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HAND-ATLAS SERIES

VOL. V.

BACTERIOLOGY

LEHMANN—NEUMANN

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ATLAS AND ESSENTIALS

OF

BACTERIOLOGY

BY

PROF. K. B. LEHMANN

CHIEF OF THE HYGIENIC INSTITUTE IN WÜRZBURG

AND

DR. RUDOLF NEUMANN

ASSISTANT IN THE HYGIENIC INSTITUTE IN WÜRZBURG

WITH 63 CHROMO-LITHOGRAPHIC PLATES, COMPRISING
558 FIGURES, AND NUMEROUS ENGRAVINGS

LONDON
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1897

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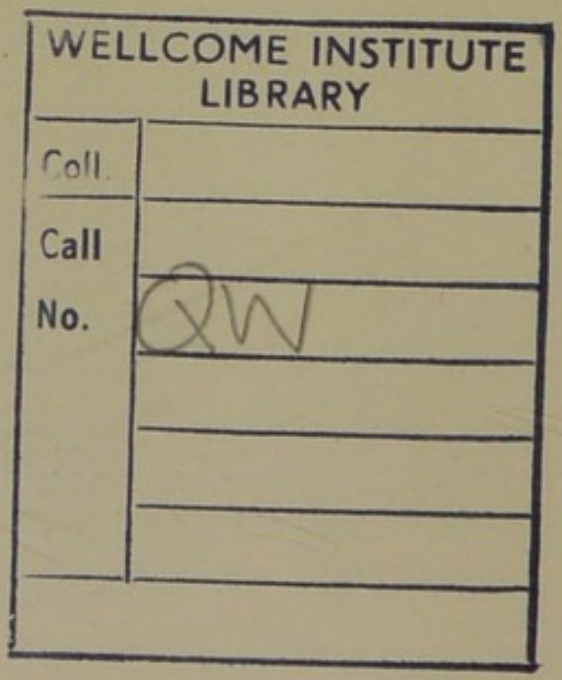

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Bacteria in soft chancre.

LIST OF ABBREVIATIONS.

A. H. = Archiv für Hygiene, Munich. Oldenbourg since 1883.

A. G. A. = Arbeiten aus dem kaiserlichen Gesundheitsamt, Berlin, Springer, since 1885.

A. K. = Arbeiten aus dem bakteriologischen Institut der technishen Hochschule zu Karlsruhe. Edited by Klein and Migula, since 1894.

A. P. = Annales de l'Institut Pasteur, Paris, Masson, since 1887.

C. B. = Centralblatt für Bakteriologie und Parasitenkunde, Jena, Fischer. Since 1894 this publication has been divided into two parts:

C. B., Part I., devoted to medico-hygienic questions.

C. B., Part II., devoted to zymotechnical, agricultural, and phytopathological studies.

Z. H. = Zeitschrift für Hygiene, Leipsic, Veit, since 1886.

Flügge = Flügge: Die Mikroorganismen, second edition, Leipsic, 1886.

Kitt, B. K. = Kitt: Bakterienkunde für Tieraerzte, second edition, Vienna, 1893.

Zimmermann 1 and 2 = O. E. R. Zimmermann: Die Bakterien unserer Trink- und Nutzwasser, Chemnitz, Part I., 1890; Part II., 1894.

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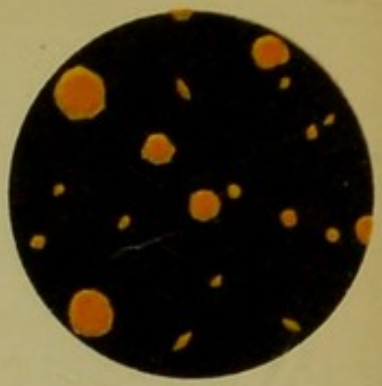

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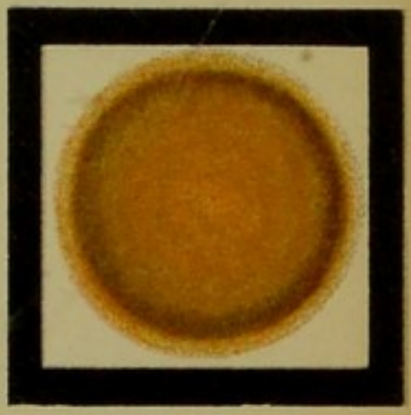

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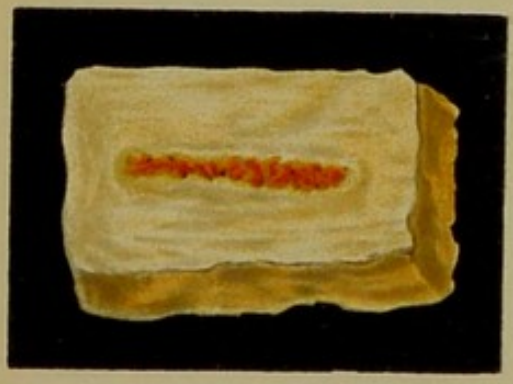

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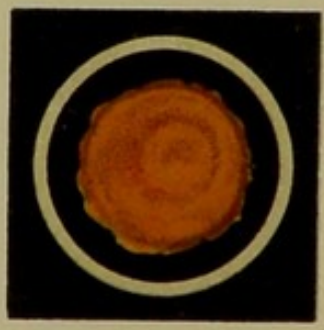

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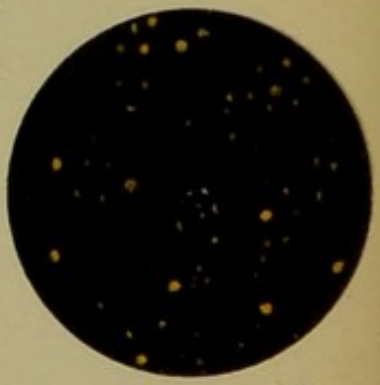

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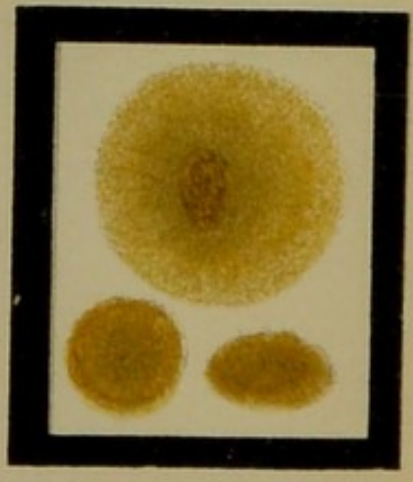

VIII.

Explanation of Plate 1.

MICROCOCCUS PYOGENES *α* AUREUS. Rosenbach, Lehmann and Neumann.

(Staphylococcus aureus Ros.)

I. Gelatin stick culture, six days at 22°.
II. Agar streak culture, five days at 22°.
III. Agar stick culture, five days at 22°. Stick canal.
IV. Agar stick culture, five days at 22°. Surface.
V. Agar plate culture (natural size), six days at 22°. Superficial and deep colonies.
VI. Agar plate, six days at 22°. ×60. Superficial small colony.
VII. Gelatin plate (natural size), four days at 22°. Superficial and deep colonies.
VIII. Gelatin plate, four days at 22°. ×60. Superficial and deep colonies.
IX. Potato culture, six days at 22°.
X. Microscopical preparation (× 1,000) of agar culture, two days at 22°.
XI. Microscopical preparation; individual cocci, before and after division. ×1,500.

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MICROCOCCUS PYOGENES γ ALBUS. Rosenbach.

(Staphylococcus albus.)

I. Agar streak culture, four days at 22°.
II. Gelatin stick culture, five days at 22°.

MICROCOCCUS PYOGENES β CITREUS. Rosenbach.

(Staphylococcus citreus.)

III. Agar streak culture, six days at 22°.

MICROCOCCUS CANDICANS. Flügge.

IV. Gelatin stick culture, six days at 22°.
V. Gelatin plate, eight days at 22°.
VI. Gelatin plate, six days at 22°. Left side, superficial colony; right side, deep colony. ×50.
VII. Potato culture, ten days at 22°.
VIII. Microscopical preparation of agar culture (×700), two days.

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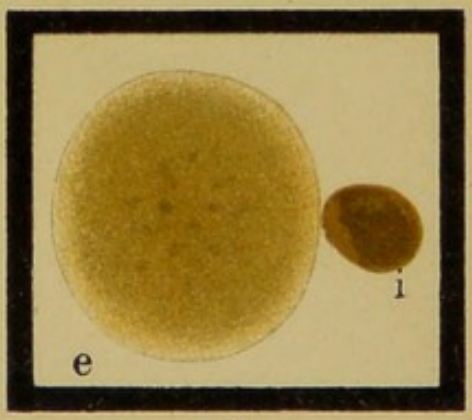

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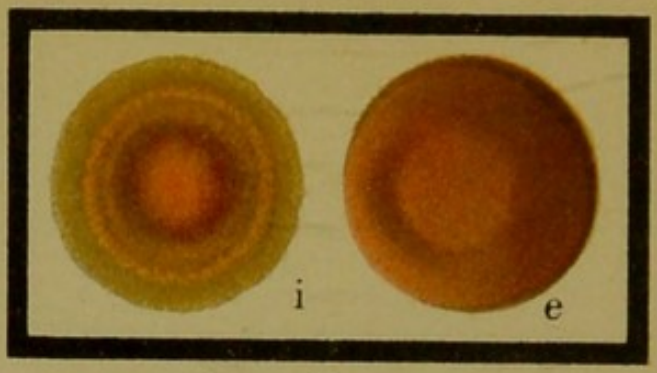

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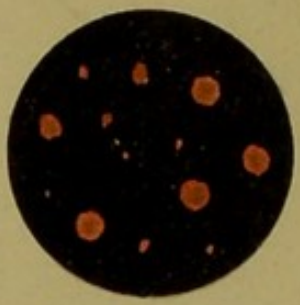

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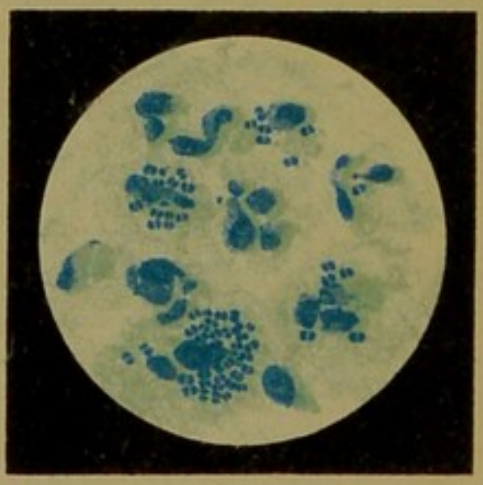

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Explanation of Plate 3.

MICROCOCCUS AGILIS. Ali-Cohen.

I. Gelatin stick culture, six days at 22°.

II. Gelatin plate, seven days at 22°. ×50. On right side, superficial colony; on left side, deep-seated colony.

III. Agar plate, seven days at 22°. Natural size.

IV. Microscopical preparation (×600) from an agar culture two days old. The individual cocci vary greatly in size, and are more irregular than appears in the plate.

V. Potato culture, ten days at 22°.

MICROCOCCUS GONORRHŒÆ. Neisser, Bumm.

VI. Smear preparation from gonorrhœal pus. ×1,000. The large blue cells are pus cells.

VI. *a*. Smear preparation from gonorrhœal pus. × 1,200. Semi-schematic.

VI. *b*. Diplococcus gonorrhœæ much enlarged. Schematic.

STREPTOCOCCUS MENINGITIDIS CEREBROSPINALIS.

(Weichselbaum) Lehmann and Neumann.

VII. Smear preparation from meningeal exudation; pus cells with transversely divided diplococci. (Copied from Jaeger: *Zschr. f. Hyg.*, Vol. XIX., Pl. VI., Fig. 3.) About ×1,200.

VIII. Microscopical preparation; pure culture, formation of tetrads. About ×1,200. (Copied from Jaeger: *Zschr. f. Hyg.*, Vol. XIX., Pl. VII., Fig. 6.)

VI. *a*

VI. *b*

Explanation of Plate 4.

MICROCOCCUS ROSEUS. (Bumm) Lehmann and Neumann.

I. Gelatin stick culture, twenty days at temperature of room.

II. Agar streak culture, thirty days at temperature of room. The white reflex on the right side is not always so pronounced.

III. Agar stick culture, ten days 22°. Puncture canal.

IV. Agar stick culture, ten days 22°. Surface.

V. Agar plate, twelve days at 22°. ×50. Above, a superficial, below, a deep-seated colony.

VI. Agar plate. More delicate structure. Fourteen days at 22°. ×50. Above, a superficial colony, below, deep-seated colonies.

VII. Gelatin plate, eight days at 22°. ×50. Superficial and deep colonies.

VIII. Microscopical preparation from agar culture (× 1,000), three days. The cocci are dividing.

IX. Potato culture of diplococcus roseus placed on an anthrax culture, ten days at temperature of room.

X. Potato culture, twenty days at temperature of room.

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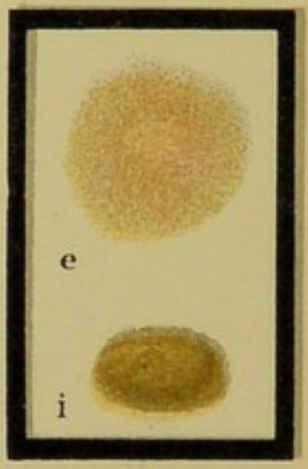

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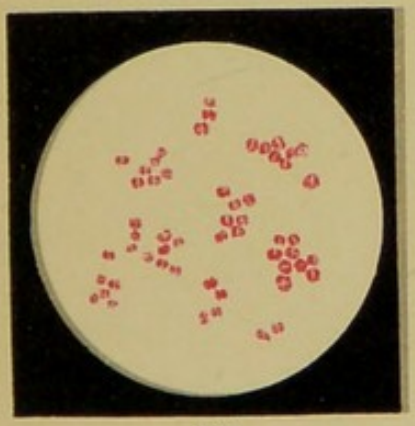

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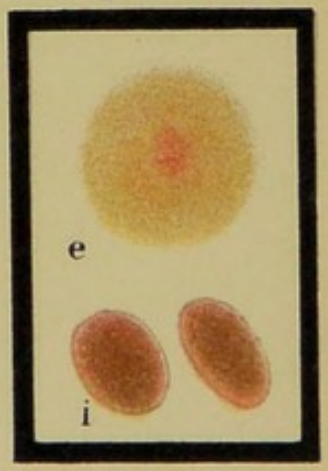

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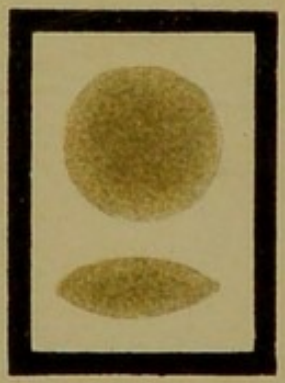

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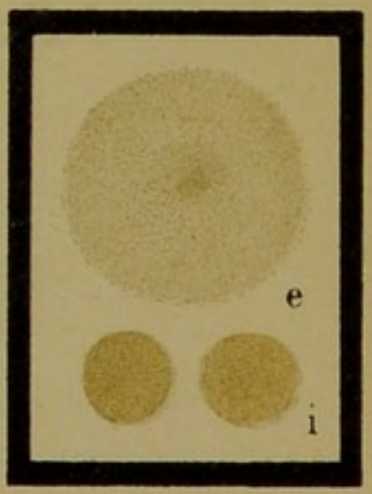


VIII.

IX.

X.

Explanation of Plate 5.

STREPTOCOCCUS LANCEOLATUS. Gamaleia.

(Diplococcus pneumoniæ A. Fraenkel.)

(Pneumococcus.)

I. Gelatin stick culture, ten days at 22°.

II. Agar streak culture, four days at 37°.

III. Agar stick culture, four days at 37°. Puncture canal.

IV. Agar stick culture, four days at 37°. Surface.

V. Agar plate, three days at 37°. Natural size.

VI. Agar plate, three days at 37°. ×50. Superficial colony. The dark colony is situated near the surface.

VII. Agar plate, three days at 37°. ×50. Deep-seated colonies.

VIII. Gelatin plate, eight days at 22°. The upper colony superficial, the two lower ones deep seated.

IX. Smear preparation from pneumonia sputum. × 1,000.

X. Pure culture from agar plate three days old. × 1,000.

XI. Microscopical preparation.

(*a*) Diplococci, single and arranged in chains. High magnifying power.

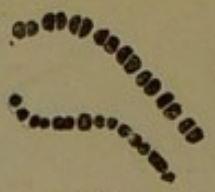
(*b*) Diplococci surrounded with gelatinous capsule.

XI.

Explanation of Plate 6.

STREPTOCOCCUS PYOGENES. Rosenbach.

I. Agar streak culture, ten days at 37°.
II. Gelatin stick culture, six days at 22°. The colony is not often found in such a vigorous state.
III. Agar stick culture, six days at 37°. Puncture canal.
IV. Agar stick culture, six days at 37°. Surface.
V. Gelatin plate, six days at 22°.
VI. Gelatin plate, six days at 22°. ×70. Somewhat abnormal shape with ragged edges. The larger colonies superficial, the smaller ones deep.
VII. Gelatin plate, six days at 22°. ×70. More frequent form. Upper one superficial, lower one deep.
VIII. Agar plate, eight days at 37°. ×50. Larger colony superficial, smaller colonies deep.
IX. Microscopical preparation from a bouillon culture, two days at 37°. ×700. The individual cocci are usually more regularly rounded.
X. Microscopical preparation from an agar culture, two days. Shorter chains. ×1,000.
XI. Microscopical preparation. Called streptococcus conglomeratus. Smear preparation from the blood of the spleen from a case of scarlatina. Copied from Kurth (Kaiserl. Gesundheitsamt, Vol. VII.).
XII. Streptococci chains, before and during division. High magnifying power.



XII.

