult to recognize a given 'species' described in Eisenberg and other authorities: in fact, the same 'species' recurs under different names, because the conditions preceding and during its cultivation in the laboratory have differed more or less.

The only way out of this difficulty will be, I think, to cultivate each form from the begining for a sufficiently long period under conditions as accurately known as possible, and strictly according to some carefully arranged plan agreed on by bacteriologists in council beforehand.

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A FALSE BACTERIUM[1].—During my investigations of the bacterial flora of the Thames, a form has turned up which well illustrates the truth that the methods of tube-plate-cultures of minute organisms may lead one astray, and that in order to settle the question of the nature of such forms we must employ the methods of direct cultivation from a single germ under powers of the microscope: that, in fact, we must supplement the macroscopic gelatine-plate-cultures of Koch and his followers by the original *microscopic* gelatine-cultures of Klebs, Brefeld, and De Bary, which preceded and suggested the now usual methods.

[1] Read before the Botanical Section of the British Association at Ipswich.

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