

**The differentiation of *Bacillus diphtheriae*, *Bacillus xerosis*, and *Bacillus pseudo-diphtheriae*, by fermentation tests in the serum-water media of HSS / by Arnold Knapp.**

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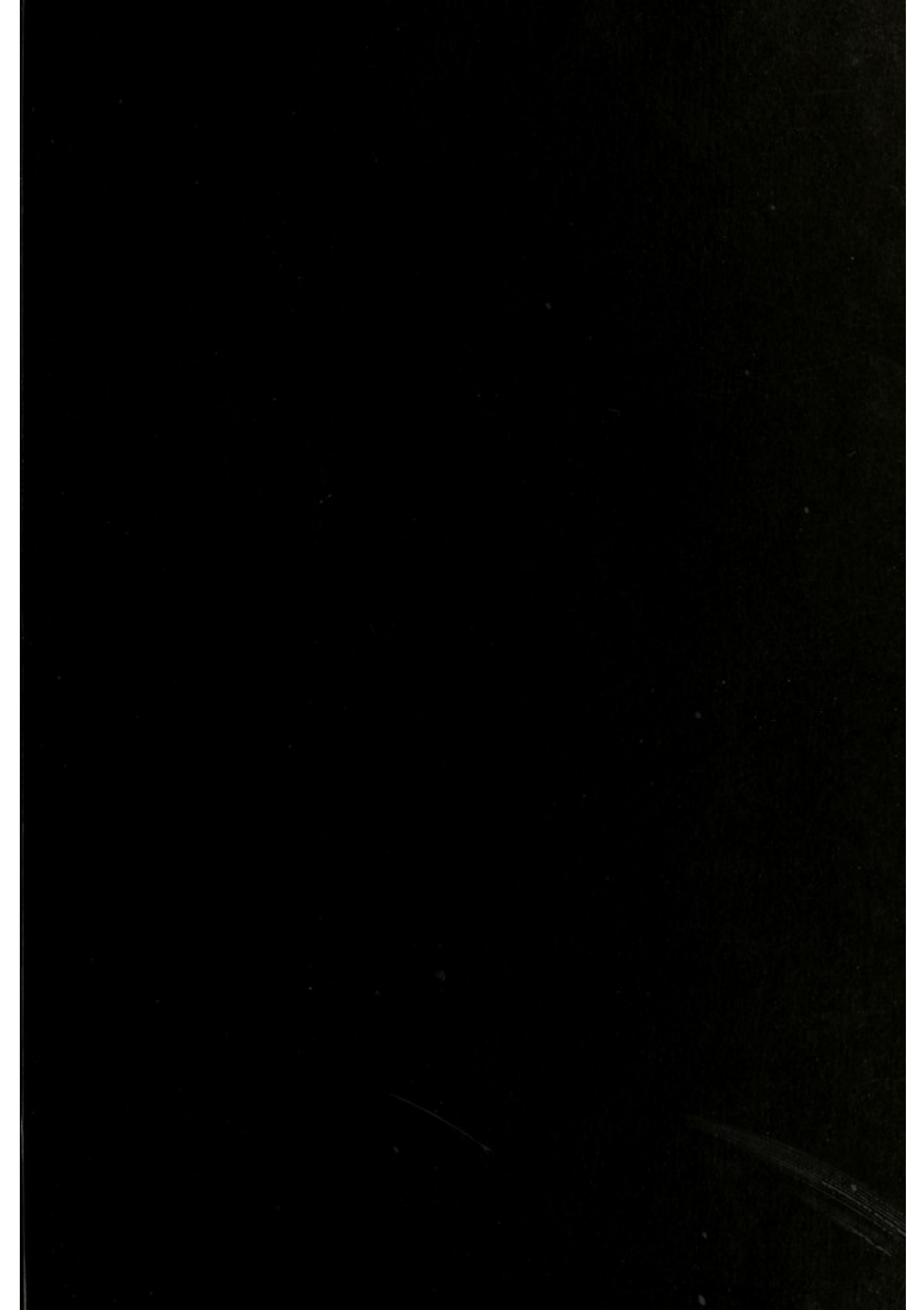
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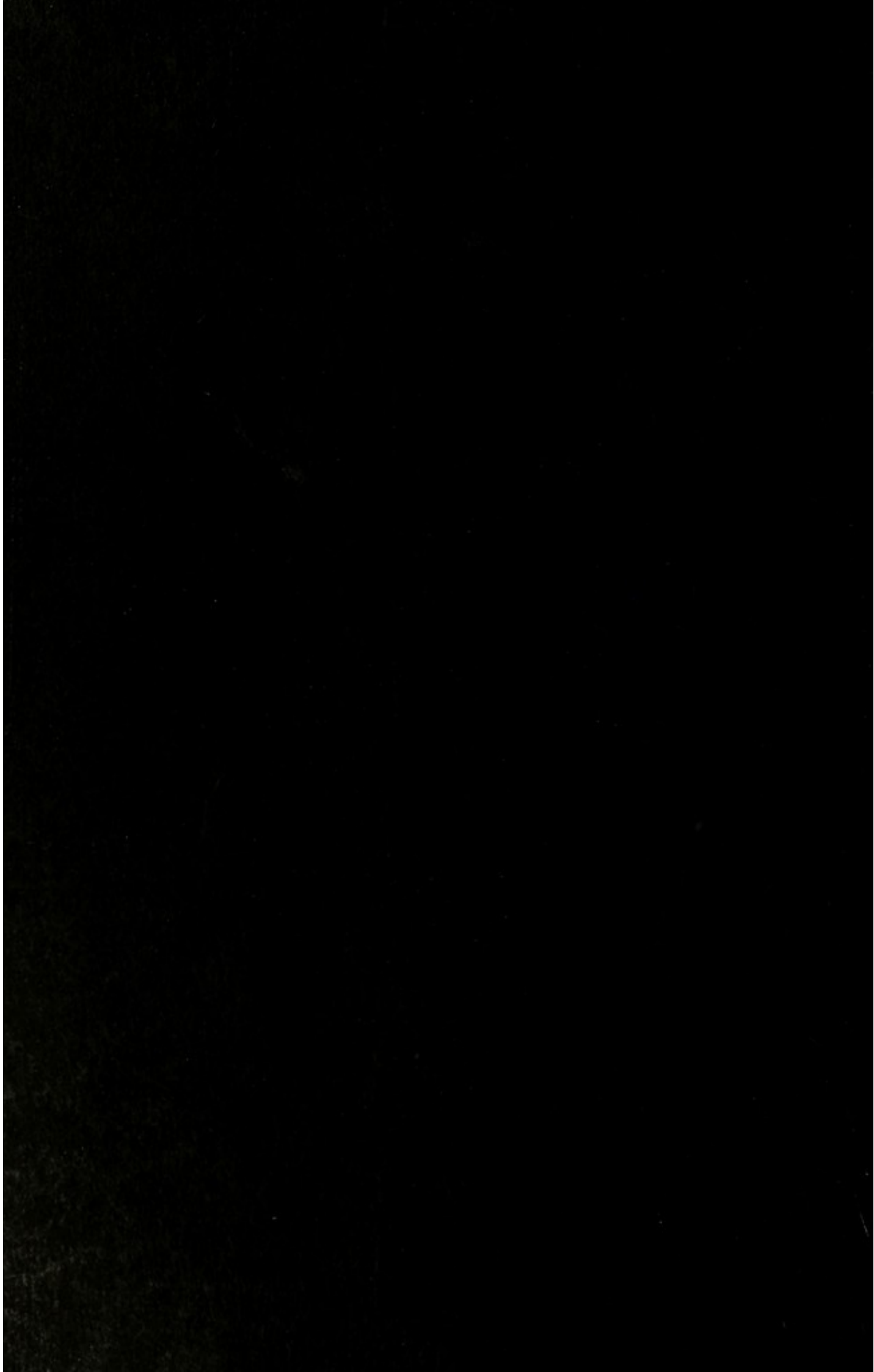
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THE DIFFERENTIATION OF BACILLUS DIPHTHERIÆ, BACILLUS XEROSIS, AND BACILLUS PSEUDO-DIPHTHERIÆ, BY FERMENTATION TESTS IN THE SERUM-WATER MEDIA OF HISS.\*