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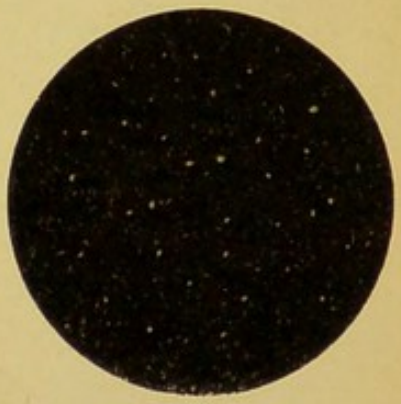

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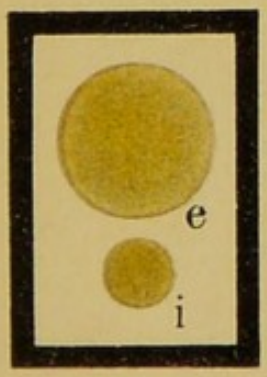

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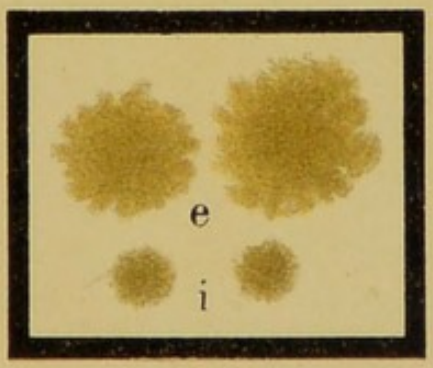

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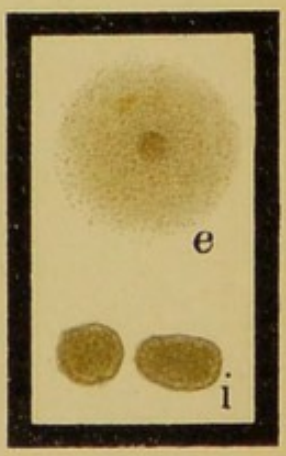

VIII.

IX.

X.

XI.

Lith.Anst.v.F.Reichhold, München.

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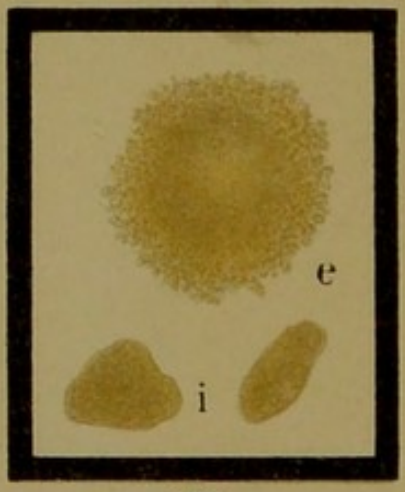
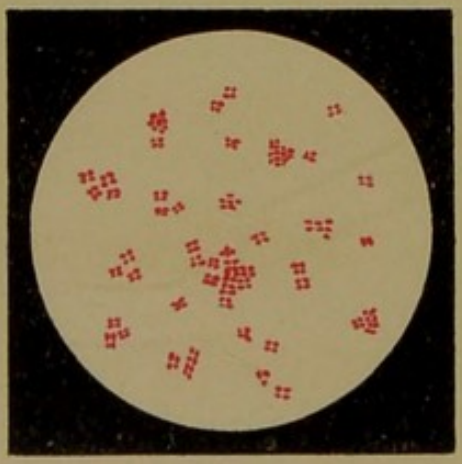
VI.

VII.



VIII.



IX.

X.

Lith. Anst. v. F. Reichhold, München

Explanation of Plate 7.

MICROCOCCUS TETRAGENUS. Koch, Gaffky.

I. Agar streak culture, five days at 37°.

II. Gelatin stick culture, ten days at 22°. Puncture canal. The "nail-head" shape is characteristic.

III. Gelatin stick culture, ten days at 22°. Surface. The color is too brown in the plate; should have been white.

IV. Agar stick culture, six days at 37°. The puncture does not always turn out so vigorous.

V. Agar stick culture, six days at 37°. Surface.

VI. Agar plate, five days at 37°. Natural size.

VII. Gelatin plate, eight days at 22°. In nature the colonies are pure white. Natural size.

VIII. Gelatin plate, eight days at 22°. ×60. The larger colony is superficial, the smaller ones are deep.

IX. Microscopical preparation from an agar culture (×800) two days old. We do not always find tetrads alone. There are numerous individual cocci.

X. Potato culture, seven days at 37°.

XI. Microscopical appearances. Tetrads before, during, and after division highly magnified.

XI.

Explanation of Plate 8.

MICROCOCCUS LUTEUS. Cohen with Lehm. and Neum.

I. Gelatin stick culture, six days at 22°.

II. Gelatin plate, three days at 22°. ×50. On right side superficial, on left side deep-seated colony.

III. Microscopical preparation (×1,000) from an agar plate two days old. The micrococci are often aggregated into tetrads.

IV. Agar plate (natural size), five days at 22°. The colonies are sometimes more yellow.

V. Potato culture, six days at 22°. Sometimes has a dull lustre.

SARCINA PULMONUM. Virchow, Hauser.

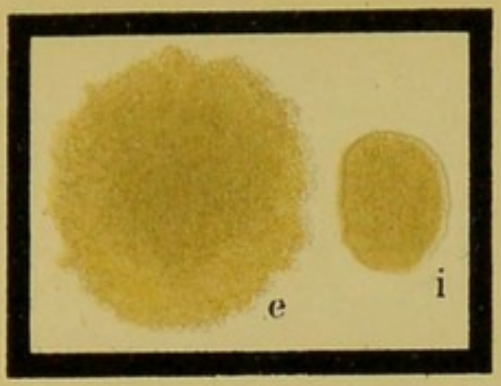
VI. Gelatin stick culture, twenty days at 22°. In reality the puncture is grayer in color.

VII. Agar streak, twenty days at 22°.

VIII. Gelatin plate, twenty days at 22°. On the right, superficial colony; on the left, deep-seated one.

IX. Potato culture, twenty days at 22°.

I.



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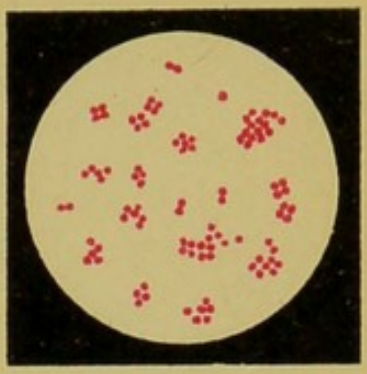
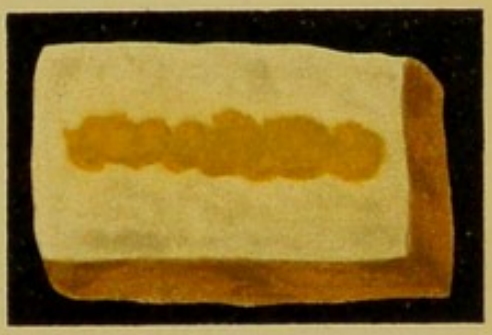


III.

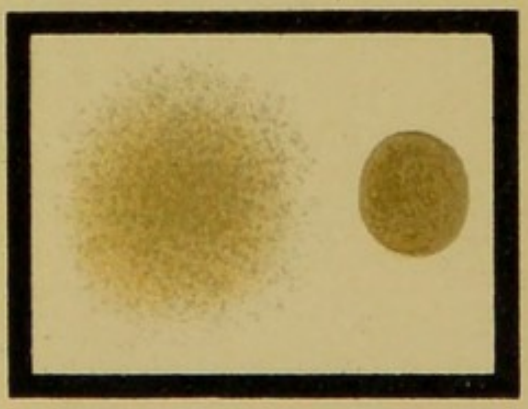
IV.



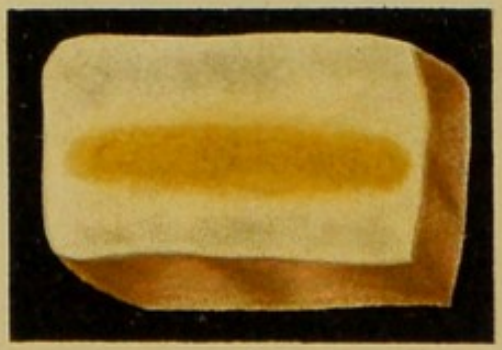
V.

VI.

VII.



VIII.

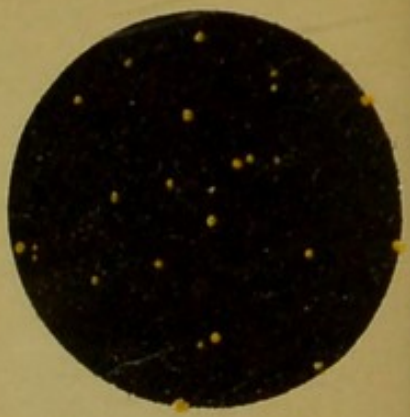


IX.

I.

II.

III.



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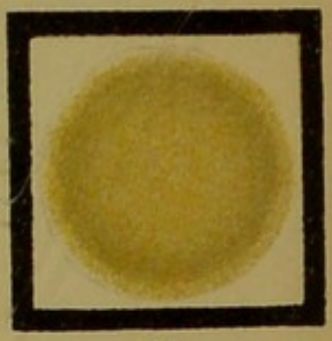
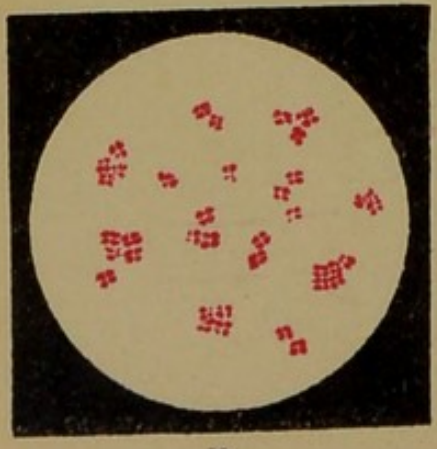


VI.

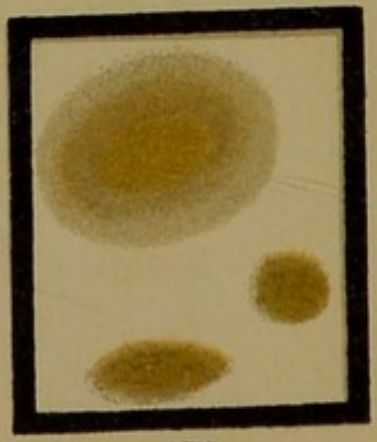
IX.

IV.

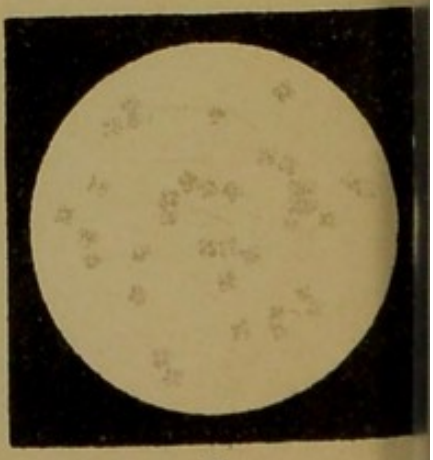
VII.



X.



VIII.

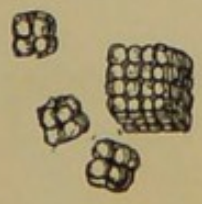


XI.

Explanation of Plate 9.

SARCINA FLAVA. De Bary with Lehm. and Stubenrath.

I. Gelatin stick culture, ten days at 22°.
II. Agar streak culture, six days at 22°.
III. Agar stick culture, six days at 22°. Puncture canal.
IV. Agar stick culture, six days at 22°. Surface.
V. Gelatin plate, five days at 22°. Natural size.
VI. Gelatin plate, five days at 22°. ×60. Superficial colony.
VII. Agar plate, six days at 22°. Natural size.
VIII. Agar plate, six days at 22°. ×60. Upper colony superficial, lower colony deep seated.
IX. Potato culture, ten days at 22°.
X. Microscopical preparation. Pure culture from an agar plate. ×1,000. Stained with fuchsin and decolorized with acetic acid.
XI. Microscopical preparation. Pure culture from bouillon. Unstained. ×1,000.
XII. Sarcina in the shape of bales (regular combination of individual packages).
XIII. Sarcina in heaps of packages (irregular mass of single regular or irregular packages).



XII.

XIII.

Explanation of Plate 10.

SARCINA AURANTIACA. Flügge.

I. Gelatin stick culture, six days at 22°.

II. Agar streak culture, five days at 22°. The color is not so red in all cases, usually it is a bright orange. Likewise in the agar stick and potato cultures.

III. Agar stick culture, six days at 22°. Puncture canal.

IV. Agar stick culture, six days at 22°. Surface.

V. Gelatin plate, five days at 22°. Natural size. The gray rim around the colony indicates the depression.

VI. Gelatin plate, five days at 22°. ×60. A young colony. The gray ring indicates the zone of depression.

VII. Agar plate, five days at 22°. Natural size.

VIII. Agar plate, five days at 22°. ×60. Upper colony superficial, lower colonies deep seated. The superficial colonies are usually opaque toward the middle.

IX. Potato culture eight days old.

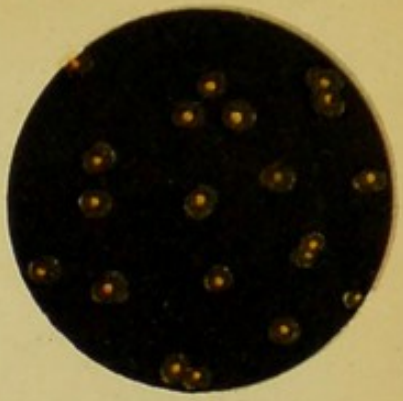
X. Microscopical preparation. Pure culture of agar. ×1,000. Colored with fuchsin and decolorized with acetic acid.

XI. Microscopical preparation. Pure culture from bouillon. ×1,000. Unstained. Semi-schematic.

I.

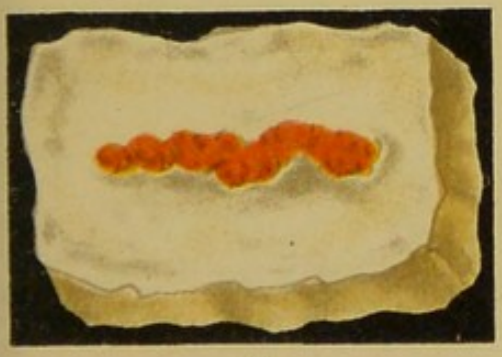
II.

III.



V.

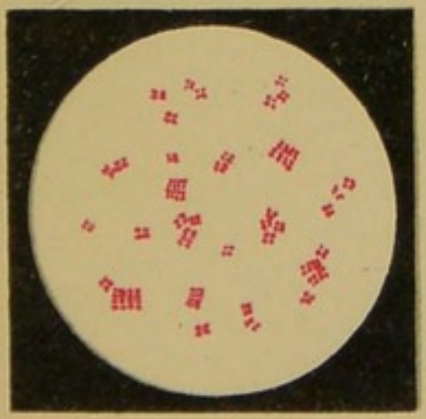
VI.



IX.

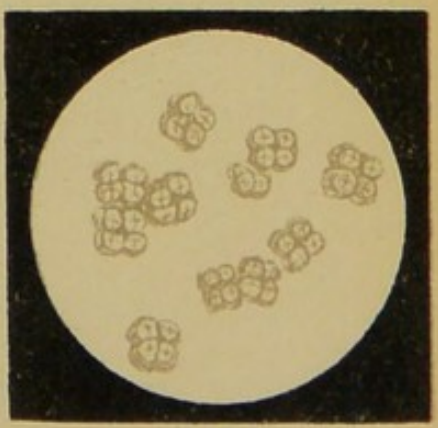
IV.

VII.



X.

VIII.



XI.

I.

II.

III.

IV.

V.

VI.

VII.

VIII.

Explanation of Plate 11.

SARCINÆ DIVERSÆ.

I. Sarcina cervina Stubenrath. Agar streak culture, fifteen days at 22°, isolated from gastric contents.

II. Sarcina pulmonum Virchow. Agar streak culture, fifteen days at 37°.

III. Sarcina erythromyxa Král. Agar streak culture, thirty days at 22°, isolated from beer.

IV. Sarcina lutea Flügge. Agar streak culture, ten days at 22°, isolated from stomach.

V. Sarcina aurantiaca Flügge. Agar streak culture, ten days at 22°, isolated from dough.

VI. Sarcina rosea Schroeter and Zimmermann. Agar streak culture, twenty-five days at 22°, isolated from "weissbeer."

VII. Micrococcus badius Lehman and Neumann. Agar streak culture, fifteen days at 22°, isolated from the atmosphere.

VIII. Sarcina canescens Stubenrath. Agar streak culture, twenty days at 22°, isolated from stomach.

Explanation of Plate 12.

BACTERIUM PNEUMONIÆ. Friedländer.

(Friedländer's pneumonia bacillus.)

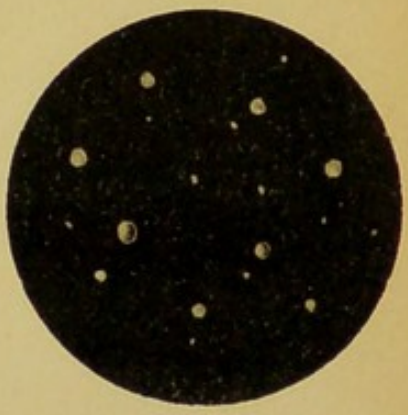
I. Agar streak culture, four days at 22°.
II. Gelatin stick culture, ten days at 22°.
III. Agar stick culture, four days at 22°. Puncture canal.
IV. Agar stick culture, four days at 22°. Surface.
V. Gelatin plate, three days at 22°. Natural size.
VI. Agar plate, two days at 22°. ×60. The brown, whetstone-shaped colony is deep seated.
VII. Gelatin plate, three days at 22°. ×50. Above, superficial colony; below, deep-seated one.
VIII. Agar plate, four days at 22°. Natural size. The delicate gray colonies and the smallest ones are deep seated. One colony has been colored too yellow.
IX. Microscopical preparation. Pure culture (×800) from an agar plate. Stained with fuchsin.
X. Microscopical preparation. Smear preparation from sputum. ×800. Fuchsin stain.
XI. Potato culture, six days.

