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**Dissociation of Yeast and Bacteria Within the Stomach and Duodenum.**

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During experiments upon the intestinal absorption of *B. prodigiosus* and various types of yeasts in rats, it was found that many of the plate cultures of the contents of different levels of the gastrointestinal tract were sterile. To determine whether these plates were actually sterile or whether forms of microbes were present in a phase which did not grow appreciably, we subjected such apparently sterile cultures to the serial plating technic described by Hauduroy.<sup>1</sup> About 0.5 cc. of sterile nutrient broth is taken up in a capillary pipette, the slender part of which is about 3 inches long. After discharging all the broth onto the surface of the plate from which transfer is to be made, the pipette is held so that the side of the Bunsen flame just touches it about 2 inches from the tip. Softening of the glass and the weight of the tip operate to bend the pipette into a spreader which is used to scrape the discharged broth back and forth across the surface of the plate, thereby washing off whatever microbic elements may be present. The same pipette is used to draw up the washings, to discharge them upon the fresh plate, and to spread them over its surface. We have found this technic superior to that of using 2 pipettes and 2 spreaders for this simple operation. Practically all of the apparently sterile plates selected for this study were derived from the stomach or duodenum of the animal; an occasional plate made from the first part of the jejunum was also sterile. These plates were all observed for at least 48 hours (many of them for 60-72 hours) and did not show detectable growth under a hand lens or low power of the microscope.

Table I shows the level from which the material was taken, the strain of yeast used, and the number of the serial transfer in which the first evidence of growth was obtained. The first sign of growth was a dull area over the inoculated part of the plate; smears taken from this area showed large fusiform rods, granular debris, and long square-ended filaments. All of these forms retained the Gram stain either uniformly or in irregular areas. Certain of the growths

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<sup>1</sup> Hauduroy, Paul, *C. R. de la Soc. de Biol.*, 1927, **97**, 1392.

TABLE I.

Sample	Level	Yeast	First Sign of Growth
			Transfer
1	Stomach	No. 16	3
2	"	18	2
3	"	17	2
4	"	17	2
5	"	18	4
6	Duodenum	16	2
7	"	17	2
8	"	16	2
9	"	18	5
10	Jejunum	M18	2
11	"	16	3

consisting entirely of the filamentous forms gave rise to typical yeast forms on transfer to acid broth (pH 5.0).

In other experiments, in which *B. prodigiosus* was the test organism, the first appearance of growth was on the fifth serial transfer. This appeared first as a dull area; the next transfer gave more definite evidence of growth in the form of a thicker grayish film. Smears of this material showed spindle-shaped rods with very slender tapering ends; the whole organism being about twice as long as the original "normal" *B. prodigiosus*. These spindle-shaped organisms were amphophilic to the Gram stain. On further serial transfers the appearance of minute colonies was observed under the hand lens; these were flat, round in outline, with smooth margin, and an internal arrangement suggesting a tangled mass of threads. After 10-12 serial transfers the colony size approached more nearly that of the original culture, but the colonies were more raised and glistening. Feeble production of red pigment was the next change to be observed in the subsequent transfers.

In both the yeast and *B. prodigiosus* experiments, litmus lactose agar plates were found to be superior to other plating media in bringing about the transformation to the approximate form and growth characteristics of the original organisms.