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The differentiation of the true diphtheria bacillus from bacilli which closely resemble it except in virulence has been the subject of considerable study in recent years on account of its importance from a clinical and biological standpoint. These similar bacilli are:

I. The xerosis bacillus, which was first described by Kutschbert and Neisser<sup>1</sup> in 1883. It has since become recognized as an almost constant inhabitant of the conjunctiva in health and disease. Schanz,<sup>2</sup> Pes,<sup>3</sup> Peters,<sup>4</sup> and others regard the Bacillus xerosis and Bacillus diphtheriæ as identical, the xerosis bacillus being a non-virulent variety of Bacillus diphtheriæ. Axenfeld<sup>5</sup> and Franke<sup>6</sup> are of an opposite opinion.

II. The pseudo-diphtheria bacillus, which was first described by Hoffmann-Wellenhof<sup>7</sup> and by Lœffler<sup>8</sup> in 1887. It was found in the throats of non-diphtheritic subjects, and resembled the diphtheria bacillus, but was non-virulent. Roux and Yersin<sup>9</sup> regarded it as a non-virulent diphtheria bacillus.

Many laborious investigations have shown some points of dissimilarity in certain cultures of these organisms, in the morphology, the reaction to special stains, acid production, and growth on various media. These differences are, however, not constant, so that at present it is generally accepted that these bacteria cannot, with certainty, be distinguished morphologically or culturally from one another.