I.

II.

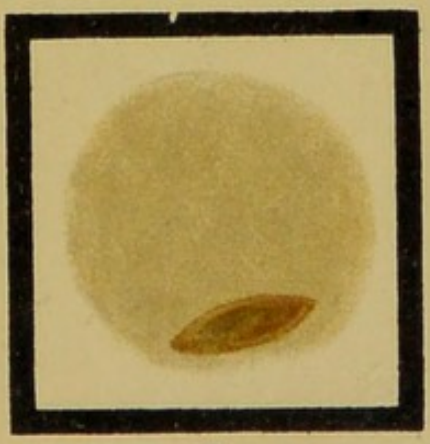
III.

IV.



V.

IX.



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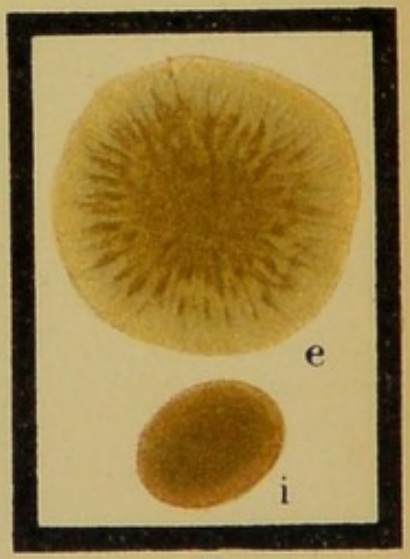


VII.

XI.

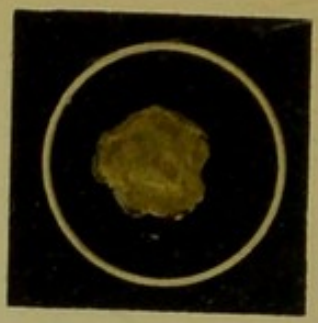
X.

VIII.

I.

II.

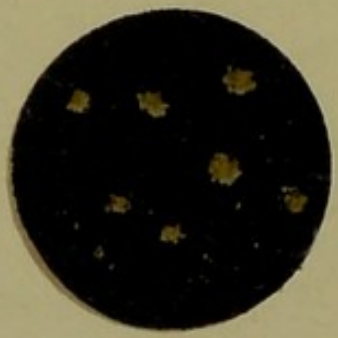
III.



IV.

V.

IX.



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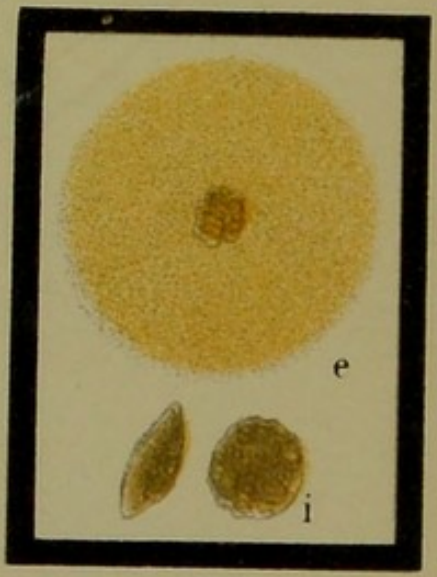

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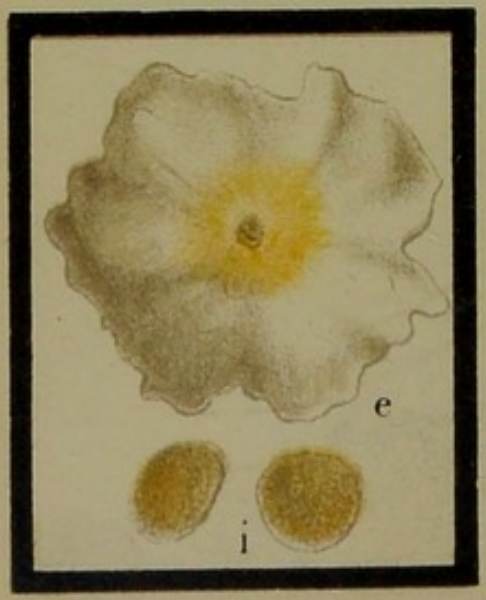


VIII

X

Explanation of Plate 13.

BACTERIUM ACIDI LACTICI. Flügge.

(Lactic-acid bacillus.)

I. Gelatin stick culture, five days at 22°. In nature the puncture canal is a little whiter.

II. Agar streak culture, five days at 22°.

III. Agar stick culture, three days at 22°. Puncture canal.

IV. Agar stick culture, three days at 22°. Surface.

V. Agar plate, three days at 22°. Natural size.

VI. Agar plate, three days at 22°. ×50. Upper colony superficial, lower ones deep seated. *Vide* Pl. 14, VII.

VII. Gelatin plate, two days at 22°.

VIII. Gelatin plate, two days at 22°. ×50. Upper colony superficial, lower colonies deep seated. The superficial colony may vary extremely in its growth. *Vide* Pl. 15, IV., VII.; Pl. 16, IX., VIII.; Pl. 17, I., II.

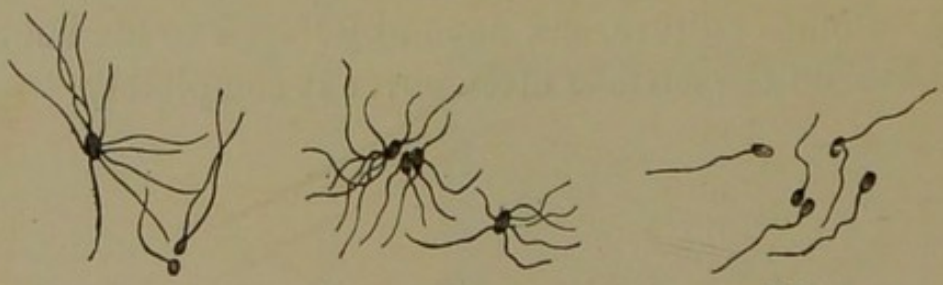
IX. Microscopical preparation. Pure culture from an agar colony. ×800.

X. Potato culture, six days at 22°. The air bubbles on the surface often cover it completely.

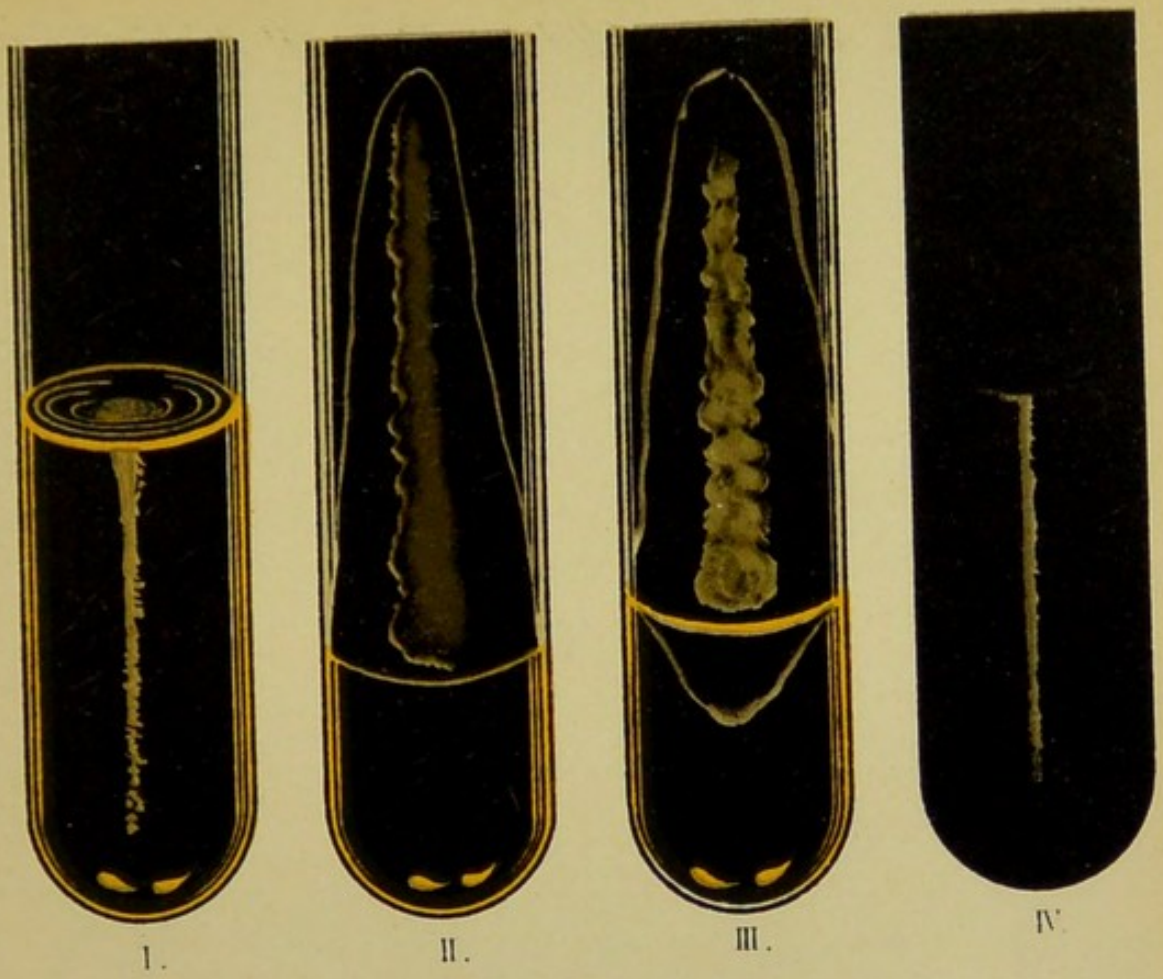
Explanation of Plate 14.

BACTERIUM COLI COMMUNE. Escherich.

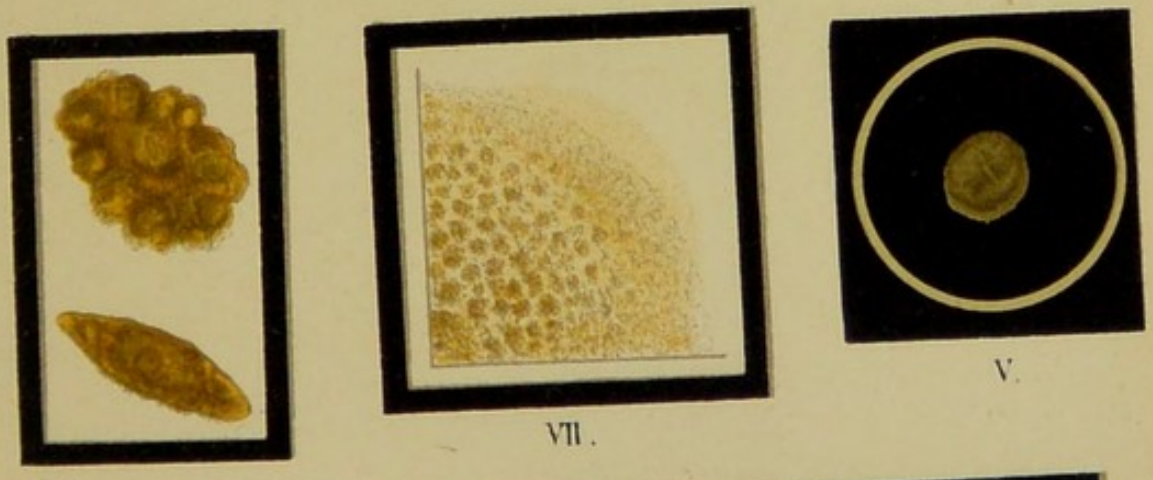
I. Gelatin stick culture, ten days at 22°.
II. Gelatin streak culture, four days at 22°. In nature, transparent and iridescent like mother-of-pearl. *Vide* Pl. 16, VI.
III. Agar streak culture, four days at 22°. *Vide* Pl. 16, V.
IV. Agar stick culture, two days at 22°. Puncture canal.
V. Agar stick culture, two days at 22°. Surface.
VI. Agar plate, four days at 22°. ×60. Deep-seated colonies. *Vide* Pl. 13, VI.
VII. Agar plate, four days at 22°. ×60. A part of a superficial colony. During growth occasionally exhibits forms like bacillus acidi lactici. *Vide* Pl. 13, VI.; Pl. 17, V., VI.; Pl. 18, IV.; Pl. 12, VIII.
VIII. Agar plate, three days at 22°. Natural size.
IX. Potato culture, five days at 22°. May also appear of a lighter or darker color.
X. Bacteria with long flagella from bacterium brassicæ acidæ. ×1,000. Stained according to Löffler's method.
XI. Bacteria with flagella, from the bacterium of pigeon diphtheria. ×1,000. Stained by Löffler's method.
XII. Bacteria with one flagellum, rarely with two flagella, from bacterium of the deer plague. ×1,000. Stained by Löffler's method.



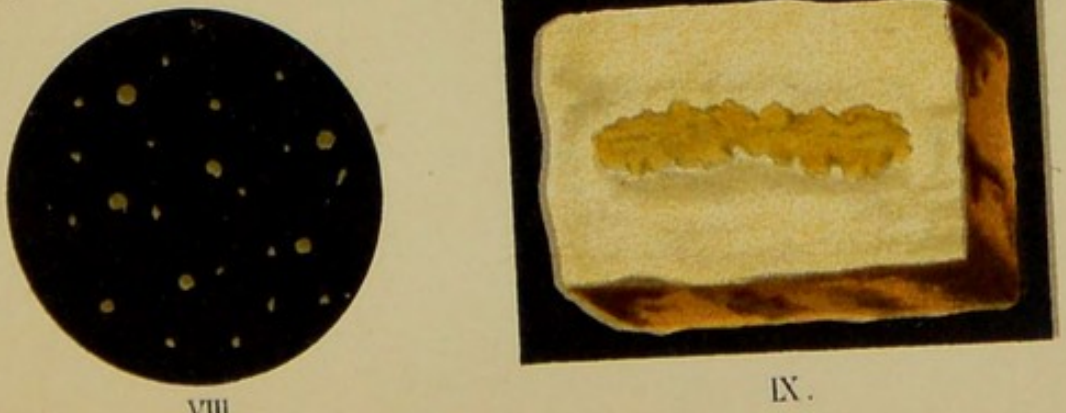
X. XI. XII.



I. II. III. IV.

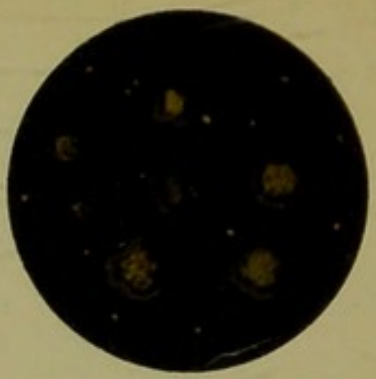


V. VI. VII.



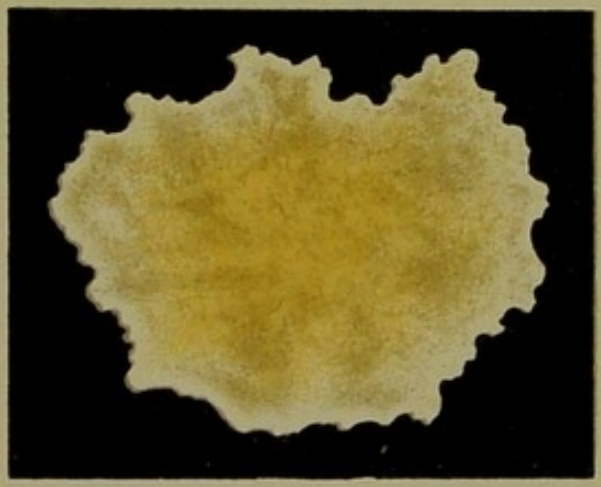
VIII. IX.

I.



II.

III



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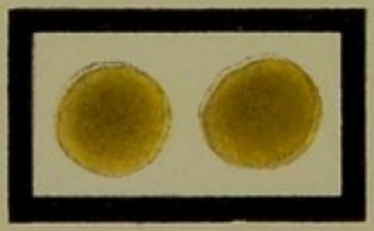


V.

VII.

VI.

VIII.

Explanation of Plate 15.

BACTERIUM COLI COMMUNE. Escherich.

I. Gelatin plate, eight days at 22°. ×60. Coli cultivated from pus. Deep-seated colonies. Abnormal shapes.

II. Gelatin plate, four days at 22°. Natural size.

III. Gelatin plate, one day at 22°. ×90. Superficial colony. *Vide* Pl. 13, VIII.; Pl. 16, VIII.

IV. Gelatin plate, four days at 22°. ×60. Superficial colony. *Vide* Pl. 16, IX.; Pl. 17, I., II.

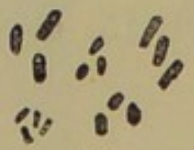
V. Gelatin plate, four days at 22°. ×60. Deep-seated colony.

VI. Gelatin plate, ten days at 22°. ×90. Superficial colony.

VII. Gelatin plate, ten days at 22°. ×90. Superficial colony.

VIII. Microscopical preparation. Pure culture from an agar plate. ×500.

IX. Bacteria of various kinds of coli. ×1,000. Great differences in size.



IX.

Explanation of Plate 16.

BACTERIUM TYPHI. Eberth, Gaffky.

(Typhoid bacillus.)

