feces found 28 per cent. of *communior*, 60 per cent. *communis*, 4 per cent. *aerogenes*, and 8 per cent. *acidi lactici*.

The results of an analysis of thirty-two strains isolated in my laboratory from human feces were as follows:

	Per Cent.
B. coli communior	65
B. coli communis	28
B. aerogenes	3.5
B. acidi lactici	3.5

The saccharose fermenting species, then, may predominate in water recently contaminated with human feces.

40 (736)

Production of creatinine by bacteria.

By J. G. FITZGERALD and CARL L. A. SCHMIDT.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California, Berkeley, California.*]

Very little work has been done to determine whether creatinine is produced by bacteria growing on media free from creatin and creatinine. Germán,[1] in a brief review of the literature on this subject, makes mention of only three contributions. In his paper, Germán gives the result of an investigation of thirty-five species of bacteria to determine whether they were able to produce creatinine. In summing up, he says that this characteristic might be of value in the differentiation of closely allied species.

Our work was undertaken to ascertain: (1) Whether creatinine production is of any value in the differentiation of groups of closely allied species; (2) to determine the method best suited for studying creatinine production by bacteria; (3) to ascertain what amounts of creatinine were produced so that certain quantitative studies might be undertaken.

A medium composed of 2 per cent. Witte's peptone and .5 per cent. salt furnishes a creatin and creatinine free medium. Twenty-eight strains of bacteria, belonging to the mucosus capsulatus group of microörganisms, including *B. rhinoscleromatis*, *B. ozænæ* and *B. lactis aerogenes*, were grown on the above medium for eight

[1] Germán, *Centralbl. f. Bakt.*, I. Abt. Orig., Bd. 63, Heft 7, 1 June, 1912.

days. Ten cubic centimeters were then tested for creatinine by Weyl's method (adding several c.c. of a 10 per cent. NaOH solution and several drops of a freshly prepared sodium nitroprusside solution, a positive reaction being indicated by the immediate appearance of a dark red zone which soon turns to a greenish color) and 50 c.c. were tested by the Folin method (adding 7.5 c.c. saturated picric acid solution and 2.5 c.c. sodium hydrate, a positive reaction being indicated by the production of an orange-red color similar to the color produced by a potassium bichromate solution). As controls, a strain of each of *B. coli communis*, *B. proteus*, *V. choleræ asiatica*, *M. aureus*, *M. albus*, and *V. Metchnikovii* were examined at the same time. A sterile peptone solution itself gives with the Folin method a slight color which to a certain extent interferes with the test; this, however, can be eliminated by comparing the solution to be tested with the control. In only one case, that of *B. proteus*, was the Folin reaction decidedly positive, while *V. choleræ asiatica*, and several others gave a somewhat doubtful positive result. With the Weyl reaction a much more accurate determination of the presence of creatinine can be made.

As regards the *Mucosus capsulatus* group, we found that creatinine production is no criterion in differentiating the species, since nearly all the strains gave negative reactions. That the amount of creatinine which is produced as a result of bacterial metabolism is very small is indicated in the results obtained by the Folin method. That certain bacteria produce creatinine more readily and in larger amounts than others is shown by the more strongly positive reactions given by *B. proteus*. Germán's results tend to show the same thing. His inability to get a positive creatinine reaction with certain organisms in twenty-four hours, which after a longer period of time gave positive reactions, does not show that no creatinine was produced during the first twenty-four hours, but rather that the amounts produced were so small that the test appeared to be negative. That there are certain bacteria which either do not produce any creatinine when grown on a peptone medium or produce amounts of creatinine so small that the Weyl reaction appears negative is shown both by Germán's and our results.

We were unable in our work to parallel Germán's results in the case of all the species which he claims produce creatinine. This we believe to be due to the fact that the ability to produce creatinine is a characteristic which may easily be lost by bacteria just as is the power of producing indol.

41 (737)

Regeneration of bone from periosteum.

By S. L. HAAS.

[*From the Pathological Laboratory of Stanford University.*]

In an endeavor to establish the factor that periosteum plays in the regeneration of bone, the following experiments were performed on the ribs of rabbits.

Several experiments consisting merely of a subperiosteal resection of the rib showed after 20 days a cartilaginous-like material filling the entire space; while after a longer time complete filling in with bone.

The next endeavor was to isolate the periosteum so as to prevent bony elements from growing in to the periosteal space.

In one experiment the rib ends were capped with lead—after 12 days no evidence of regeneration. This method was discarded as it was thought better to try and raise the rib from its periosteal covering without severing the ends, and isolate it by sewing muscle beneath the raised rib.

In 10 experiments of this nature the results uniformly showed a tendency for bone to grow in at the angle where the rib was raised from its periosteal bed. In one experiment after 59 days the whole area was filled in by bone, although in another after 8 months there was only a small spicule of bone growing in at the sternal side. In only one experiment after 26 days was an isolated island of bone found, free from connection with bone elements.

Although this does not prove the point sought, it at least emphasizes the tendency of bone to grow in the direction of existing periosteum.

At this stage it was noticed that there was a difference at times in bone regeneration and it was suggested to try the effect