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In 1897 M. Neisser<sup>10</sup> described a method of staining by which, under special conditions, *Bacillus diphtheriæ* showed typical granules, which were said to be absent in *Bacillus xerosis*. It now seems that this is not the case, the granules being demonstrable in the xerosis bacilli also, but at a later period of their cultural development (Schanz<sup>11</sup>). Heinersdorf,<sup>12</sup> however, never obtained a double stain characteristic of true diphtheria bacilli in sixty cultures of *Bacillus xerosis* of less than twelve hours' growth. In animal inoculations with cultures and diphtheria anti-toxin (Spronck,<sup>13</sup> quoted from Denny<sup>14</sup>) the lesions regularly produced by the slightly virulent diphtheria bacilli were prevented, while in the case of inoculations of *Bacillus pseudo-diphtheriæ* and *Bacillus xerosis* the production of lesions was not prevented.

Denny studied the changes in the morphology of these three species during their growth on blood serum, and also the influence exerted on the morphology by variations of temperature, reaction of media, and symbiosis, and found that these species were always indistinguishable morphologically at certain stages and under certain conditions. He was, however, able to find distinguishing characteristics if all parallel developmental stages were observed.

Schwoner<sup>15</sup> succeeded in differentiating the diphtheria bacillus and the pseudo-diphtheria bacillus by agglutination in specific sera.

The determination of the pathogenicity of these bacilli, and the toxicity of their culture fluids is, however, generally considered the final and only reliable means of differentiation.

PERSONAL EXPERIMENTS. — The large numbers of xerosis bacilli encountered in a study of conjunctival lesions which was being carried on by the writer led, at Dr. Hiss' suggestion, to an investigation of the ability of *Bacillus xerosis* to ferment sugars with acid production and to a comparison of its fermentative powers with those of the true bacillus diphtheriæ and *Bacillus pseudo-diphtheriæ*.

The method of study followed has been employed by Hiss

in differentiating the pneumococcus and streptococcus (Centralblatt f. Bakt., 1902), and more recently the organisms of dysentery and typhoid fever (Medical News, Feb. 14, 1903). This method consists in the use of serum-water media. The medium is composed of beef serum, 1 part, distilled water, 3 parts, and is practically sugar free. After heating to 100° C. for a short time to destroy the enzymes of the blood, one per cent additions of the following sugars are made: dextrose, mannite, maltose, lactose, saccharose, and dextrin. Finally litmus solution (Merck's pure litmus, five per cent solution in water) is added in the proportion of one per cent. The media are tubed and sterilized at 100° C. for ten minutes on three consecutive days.

The growth of organisms in these media may, according to their physiological peculiarities, be associated with the production of acid, a consequent reddening, and coagulation of the media containing certain of these sugars. Thus, if different sugars are affected, a means of differentiation is furnished.

By this method twenty-seven cultures of diphtheria bacilli, ten cultures of xerosis bacilli, and four cultures of pseudo-diphtheria bacilli were tested.\*

The following constant results were obtained after twenty-four to forty-eight hours' growth at 37° C.

Pseudo-diphtheria bacillus: None of the sugars were fermented; the media remained blue.

Diphtheria bacillus: Dextrose, mannite, maltose, and dextrin were fermented with acid production, the medium becoming red and coagulating. Saccharose was not fermented.

Xerosis bacillus: Dextrose, mannite, maltose, and saccharose were fermented with acid production; the medium turned red and coagulated. Dextrin was not fermented.

These results do not change after many days at 37° C.

A peculiarity of the growth of the xerosis bacillus was the formation of a very thin scum or pellicle on the surface of the media. This was absent in the cultures of the two other species.

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\*For some of these cultures I am indebted to Dr. W. H. Park of the New York Department of Health, and to Dr. Mary Heffernan of the University of Chicago.



CONCLUSIONS. — We thus see from these experiments that the diphtheria culture and the cultures of *Bacillus xerosis* ferment dextrose, mannite, and maltose. Their behavior in the presence of these carbohydrates offers, therefore, no point of differentiation.

The results with saccharose are, however, different. The xerosis bacilli ferment saccharose, while the true diphtheria bacillus, so far as our experience goes, does not.

In the case of dextrin we also find a difference. Dextrin is fermented by the diphtheria bacilli, but not by the xerosis bacilli.

Pseudo-diphtheria bacilli do not ferment any of the sugars tested. Hence a study in saccharose and dextrin media will, it seems, serve to differentiate these three types or species of organisms with which we have experimented. If the organism does not ferment either of these sugars, it is the pseudo-diphtheria bacillus; if it ferments saccharose, it is the xerosis bacillus; if dextrin is fermented, it is the true diphtheria bacillus.

Although the series of experiments on which this differentiation is based is not extensive enough to be exhaustive, still the results have been so uniform that we feel justified in calling attention to them as indicating a method of differentiation, which, it is hoped, may prove as reliable as it is simple and rapid.

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