I. Agar stick culture, three days at 22°. Puncture canal.

II. Agar stick culture, three days at 22°. Surface.

III. Gelatin stick culture, eight days at 22°. Puncture canal.

IV. Gelatin stick culture, eight days at 22°. Surface.

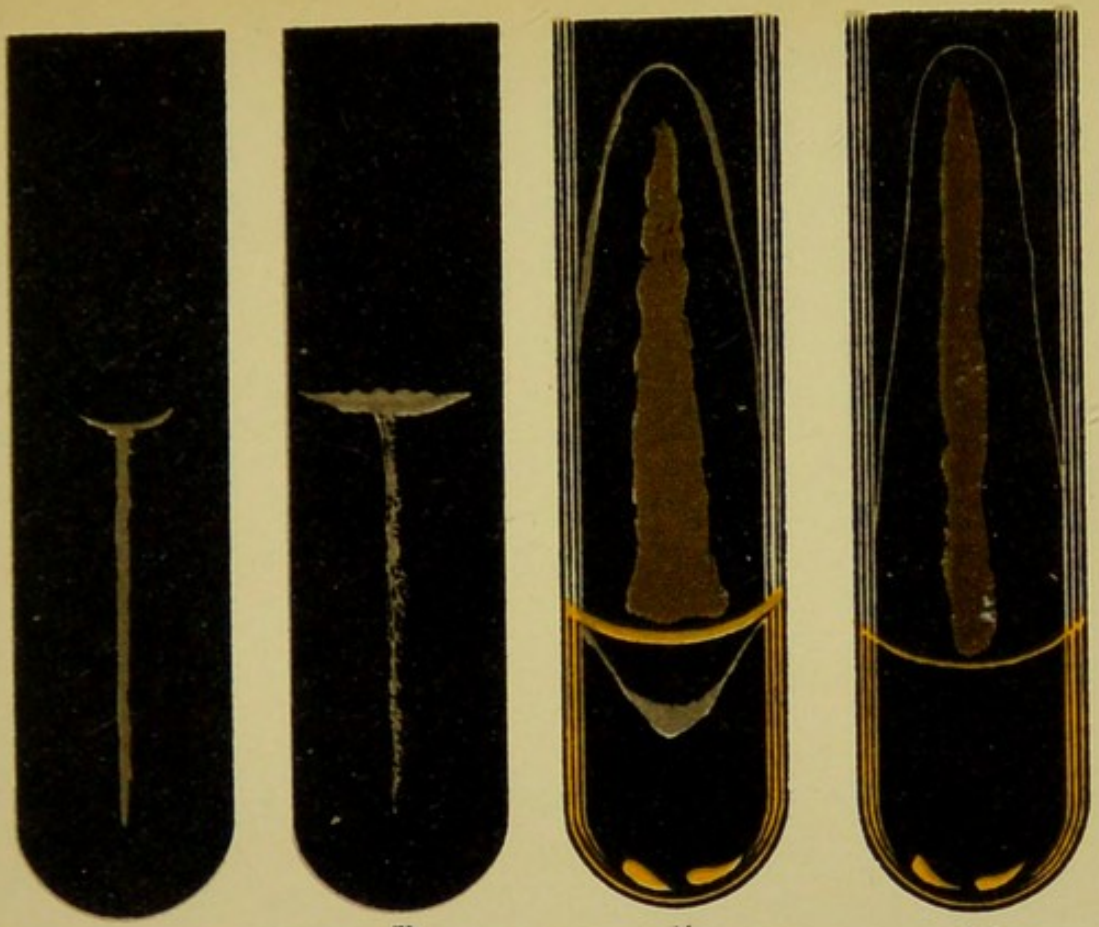
V. Agar streak culture, four days at 22°. *Vide* Pl. 14, III.

VI. Gelatin streak culture, three days at 22°. *Vide* Pl. 14, II.

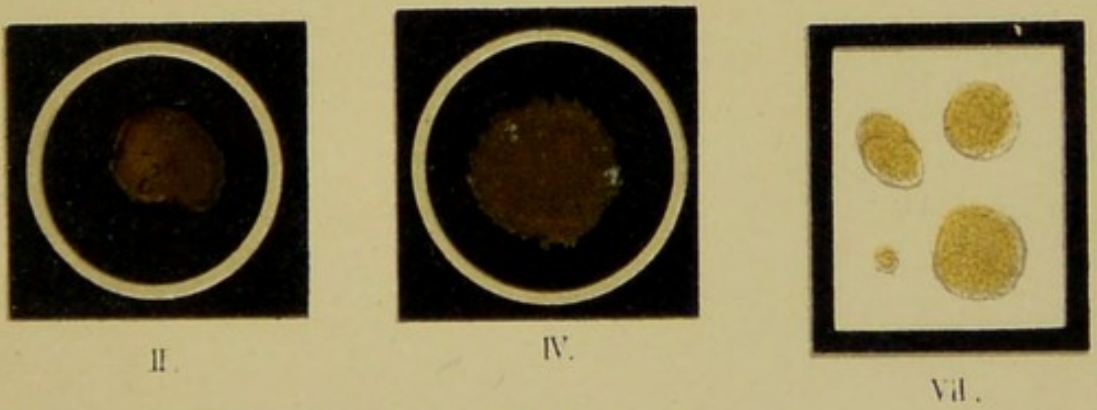
VII. Gelatin plate, one and a half days at 22°. Deep-seated colony. *Vide* Pl. 15, V.; Pl. 13, VIII.

VIII. Gelatin plate, one and a half days at 22°. Superficial colony. *Vide* Pl. 15, III.; Pl. 13, VIII.

IX. Gelatin plate, four days at 22°. Superficial colony. *Vide* Pl. 15, IV., VII.



I. III. V. VI.



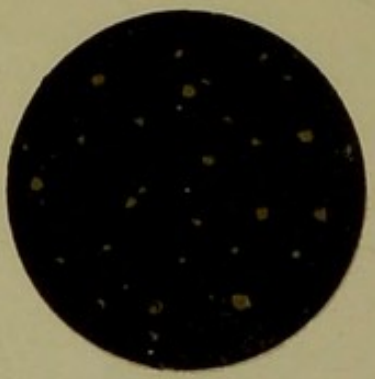
II. IV. VII.

IX. VIII.

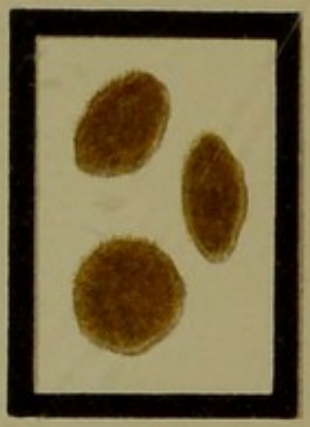
I.

II.

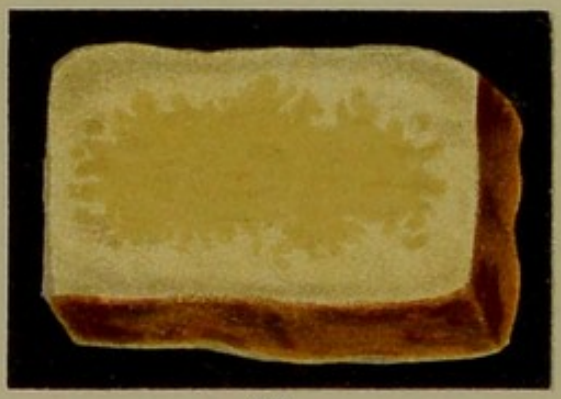
III.



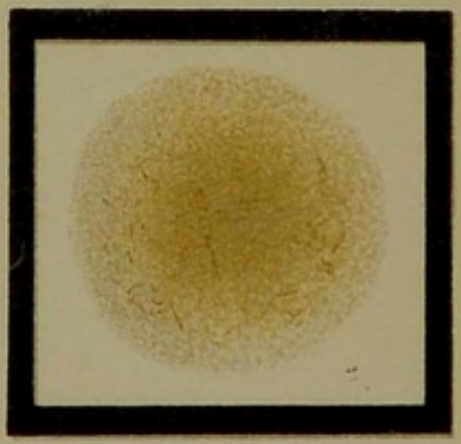
IV.



V.



VII.



VI.

VIII.

IX.

Explanation of Plate 17.

BACTERIUM TYPHI. Eberth, Gaffky.

(Typhoid bacillus.)

I. Gelatin plate, eight days at 22°. ×90. Superficial colony. *Vide* Pl. 15, VII., VI.

II. Gelatin plate, eight days at 22°. ×150. Superficial colony.

III. Gelatin plate, four days at 22°. Natural size.

IV. Agar plate, four days at 22°. Natural size.

V. Agar plate, four days at 22°. ×60. Deep-seated colonies.

VI. Agar plate, four days at 22°. ×60. Superficial colonies.

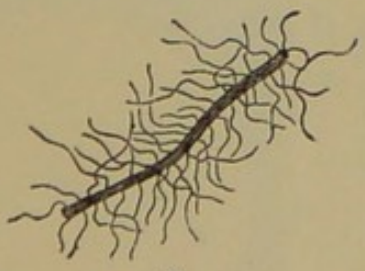
VII. Potato culture, five days at 22°.

VIII. Microscopical preparation. Pure culture from agar plate. ×1,000.

IX. Microscopical preparation. Bacilli with flagella. Copied from Fraenkel and Pfeiffer: "Atlas d. Bakterienkunde," Plate 54, Fig. 111.

X. Microscopical preparation. Long thread, thickly studded with flagella. ×1,500. Löffler's stain.

XI. Microscopical preparation of bacterium typhi murium Löffler, with flagella and capsule. ×1,500. Stained by Löffler's method.



X.

XI.

Explanation of Plate 18.

BACTERIUM SEPTICÆMIÆ HÆMORRHAGICÆ. Hüppe.

(Fowl cholera, rabbit septicæmia, etc.)

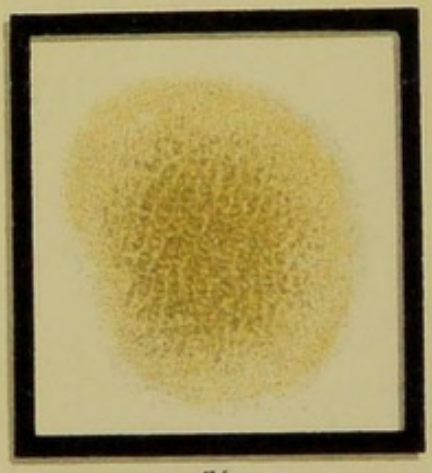
I. Gelatin stick culture, seven days at 22°.
II. Agar streak culture, seven days at 22°.
III. Agar plate, five days at 22°. Natural size.
IV. Agar plate, five days at 22°. ×60. Superficial colony. *Vide* Pl. 17, VI.; 14, VII.; 13, VI.
V. Agar plate, five days at 22°. ×60. Deep-seated colonies.
VI. Gelatin plate, five days at 22°. Natural size.
VII. Gelatin plate, five days at 22°. ×90. Deep-seated colonies.
VIII. Gelatin plate, five days at 22°. ×90. Superficial colony. *Vide* Pl. 17, I.; Pl. 16, IV., VIII.; Pl. 15, IV., III., VII.; Pl. 13, VIII.
IX. Microscopical preparation. ×1,000. Pure culture from an agar plate.
X. Individual bacteria. Highly magnified. Schematic.

X.

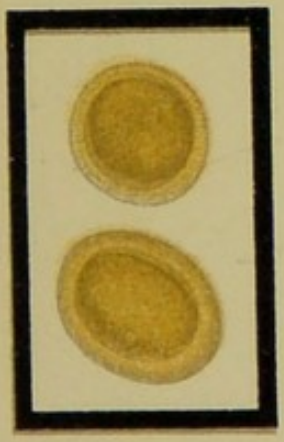
I.

II.

III.

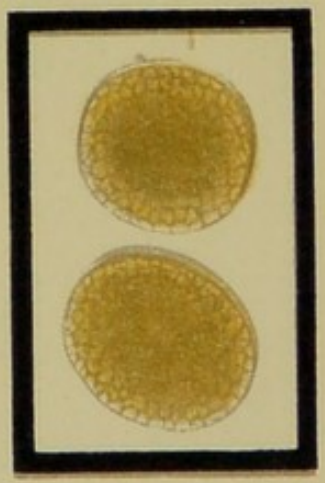


IV.



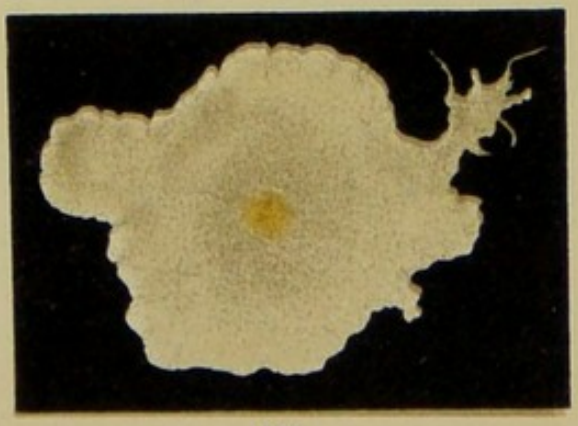
VII.

VI.



V.

IX.

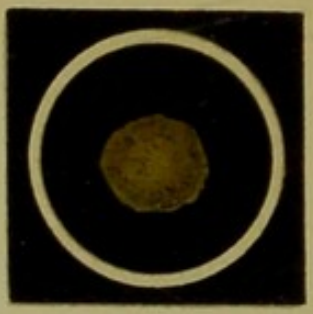


VIII.

I.

II.

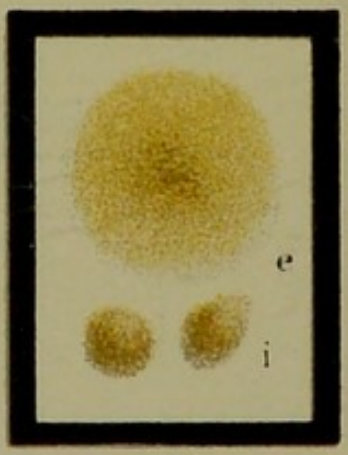
III.



IV.

V.

VI.



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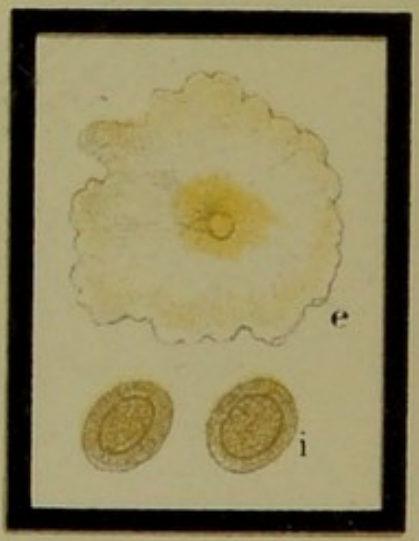

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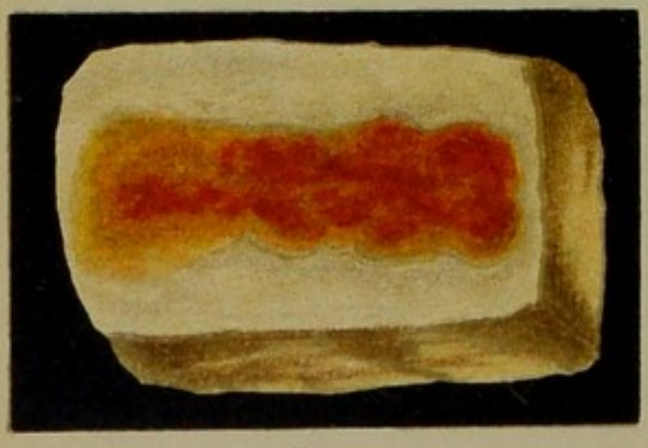
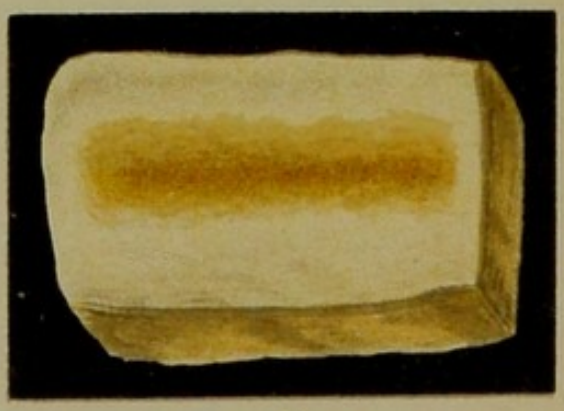


IX.



X.

Explanation of Plate 19.

BACTERIUM MALLEI. Löffler.

(Glanders.)

I. Gelatin stick culture, six days at 22°.
II. Agar streak culture, six days at 37°. The middle white line is not always so pronounced.
III. Agar stick culture, three days at 37°. Puncture canal.
IV. Agar stick culture, three days at 37°. Surface.
V. Gelatin plate, five days at 22°. Natural size.
VI. Gelatin plate, four days at 22°. ×60. Upper colony superficial, lower colonies deep seated.
VII. Agar plate, two days at 22°. ×60. Upper colony superficial, lower colonies deep seated.
VIII. Microscopical preparation. Pure culture. ×800. Fuchsin stain.
IX. Potato culture, two days at 37°.
X. Potato culture, twenty days at 37°.
XI. Individual bacteria. Highly magnified. In some places the staining fluid is absorbed poorly or not at all.

XI.

Explanation of Plate 20.

CORYNEBACTERIUM DIPHTHERIÆ. (Löffler) Lehmann and Neumann.

(Diphtheria bacillus.)

I. Glycerin-agar stick culture, twenty days at 22°. Puncture canal.

II. Glycerin-agar streak culture, eight days at 22°.

III. Glycerin-agar stick culture, twenty days at 22°. Surface.

IV. Glycerin-agar plate, eight days at 22°. ×60. Deep and superficial colonies.

V. Glycerin-agar plate, forty days at 22°. ×60. On the left side deep-seated colonies; on the right side deep and superficial colonies.

