completed in this laboratory a study of the spore bearing anaerobes present in stool specimens from 72 individuals, 60 of whom exhibited symptoms more or less definitely related to the intestine, but did not find the *B. histolyticus* in any instance. I believe, however, that it occurs in the human intestine more commonly than these results would seem to indicate, and that failure to find it in stool specimens may be due to overgrowth with other organisms of the same group, such as *B. sporogenes* or *B. welchii.*

The question of the possible harmful effect of this toxin-producing organism, when vegetating in the intestine, will be discussed in subsequent communications.

## 72 (2595)

### Changes in virulence and growth characteristics of bacterium lepisepticum following alterations in oxygen tension.

By LESLIE T. WEBSTER.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

The micro-organism of the pasteurella group associated with rabbit snuffles and its complicating pneumonias, septicemias, etc., is known as *Bacterium lepisepticum.* Quite recently, De Kruif noted that a mutation of freshly isolated strains of this microbe, which took place in extract broth cultures, was favored by high concentrations of peptone, and was inhibited by undiluted serum or beef infusion. The recently isolated virulent strain which he designated as Type D was found by him to grow diffusely in serum and plain broth, to form rather opaque, fluorescing colonies on serum agar, and to have an acid agglutination zone of pH 3 to 3.5; while the mutant, which he called Type G, exhibited granular growth in fluid media, appeared as translucent, non-fluorescing colonies on serum agar, showed an acid agglutination optimum between 3.5 and 5, and was of low virulence.

This apparent physical change in the organism, together with the general distinguishing characteristics of each type, has been

noted by us in many of our strains derived from both normal and infected rabbits. So far the pure Type D strains have shown a similar high degree of virulence for rabbits and mice, and all pure G strains have shown a similar low degree of pathogenicity for these same animals. Present studies on growth activities described in this paper, however, necessitate a different interpretation of the D to G transformation and the accompanying drop in virulence.

If numbers of Type G organisms varying from one to one billion are inoculated into tubes containing 5 cc. of extract broth pH 7.4, growth will occur in every tube. If Type D microbes are inoculated into extract broth tubes in a similar manner, multiplication will not occur in tubes which have received a seeding of less than about 50,000. However, if to the extract broth a small quantity of rabbit's blood is added, Type D, inoculated in the smallest numbers, will multiply. Analyzing this phenomenon more carefully by hourly counts after inoculation, it was seen that Type G in plain broth multiplied immediately without lag, and reached a maximum of one billion per cc. in 10 to 12 hours; and that microbes D in plain broth not only failed to grow but died unless at least 50,000 were seeded per tube. On the other hand, the addition of small amounts of rabbit's blood initiated growth of Type D when a very small number of microbes (1 to 10) was seeded and furnished conditions suitable for a logarithmic multiplication of the bacilli reaching a maximum of one billion per cc. in 8 to 10 hours.

When tubes of plain broth inoculated with Type D were examined after an incubation period of 48 hours or more, a granular film was seen at the surface of the liquid which settled rapidly when agitated. This material was found to consist of Type G bacteria. This phenomenon did not occur when blood was added to the broth. It seemed possible, therefore, that the D to G transformation might be associated in some way with oxygen tension; and to test this supposition a series of broth tubes which had been kept in boiling water for 30 minutes and then sealed with vaseline was inoculated with varying numbers of Type D microbes. It was noted that abundant growth was present in every tube, including those which had received the smallest possible inoculum, and that subsequently no D to G transformation occurred. Evidently, then, limiting the oxygen supply to extract broth tubes, or adding rabbit blood to this medium under aero-

bic conditions, facilitates growth and inhibits the D to G transformation.

This observation suggested the possibility that an oxidation reduction process, possibly such a peroxidase-peroxide system as McLeod and Avery have independently described, might be concerned in this type mutation with accompanying loss of virulence. The activity of the blood in inducing optimum growth of Type D microbes and inhibiting the D to G transformation was tested and found to function in a dilution of 1/40,000 of a cc., a quantity just sufficient to give a positive benzidine test. Washed and autoclaved red blood corpuscles in a similar high dilution to the limit of the benzidine reaction behaved in a similar manner. This evidence indicated so strongly that blood was behaving as a peroxidase, that a synthetic compound was tried, $Fe(OH_2)(NC)_5Na_3$, with strong catalytic properties, suggested by Doctor Baudisch and kindly put at our disposal by him. This preparation of iron reacted in dilutions of 0.002 mg. per cc., giving a positive benzidine reaction, furnishing optimum growth conditions for Type D, and inhibiting the appearance of G forms.

Peroxidase is present in the concentrated suspensions of Type D cells; in similar suspensions of Microbe G, the test is approximately four times as strong. So far we have not been able to demonstrate peroxide in the cultures.

*Bacterium lepisepticum* may be considered, then, as an organism so delicately adjusted in its oxygen requirements that it fails to multiply freely or to maintain certain characteristics associated with its virulence unless available oxygen is mechanically limited, or the oxygen effect is minimized by the presence of peroxidase.

## 73 (2596)

## The extraction of alkaloids from blood.

By ROBERT A. HATCHER.

[*From the Department of Pharmacology, Cornell University Medical College, New York City.*]

When certain alkaloidal salts are added to defibrinated blood of the cat the bases can be extracted by shaking the blood with chloroform. Strychnin has been recovered in this way from a