MICROBIC DISSOCIATION: DETECTION OF THE "*R*" VARIANT BY MEANS OF A SPECIFIC DROP-AGGLUTINATION.

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The characters by which the "*R*" variant of a given bacterial type is distinguished from the "*S*" variant are to-day widely known. Most of them are morphological and in most bacterial types it is not difficult to say whether a single colony is smooth or rough; but every bacteriologist is familiar with those borderline colonies, which are apparently smooth, but which later prove to be serologically rough. It sometimes happens that by special stimulation the reversion from an "*R*" culture into the "*S*" form succeeds, but that, notwithstanding the smooth appearance of the isolated colonies, the organisms persist in showing some characters which belong to the "*R*" variant, such as instability in saline (Bruce White, 1926).

More often we meet bacterial species in which the morphological differentiation of colonies of the two variants is exceedingly difficult, as in the *Brucella* group. It is now recognised that the "*R*" variant of the *Brucella* is what was previously known under the misnomer of *Br. paramelitensis* (Hadley, 1926; Zdrodowski, Brenn, Voskressenski, 1930; Pampana, 1931), and it is not easy to distinguish a colony of the latter from any colony of *Br. melitensis*.

Furthermore, we cannot exclude the possibility that, even in the most typical smooth colonies of, let us say, a *Salmonella*, a few microbic elements might exist which have undergone the rough variation. They would not alter the morphology of the colony; and to make sure that this is 100 per cent. smooth we ought to try its stability in saline or test it with Millon's reagent: both rather delicate procedures.

Recently we have described a very simple way of detecting the presence of the "*R*" variant in cultures of the most common micro-organisms (Pampana, 1931). The reagent consists of a 1:500 solution of *trypaflavine*, in normal saline. A drop of the solution is put on a slide. Close to the drop, but not in the drop, we depose a minute fraction of a loopful of the bacterial colony to be examined. We then flame the loop, and, when it is cool again, we moisten it gently with the trypaflavine and gradually emulsify the material on the slide. Finally we mix it with the whole droplet of trypaflavine solution. If the colony contained the "*R*" variant, agglutination takes place immediately or within a few seconds. The reaction is very easily read, the more so if the surface of the slide is illuminated by oblique light against a dark background.

The reaction can, of course, also be performed in test-tubes, by mixing equal volumes of trypaflavine solution (1:500) and of bacterial emulsion. I have shown, however, that drop-agglutination on a slide is more sensitive than tube-agglutination and altogether preferable. The technique of the former, as previously described, is extremely simple; but the greatest care must be taken in the gradual mixing of the bacteria with the solution because the mixing of the germ suspension with the whole droplet of trypaflavine at once may cause a pseudo-agglutination. In half an hour, 20–25 colonies can easily be tested, four to a slide.

The trypaflavine test-tube agglutination was first introduced by Alessandrini and Sabatucci (1931) with a view to differentiating the *Brucella* group and other bacterial types. I have shown that the reaction cannot be used to separate bacterial types, but that it appears to be closely connected with the dissociation of any type, namely, with the "*R*" variant.

Up to the present several workers in Italy have confirmed that the trypaflavine drop-agglutination is an efficient and true reagent for the detection of the "*R*" variant. I have studied it in all known types of *Salmonella* and in the *Brucellae*; and Sabatucci (1932) in *Micrococcus pyogenes*; Favia (1932) and Mazzetti (1932) in *B. anthracis*; Seppilli and Guiso (1932) in *Saccharomyces cerevisiae*; Cilli (1932), Pisu (1932), Seppilli and Denes (1932), Seppilli and Maschio (1932), Spinelli (1932) have all confirmed these observations. The reaction appears to have been accepted as one of the typical characters by which the "*R*" variant of any bacterial type can be differentiated from the "*S*" form.

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