VI. Glycerin-gelatin plate, twenty days at 22°. Natural size. Superficial and deep colonies.

VII. Glycerin-gelatin plate, twenty days at 22°. ×60. On the left side deep-seated colonies; on the right side superficial ones.

VIII. Potato culture, fourteen days at 22°.

IX. Microscopical preparation. Pure culture from bouillon two days old. ×700.

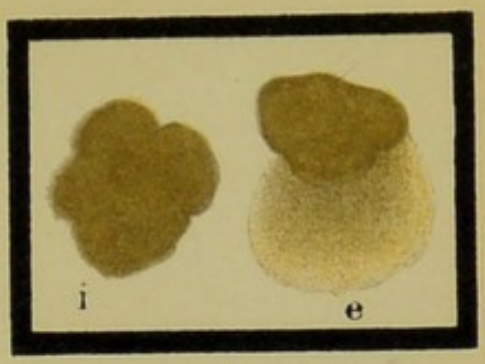
X. Microscopical preparation. Pure culture from bouillon. Involution forms. About ×1,200.

XI. Individual bacteria. Highly magnified. Schematic.

XI.

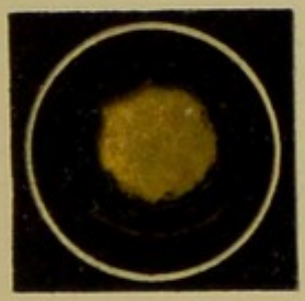
I.

II



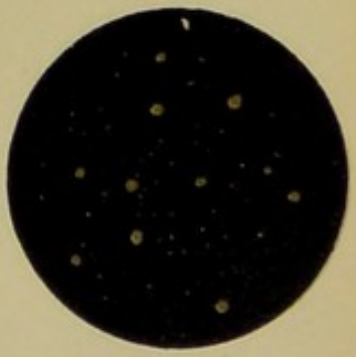
V.

IV.



III.

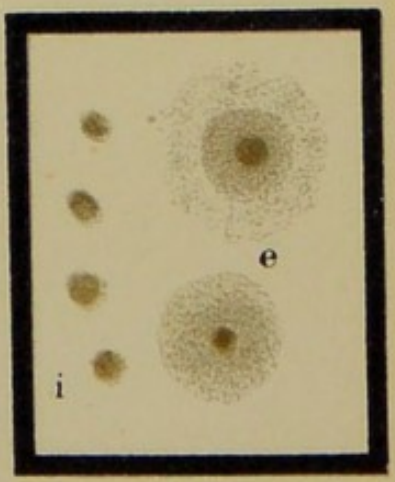
VIII.



VI.

IX.

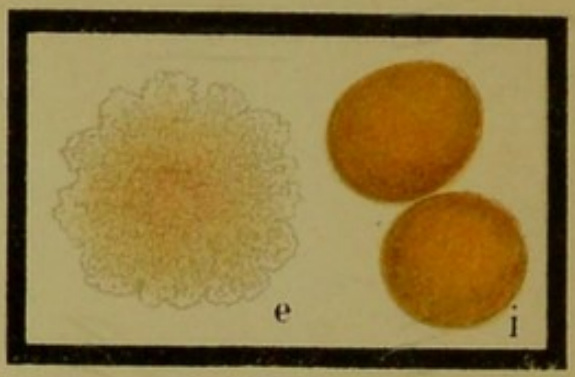
X.



VII.

I.

II.



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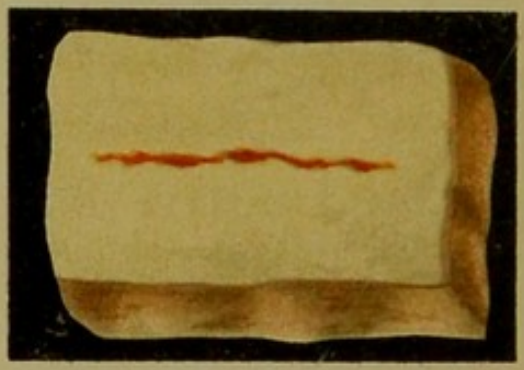

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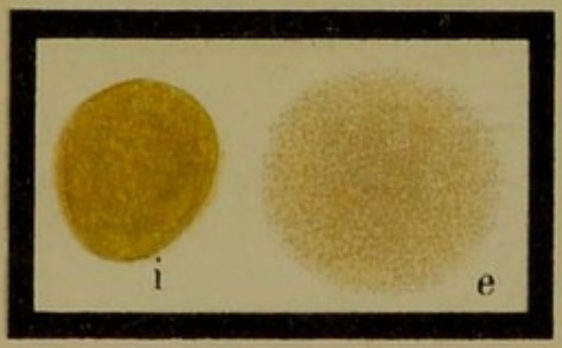

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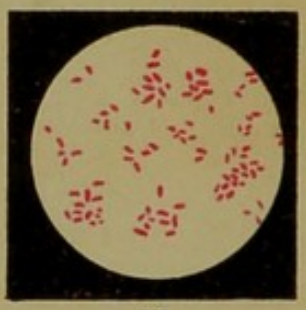
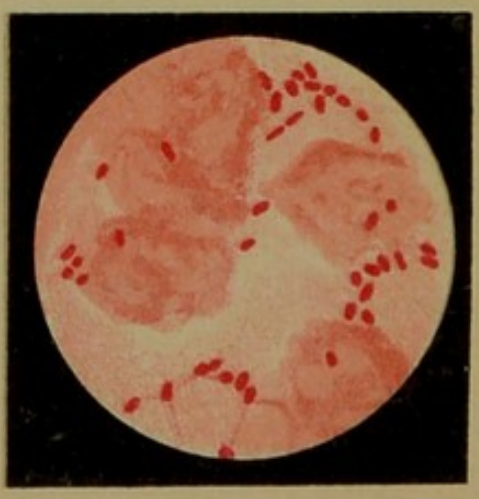


VI.

VII.



VIII.

Explanation of Plate 21.

BACTERIUM LATERICIUM. Adametz.

I. Agar streak culture, seven days at 22°.
II. Gelatin stick culture, fourteen days at 22°.
III. Gelatin plate, seven days at 22°. ×60. Deep-seated colonies on the right, superficial on the left.
IV. Potato culture, thirty days at 22°. Natural size.
V. Agar plate, seven days at 22°. Superficial colony on the right, deep one on the left.
VI. Microscopical preparation. Pure culture from agar twenty-four hours. About ×800.

BACTERIUM HÆMORRHAGICUM. (Kolb) Lehm. and Neum. (Morbus Werlhofii.)

VII. Microscopical preparation. Pure culture from bouillon three days old. (Copied from Kolb: A. G., Vol. VII., Pl. II., Figs. 1 and 2).
VIII. Smear preparation from the liver of a dog. (Copied from Kolb: *l.c.*, Vol. VII., Pl. III., Fig. 8.)

Explanation of Plate 22.

BACTERIUM PUTIDUM (Flügge) Lehm. and Neum.

(Bacterium fluorescens non-liquefaciens Autor.)

I. Gelatin stick culture, three days at 22°.

II. Gelatin plate, twenty-four hours at 22°. ×90. Deep-seated colony.

III. Gelatin plate, twenty-four hours at 22°. ×90. Superficial colony. *Vide* Pl. 13, VIII.; Pl. 15, III.

IV. Gelatin plate, four days at 22°. Natural size. Appearance of colonies upon a dark background.

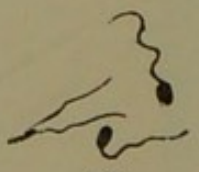
V. Potato culture, four days at 22°. Natural size. *Vide* Pl. 14, IX.

VI. Microscopical preparation. Pure culture from gelatin plate. ×800. Ordinarily threads are formed on agar.

VII. Agar plate, eight days at 22°. Natural size. Appearance of the colony on a white background.

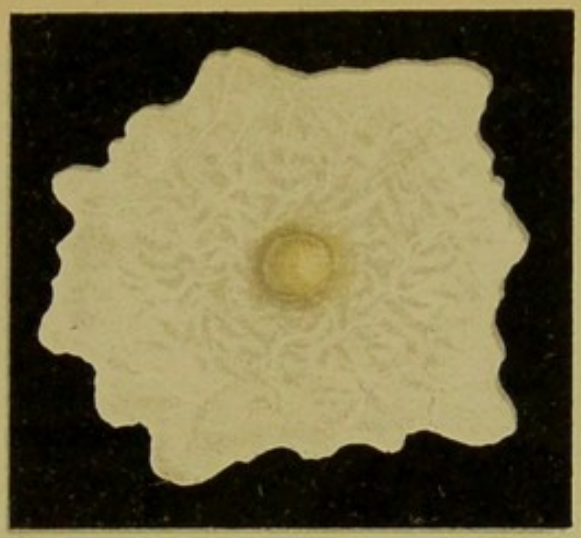
VIII. Agar plate, three days at 22°. ×60.

IX. Bacteria with one flagellum, more rarely two flagella. ×1,000. Stained according to Löffler's method.



IX.

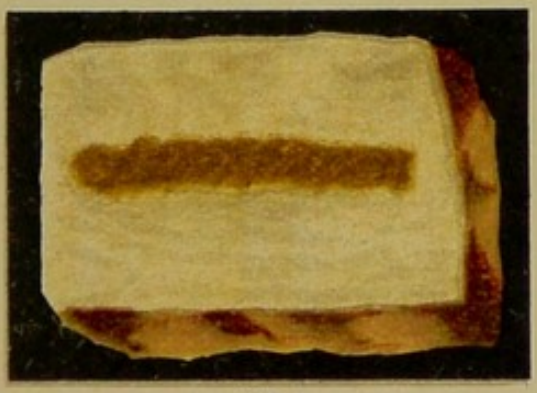
I.



II.

III.

IV.

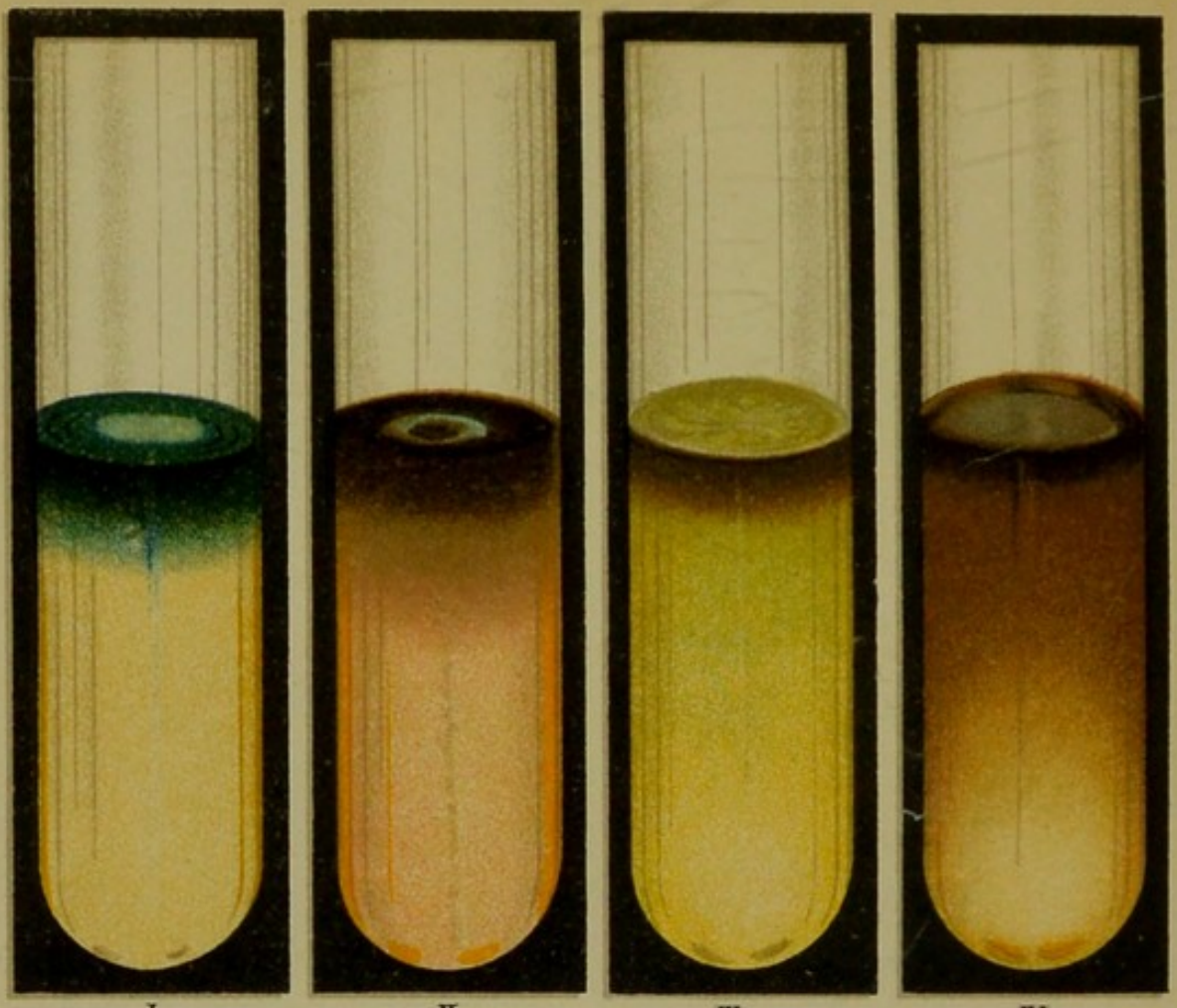


V.

VI.

VII.

VIII.



I. II. III. IV.

V.

VI.

VII.

VIII.

Explanation of Plate 23.

BACTERIUM SYNCYANEUM. (Ehrenb.) Lehm. and Neum.

(Bacillus cyanogenes Flügge; blue milk.)

I.–III. Gelatin stick cultures, six to ten days at 22°. Other shades of color are also observed.

IV. Agar stick culture, ten days at 37°.

V. Bouillon culture, four days at 37°.

VI. Milk culture, three days at 37°, upon non-sterilized milk.

VII. Microscopical preparation. Pure culture from agar plate. ×800.

VIII. Microscopical preparation. Pure culture. Flagella stained with Löffler's mordant.

IX. Bacteria with flagella; one or more at a pole. × 1,000. Stained by Löffler's method.

IX.

Explanation of Plate 24.

Bacterium syncyaneum. (Ehrenb.) Lehm. and Neum.

(Bacillus cyanogenes Flügge; blue milk.)

I.–III. Potato cultures, three to ten days at 22°. Potatoes of different kinds inoculated with the same culture. The differences in color may be still more manifold.

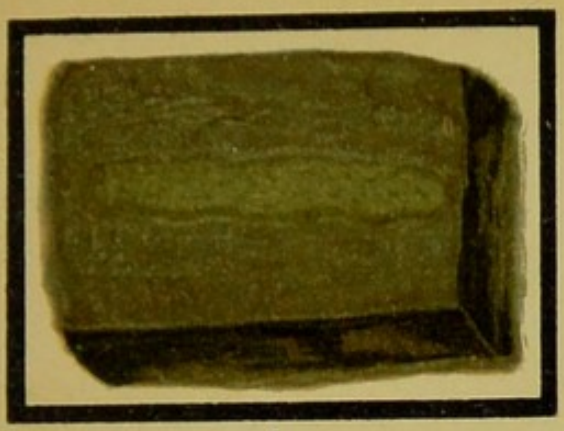
IV. Agar plate, three days at 22°. Natural size.

V. Agar plate, three days at 22°. ×60. On the right deep-seated, on the left superficial colonies.

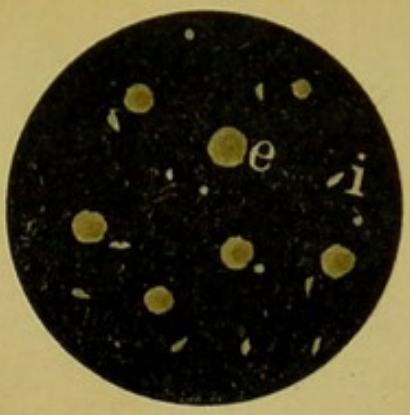
VI. Gelatin plate, three days at 22°. Natural size.

VII. Gelatin plate, eight days at 22°. Natural size. View of the colonies against a white background.

VIII. Gelatin plate, three days at 22°. ×60. Above, superficial; below, deep-seated colonies.



I.



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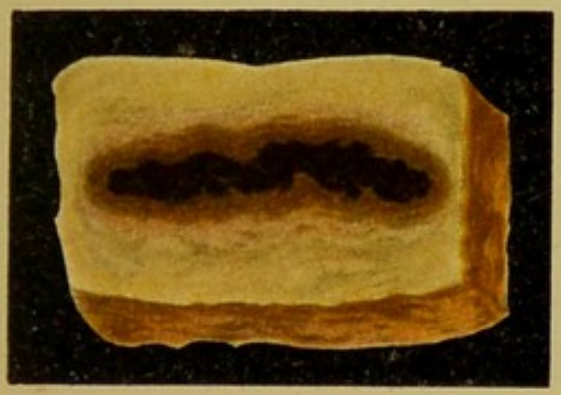

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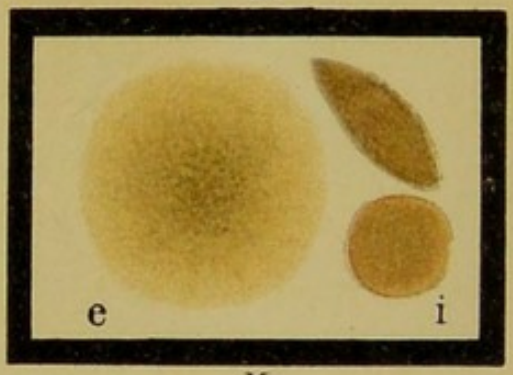

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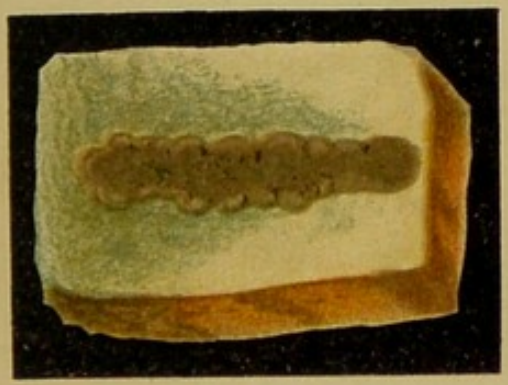

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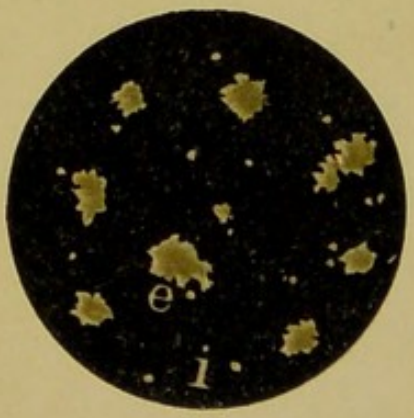

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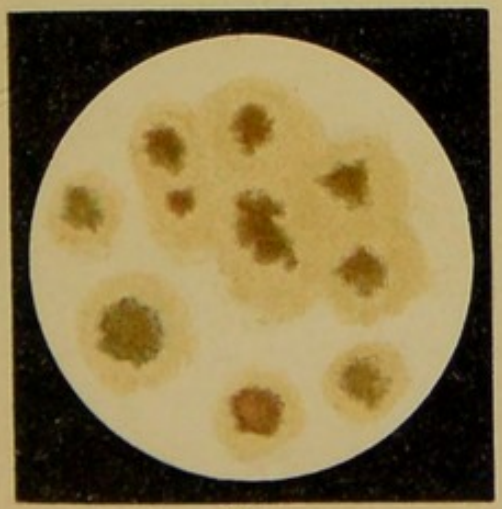
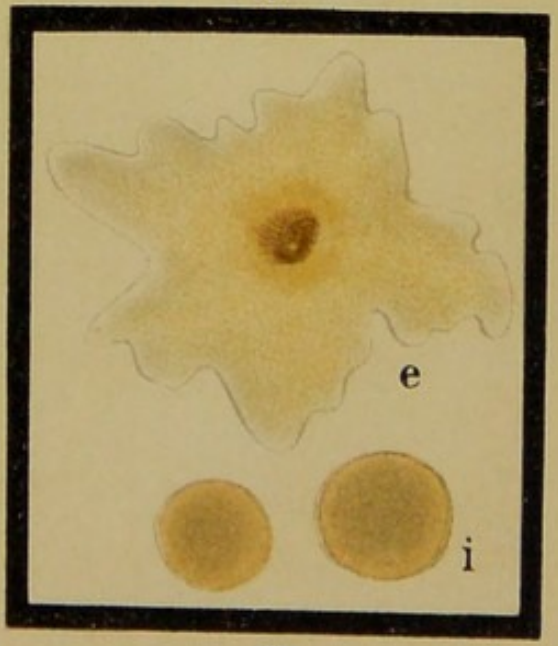


VII.



VIII.

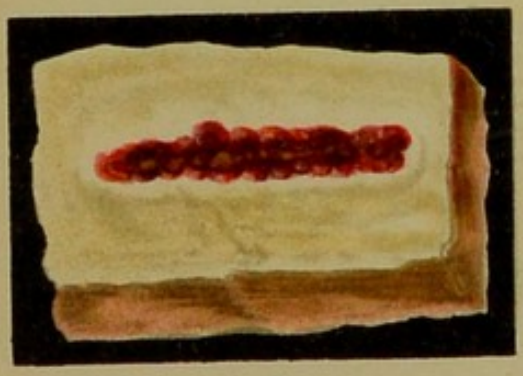
I.

II.

III.

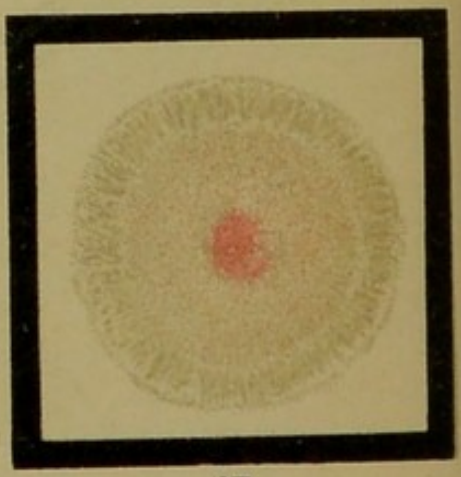
V.

VI.



IX.

IV.



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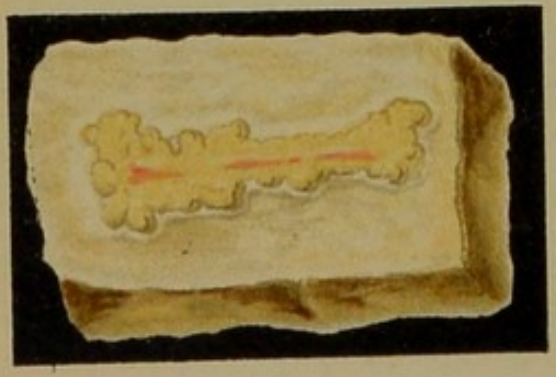


X.

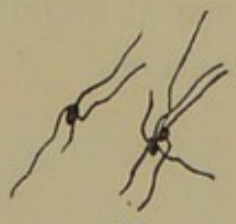
XI.

VIII.

Explanation of Plate 25.

BACTERIUM PRODIGIOSUM. (Ehrenb.) Lehm. and Neum.

I. Gelatin stick culture, one day at 22°.
II. Agar streak culture, four days at 22°.
III. Agar stick culture, four days at 22°. Puncture canal.
IV. Agar stick culture, four days at 22°. Surface.
V. Agar plate, two to four days at 22°. Natural size. Colonies with and without development of coloring matter.
VI. Agar plate, eight days at 22°. ×60. Superficial colonies reddish, deep ones yellowish.
VII. Gelatin plate, two days at 22°. ×60. Superficial colony just beginning to sink.
VIII. Gelatin plate, two days at 22°. Natural size.
IX. Potato culture, eight days at 22°. Typical, with metallic reflex on the surface.
X. Potato culture, eight days at 22°. Atypical, white deposit.
XI. Microscopical preparation. Pure culture from agar. ×800. Fuchsin stain.
XII. Bacteria with several flagella. ×1,000. Stained by Löffler's method.



XII.

Explanation of Plate 26.

BACTERIUM KILIENSE. (Breunig and Fischer) Lehm. and Neum.

(Kielwater bacillus.)

I. Agar streak culture, four days at 22°.

II. Gelatin stick culture, four days at 22°. Colony without development of coloring matter.

III. Gelatin plate, five days at 22°. Natural size. Colonies with and without development of coloring matter.

IV. Gelatin plate, five days at 22°. ×60. Superficial colony.

V. Gelatin plate, five days at 22°. ×60. Deep-seated colony.

VI. Agar plate, five days at 22°. Natural size. Colored and uncolored, superficial and deep-seated colonies.

VII. Agar plate, five days at 22°. ×60. Uncolored colonies. On the right side, superficial; on the left, deep seated.

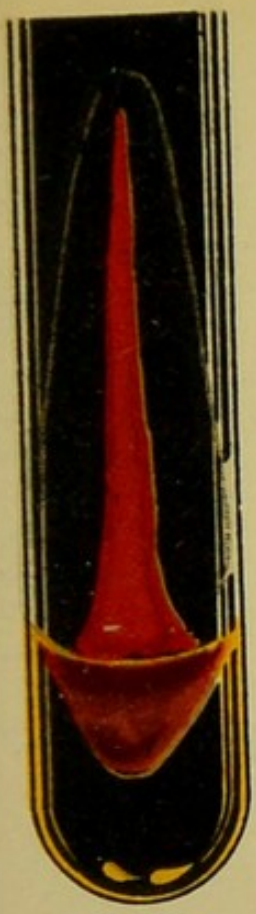
VIII. Agar plate, five days at 22°. ×60. Colored colonies. On the right, superficial; on the left, deep seated.

IX. Microscopical preparation. ×1,000. Pure culture from agar plate. Fuchsin stain.

X. Potato culture, five days at 22°.

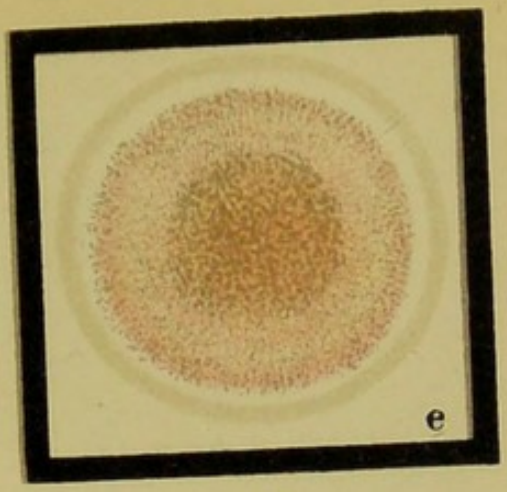
XI. Bacteria with several flagella. ×1,000. Stained by Löffler's method.

XI.



I.

II.



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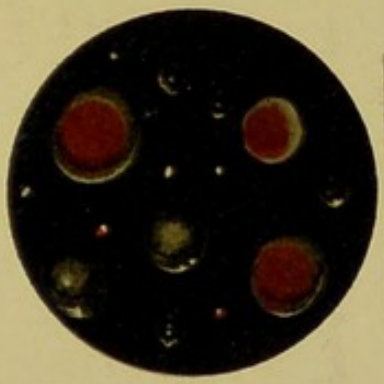
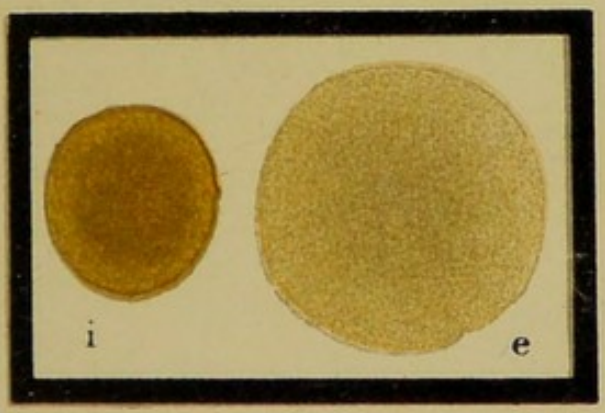


III.

V.



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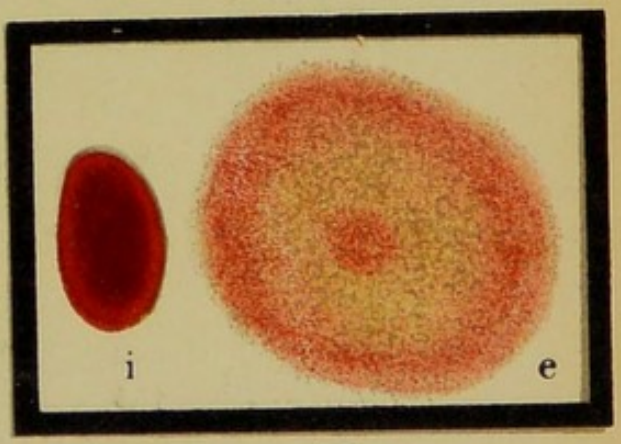
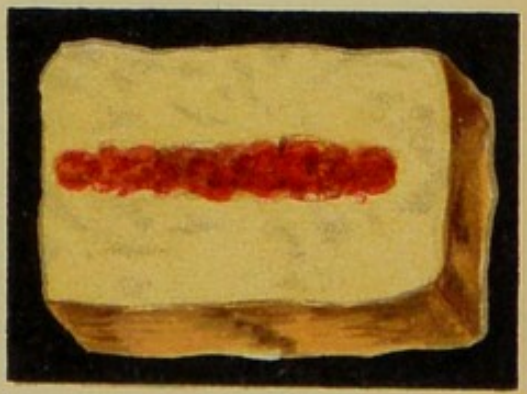


VIII.

VI.

IX.



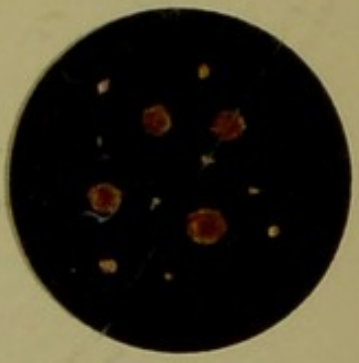
X.

I.

II.

III.

V.

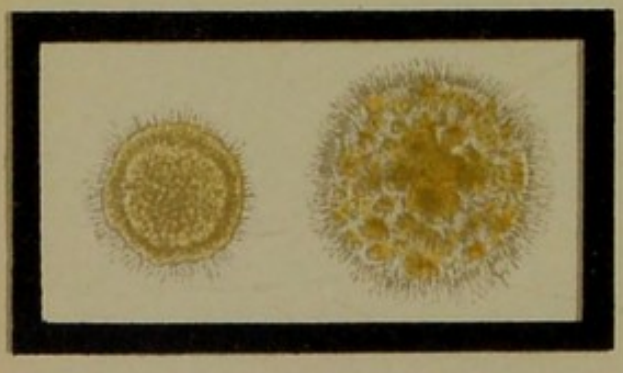


VI.

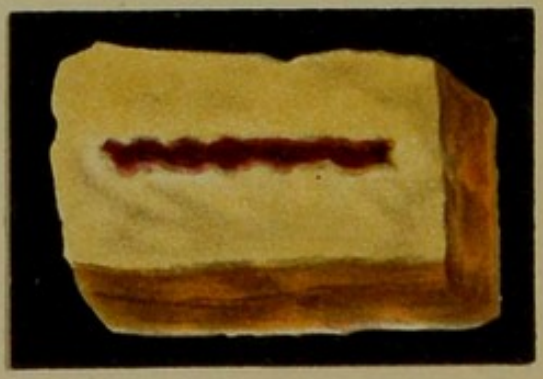
VII.

IV.

IX.



VIII.



X.

Explanation of Plate 27.

BACTERIUM JANTHINUM. Zopf.

I. Gelatin stick culture, ten days at ordinary temperature.
II. Agar streak culture, six days at ordinary temperature. The white borders at the sides also become violet after prolonged standing.
III. Agar stick culture, seven days at ordinary temperature. Puncture canal.
IV. Agar stick culture, seven days at ordinary temperature. Surface.
V. Agar plate culture (×60), four days at ordinary temperature. Superficial and deep colony. Within the former the original colony is still visible.
VI. Agar plate culture, eight days at ordinary tem perature. Natural size. The colonies often take on a dark violet color.
VII. Gelatin plate culture, six days at ordinary temperature. Natural size. The blue zones are not always so deeply colored.
VIII. Gelatin plate culture, six days at ordinary temperature. ×60. The smaller colony is near the surface, the larger one upon the surface.
IX. Microscopical preparation, from a five days' agar culture. ×700.
X. Potato culture, six days at ordinary temperature.
XI. Bacteria with flagella. ×1,000. Löffler's stain.
XII. Bacteria with flagella, from a culture obtained from Sweden, ×1,000.

XI.

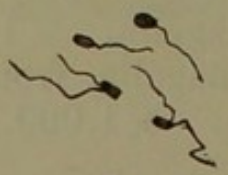
XII.

Explanation of Plate 28.

BACTERIUM FLUORESCENS. Flügge.

(Bacillus fluorescens liquefaciens. Flügge.)

I. Gelatin stick culture, two days at 22°.
II. Gelatin stick culture, eight days at 22°.
III. Agar streak culture, three days at 22°.
IV. Agar stick culture, four days at 22°.
V. Gelatin plate, two days at 22°. Part of a superficial colony. ×90.
VI. Agar plate, twenty-four hours at 22°. ×60. *e*, superficial; *i*, deep-seated colony.
VII. Gelatin plate, three days at 22°. Natural size.
VIII. Microscopical preparation. Pure culture from agar plate. ×800.
IX. Potato culture, four days at 22°. Natural size. *Vide* Pl. 22, V.; Pl. 14, IX.
X. Bacteria with flagella, usually one, more rarely two or more. ×1,000. Löffler's stain.



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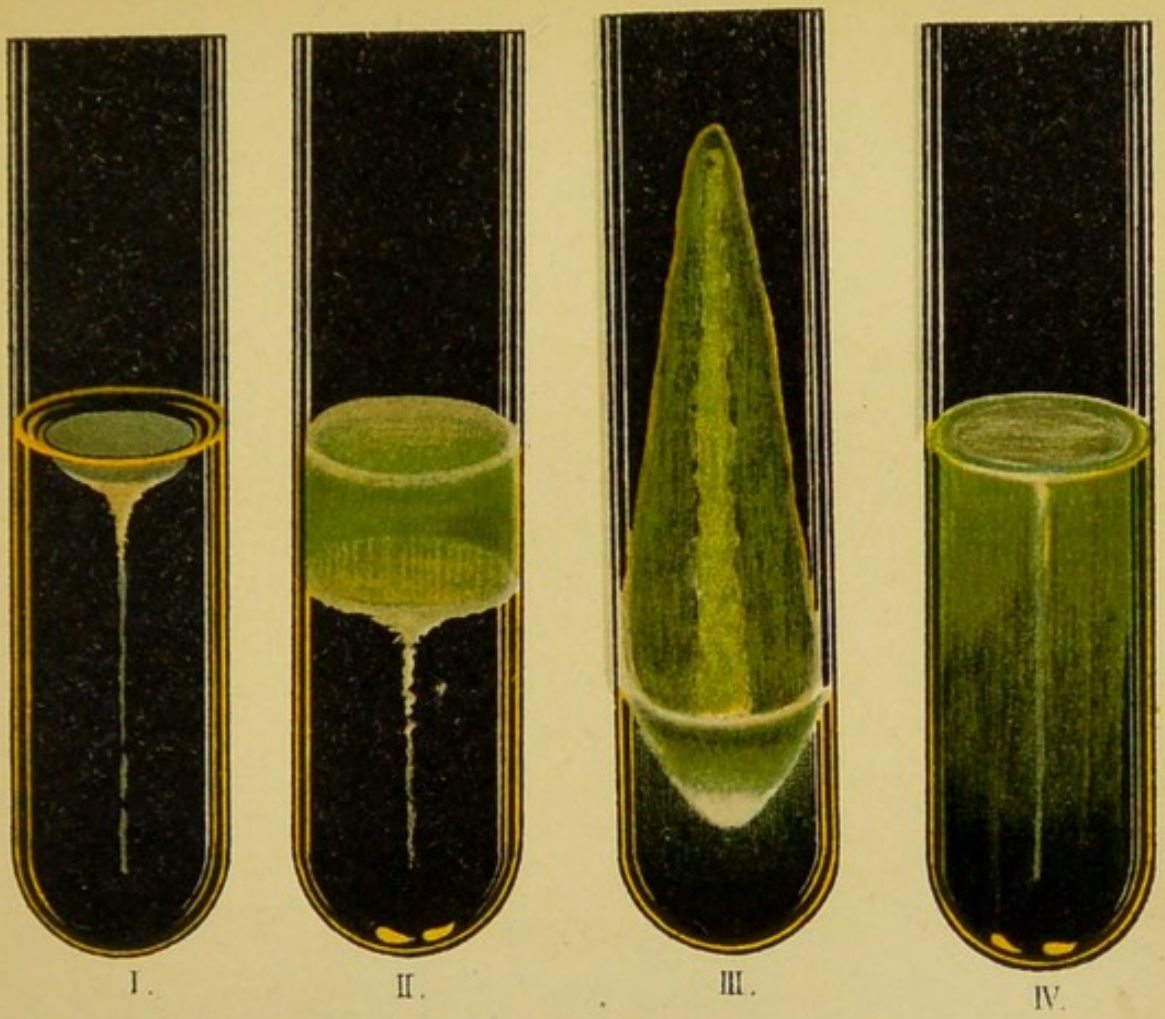
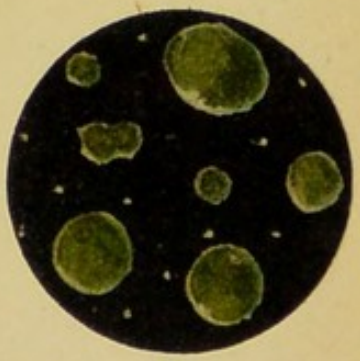


I. II. III. IV.

V

VI.



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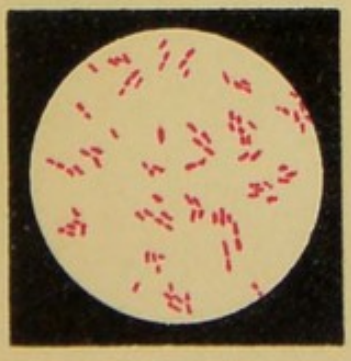
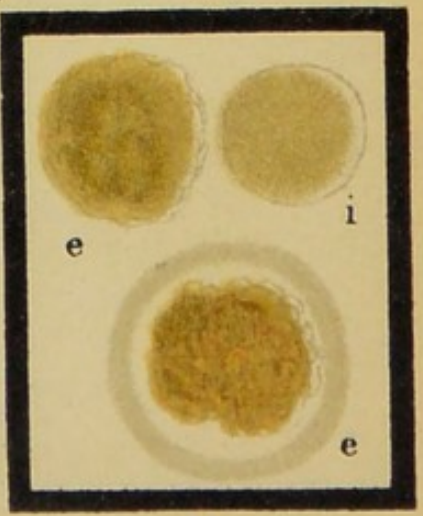


VIII.

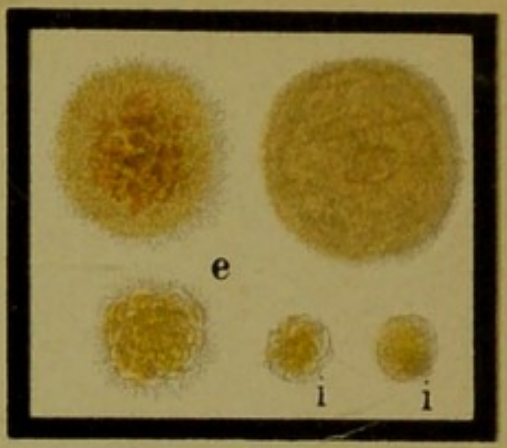
IX.



X.

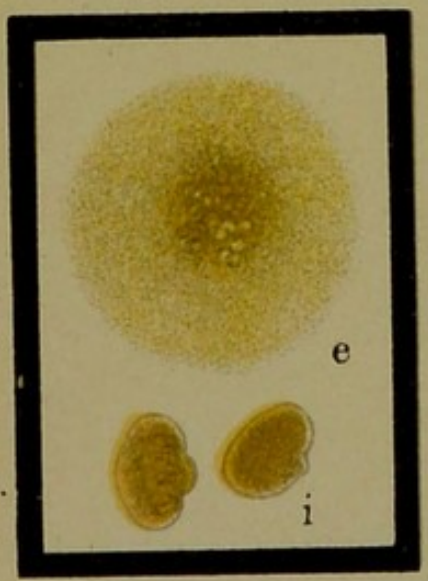
I.

II.



III.

IV.



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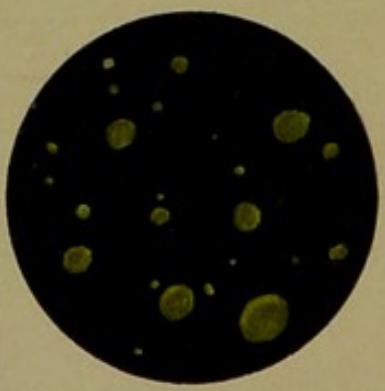

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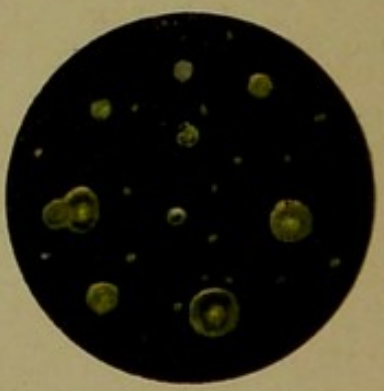

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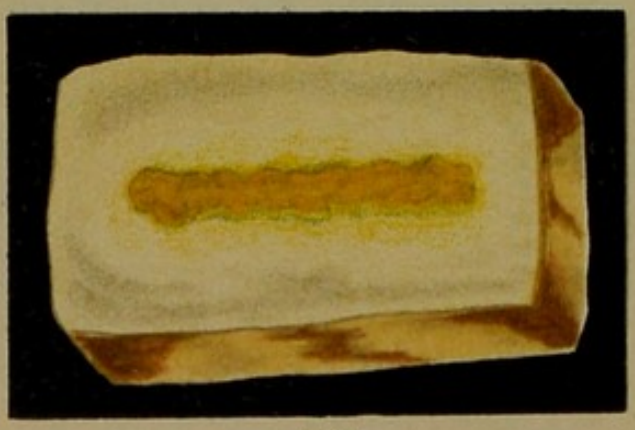

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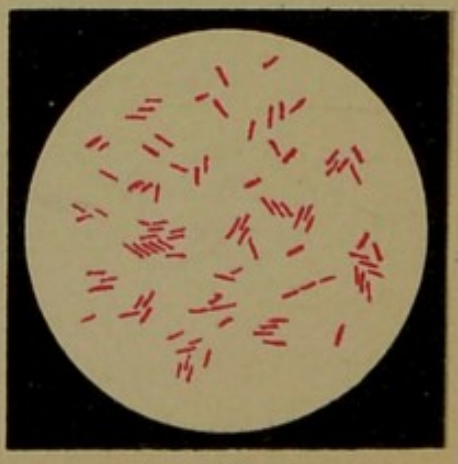


IX.

Explanation of Plate 29.

BACTERIUM PYOCYANEUM. (Flügge) Lehm. and Neum.

(Green pus.)

I. Gelatin stick culture, three days at 22°.
II. Agar streak culture, two days at 37°.
III. Gelatin plate, two days at 22°. ×60. Deep-seated colonies and some immediately beneath the surface, in younger and older stages.
IV. Gelatin plate, five days at 22°. ×60. Part of a superficial colony.
V. Gelatin plate, two days at 22°. Natural size.
VI. Agar plate, two days at 37°. Natural size.
VII. Agar plate, two days at 37°. ×60. Below, deep-seated; above, superficial colonies.
VIII. Potato culture, three days at 37°. Natural size.
IX. Microscopical preparation. Pure culture from agar plate. ×800.
X. Bacteria with one, more rarely two polar flagella. ×1,000. Löffler's stain.

X.

Explanation of Plate 30.

BACTERIUM ZOPFII. Kurth.

I. Gelatin stick culture, six days at 22°.

II. Gelatin streak culture, thirty-six hours at 37°. In reality of a gray transparent color.

III. Agar stick culture, six days at 22°. Puncture.

IV. Agar stick culture, six days at 22°. Surface.

V. Gelatin plate, seven days at 22°. Natural size.

VI. Gelatin plate, thirty-six hours at 22°. Natural size.

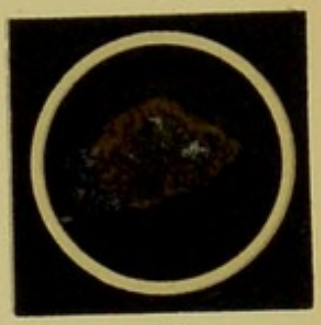
VII. Gelatin plate, twenty-four hours at 22°. ×90. Thread-like part of the colony. Deeply situated.

VIII. Gelatin plate, twenty-four hours at 22°. ×60. Superficial colony. *Vide* Pl. 32, VIII.; Pl. 33, VII.

I.

II.

III.



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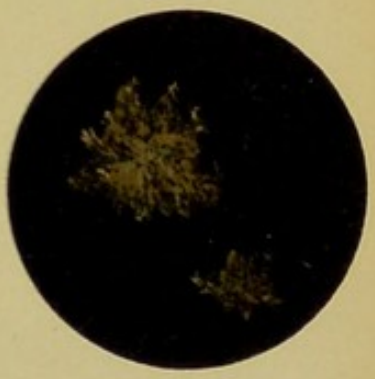
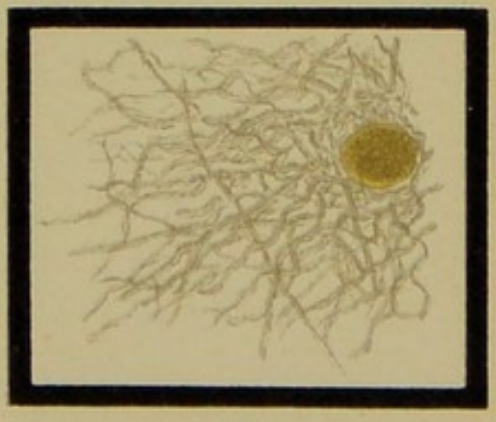


V

VI.

VIII.



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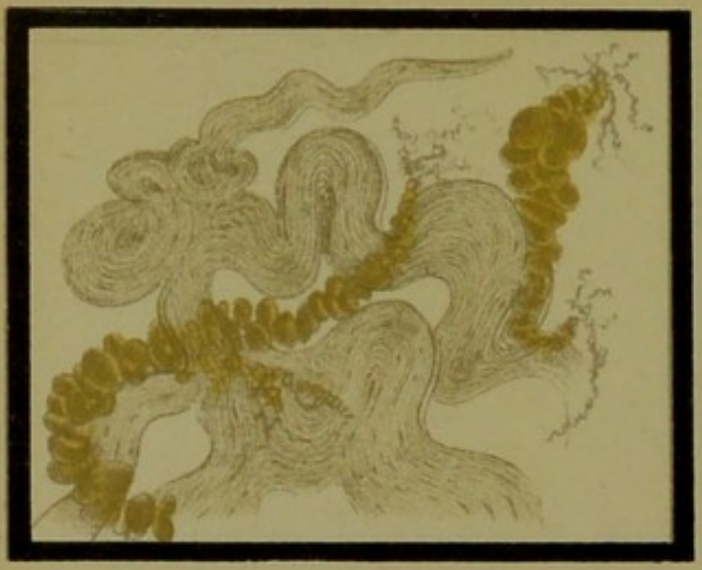
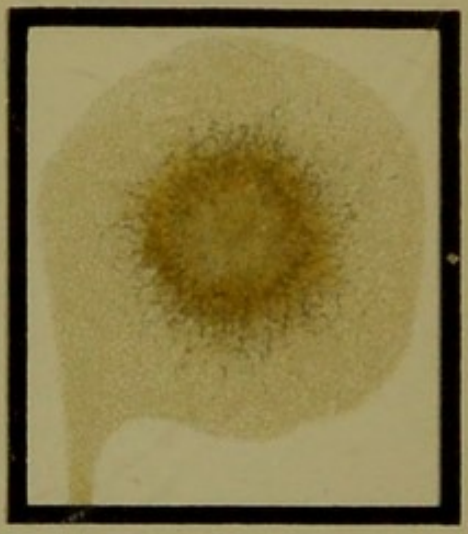


I.

II.



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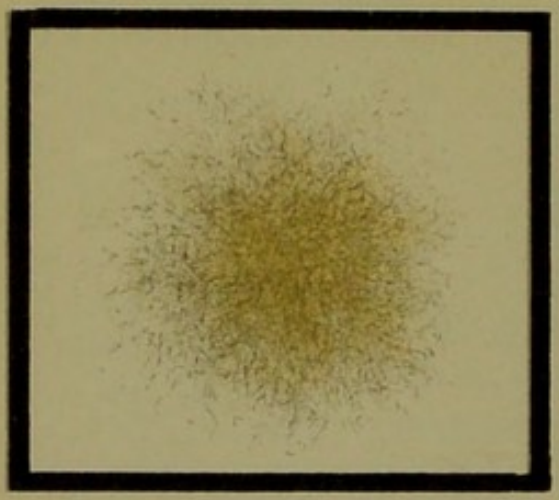

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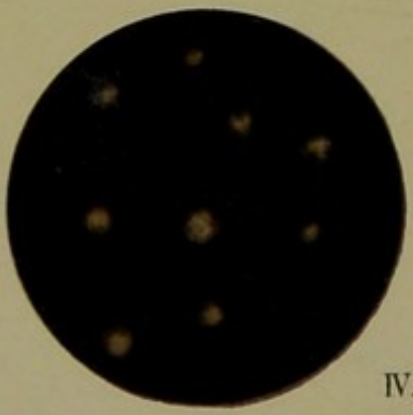

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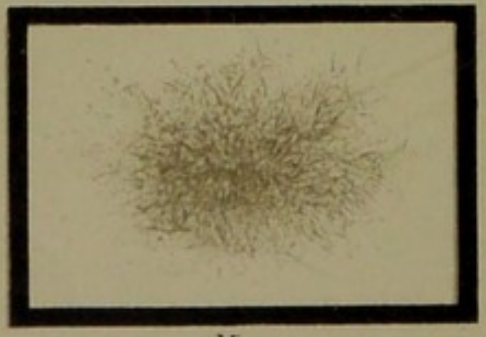

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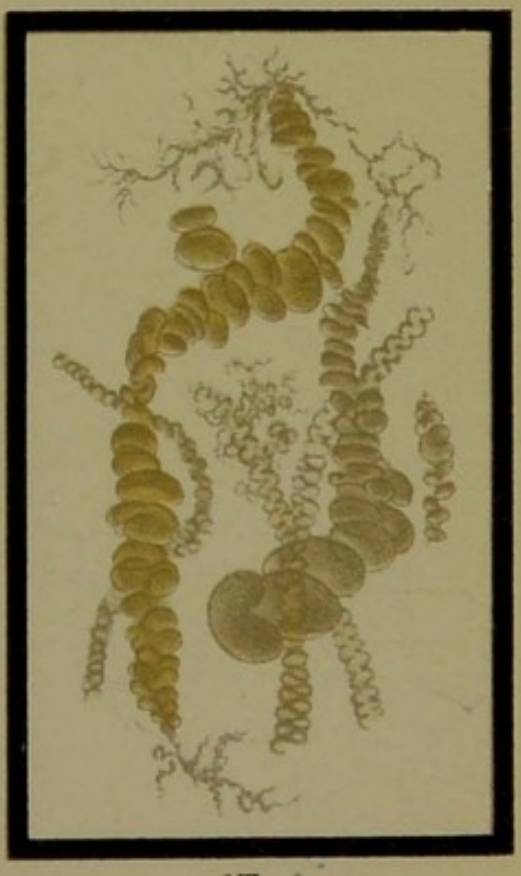


VII.

Explanation of Plate 31.

BACTERIUM ZOPFII. Kurth.

I. Gelatin plate, eight days at 22°. ×90. Border of a colony.

II. Microscopical preparation. ×1,000. Pure culture from agar plate. Stained with fuchsin.

III. Agar plate, twenty-four hours at 37°. ×60. Superficial colony surrounded by innumerable bacteria which have wandered away.

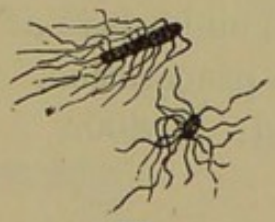
IV. Agar plate, twenty-four hours at 37°. Natural size.

V. Agar plate, twelve hours at 37°. Deep-seated and superficial colony.

VI. Agar plate, four days at 22°. Deep-seated colony.

VII. Gelatin plate, eight days at 22°. Sausage-shaped forms of a deep-seated colony.

VII. Bacteria with numerous flagella. ×1,000. Löffler's stain.



IX.

Explanation of Plate 32.

BACTERIUM VULGARE β MIRABILIS. (Hauser) Lehm. and Neum.

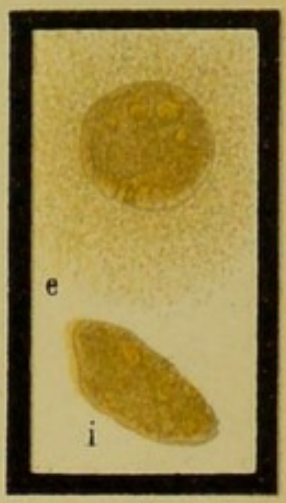
(Proteus mirabilis Hauser.)

I. Agar stick culture, two days at 22°. Puncture canal.
II. Agar stick culture, two days at 22°. Surface.
III. Gelatin stick culture, six days at 22°.
IV. Agar streak culture, two days at 22°.
V. Agar plate, seven days at 22°. Natural size.
VI. Agar plate, seven days at 22°. ×60. Above, superficial; below, deep-seated colony.
VII. Gelatin plate, two days at ×22°. 60. Deep-seated colonies.
VIII. Gelatin plate, two days at 22°. ×60. Superficial colony.
IX. Potato culture, eight days at 22°. Natural size.
X. Microscopical preparation. Pure agar culture, two days old. ×800.

I.

III.

IV.

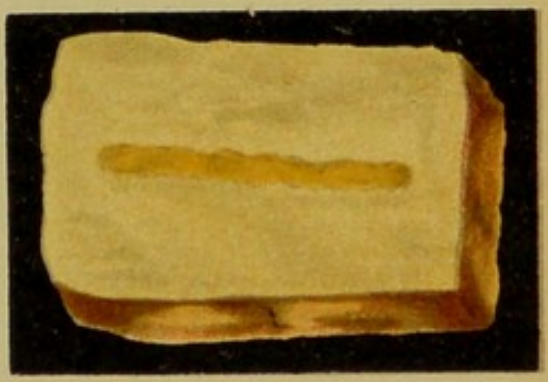


VI.

V.

II

VII.



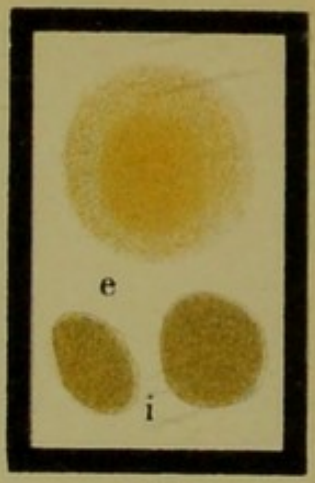
IX.

VIII.

X.

I.

II



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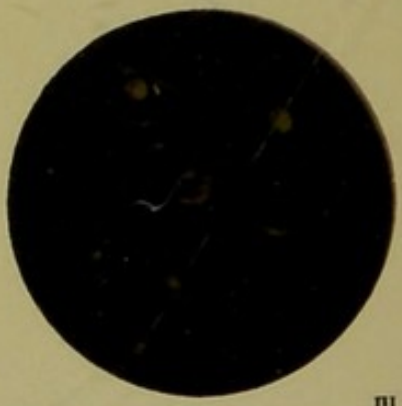
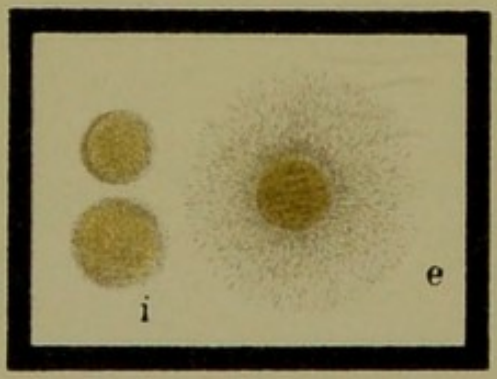


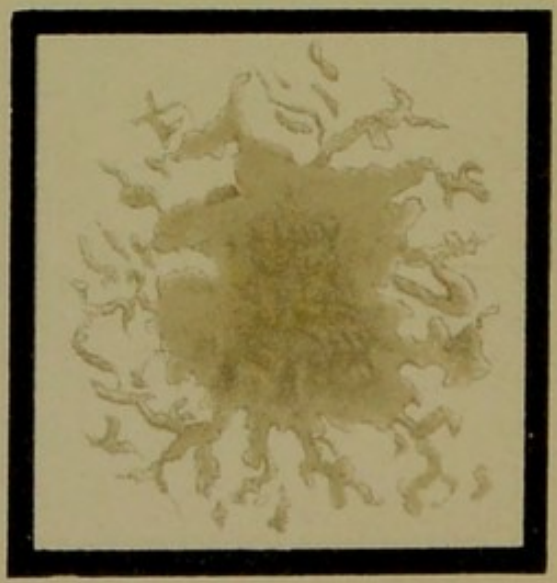
III.

V.



VI.

VIII.



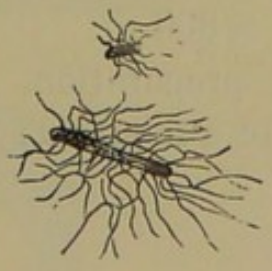
VII

Explanation of Plate 33.

BACTERIUM VULGARE Lehm. and Neum.

(Proteus vulgaris Hauser.)

I. Gelatin stick culture, twenty-four hours at 22°.
II. Agar streak culture, thirty-six hours at 22°.
III. Agar plate, thirty-six hours at 22°. Natural size.
IV. Agar plate, four days at 22°. ×60. Above, superficial; below, deep-seated culture.
V. Gelatin plate, thirty-six hours at 22°. Natural size.
VI. Gelatin plate, thirty-six hours at 22°. ×60. Right side, superficial; left side, deep-seated colonies. The lower one, emerging on the surface, is beginning to liquefy.
VII. Gelatin plate, three days at 22°. ×60. Deep-seated colony. Zoöglœa form, like bacterium Zopfii.
VIII. Microscopical preparation. ×800. Pure agar culture. Fuchsin stain.
IX. Bacteria with numerous flagella. ×1,000.



IX.

Explanation of Plate 34.

BACTERIUM ERYSIPELATOS SUUM. Migula.

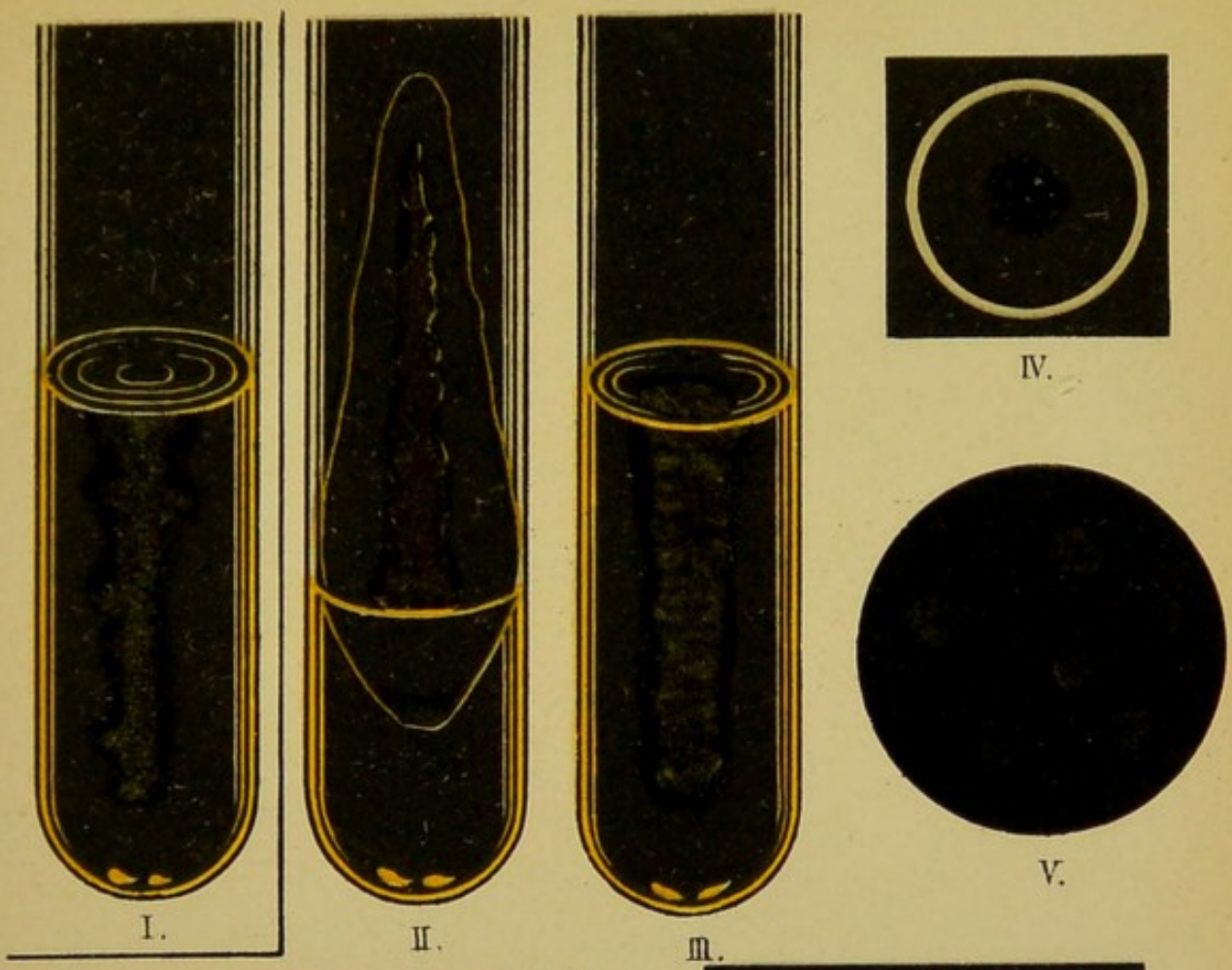
(Hog erysipelas.)

I. Gelatin stick culture, five days at 22°.

BACTERIUM MURISEPTICUM. Migula.

(Mouse septicæmia.)

II. Agar streak culture, four days at 22°.
III. Gelatin stick culture, four days at 22°.
IV. Agar stick culture, four days at 22°. Surface.
V. Gelatin plate, four days at 22°. Natural size.
VI. Gelatin plate, four days at 22°. ×60. Superficial colony.
VII. Agar plate, four days at 22°. ×60. Right side, superficial; left side, deep-seated colony.
VIII. Microscopical preparation. Pure agar culture, two days. ×800.
IX. Microscopical preparation. Smear preparation from blood of a mouse's spleen. ×800.



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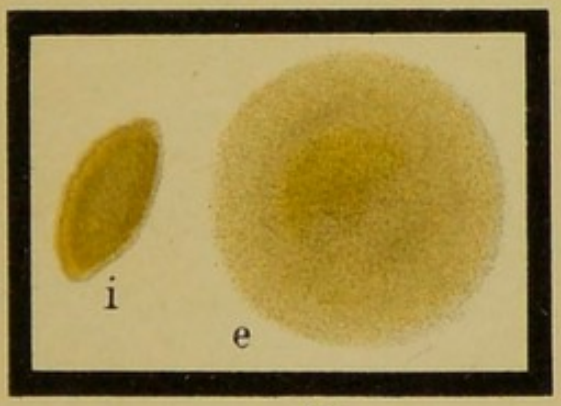

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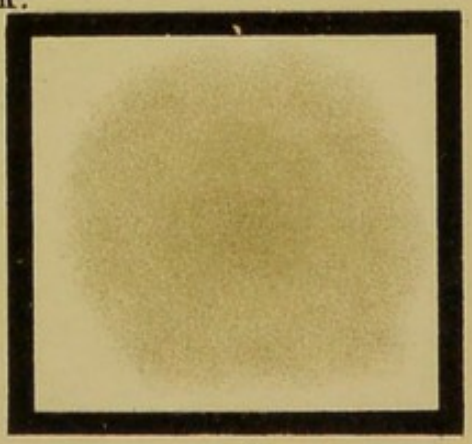
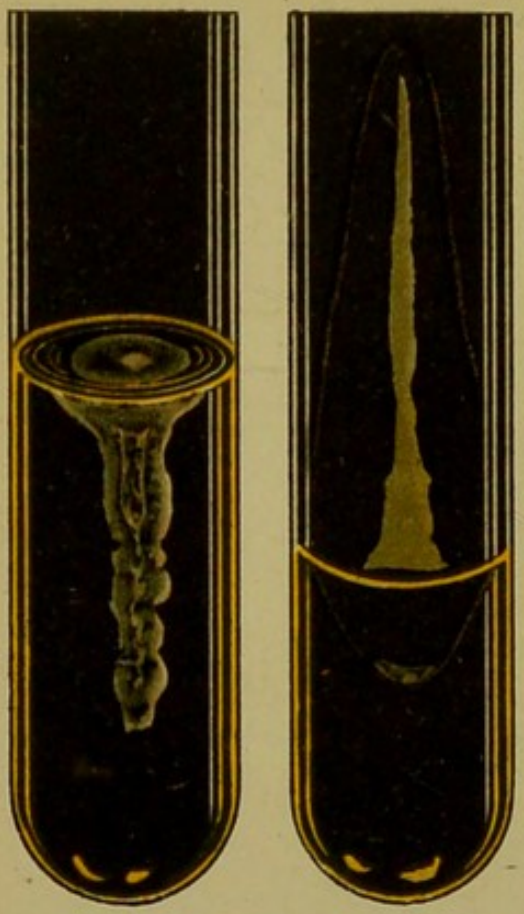


VI.

VIII.

IX.



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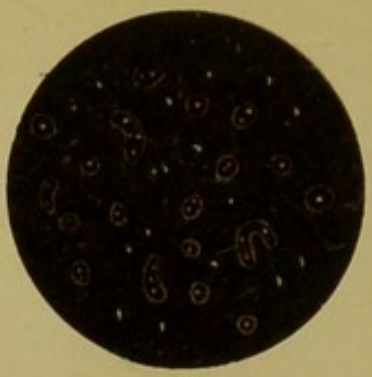

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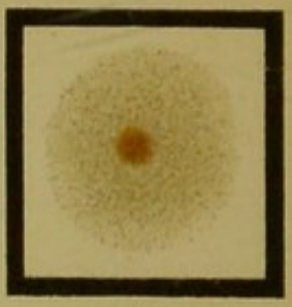

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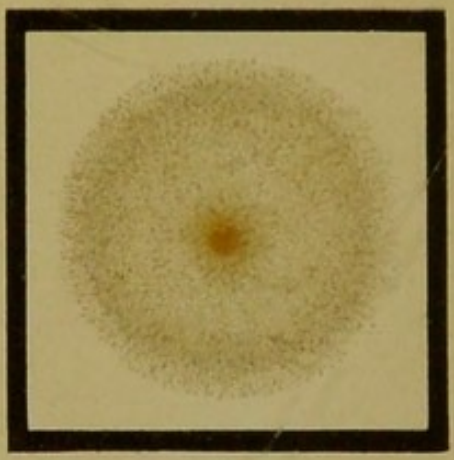

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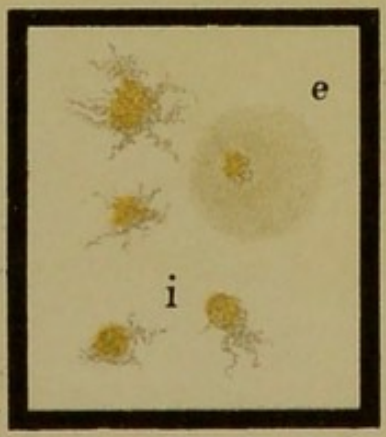

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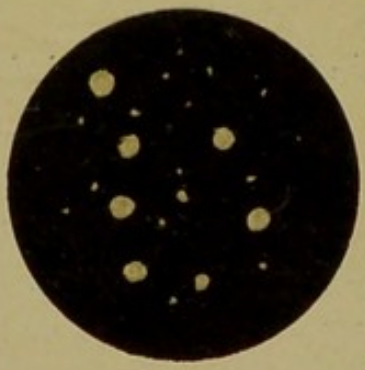

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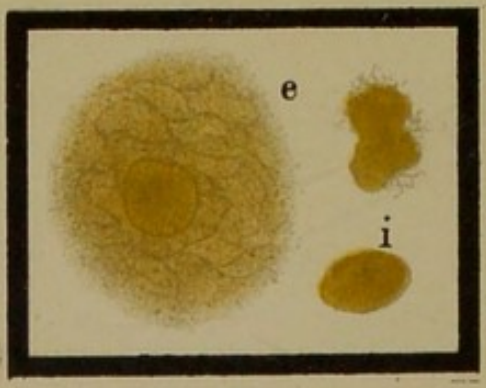

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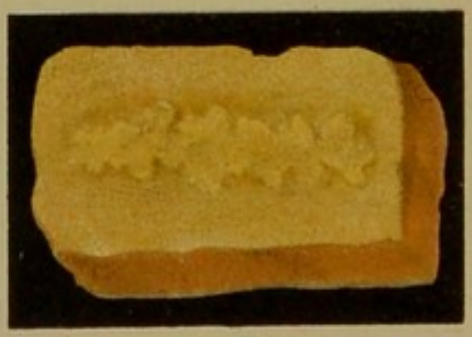


IX.

Explanation of Plate 35.

BACILLUS MEGATHERIUM. De Bary.

I. Gelatin stick culture, twenty-four hours at 22°.
II. Agar streak culture, three days at 22°.
III. Gelatin plate, thirty-six hours at 22°. Natural size.
IV. Gelatin plate, thirty-six hours at 22°. ×60. Deep-seated colony.
V. Gelatin plate, thirty-six hours at 22°. ×60. Superficial colony.
VI. Agar plate, four days at 22°. Natural size.
VII. Agar plate, one day at 22°. ×60. Right side, superficial; left side, deep-seated colony.
VIII. Agar plate, four days at 22°. ×60. Right side, deep-seated; left side, superficial colony.
IX. Potato culture, five days at 22°. Natural size.
X. Microscopical preparation. Pure agar culture. ×800.
XI. Bacilli with numerous flagella. ×1,000. Löffler's stain.

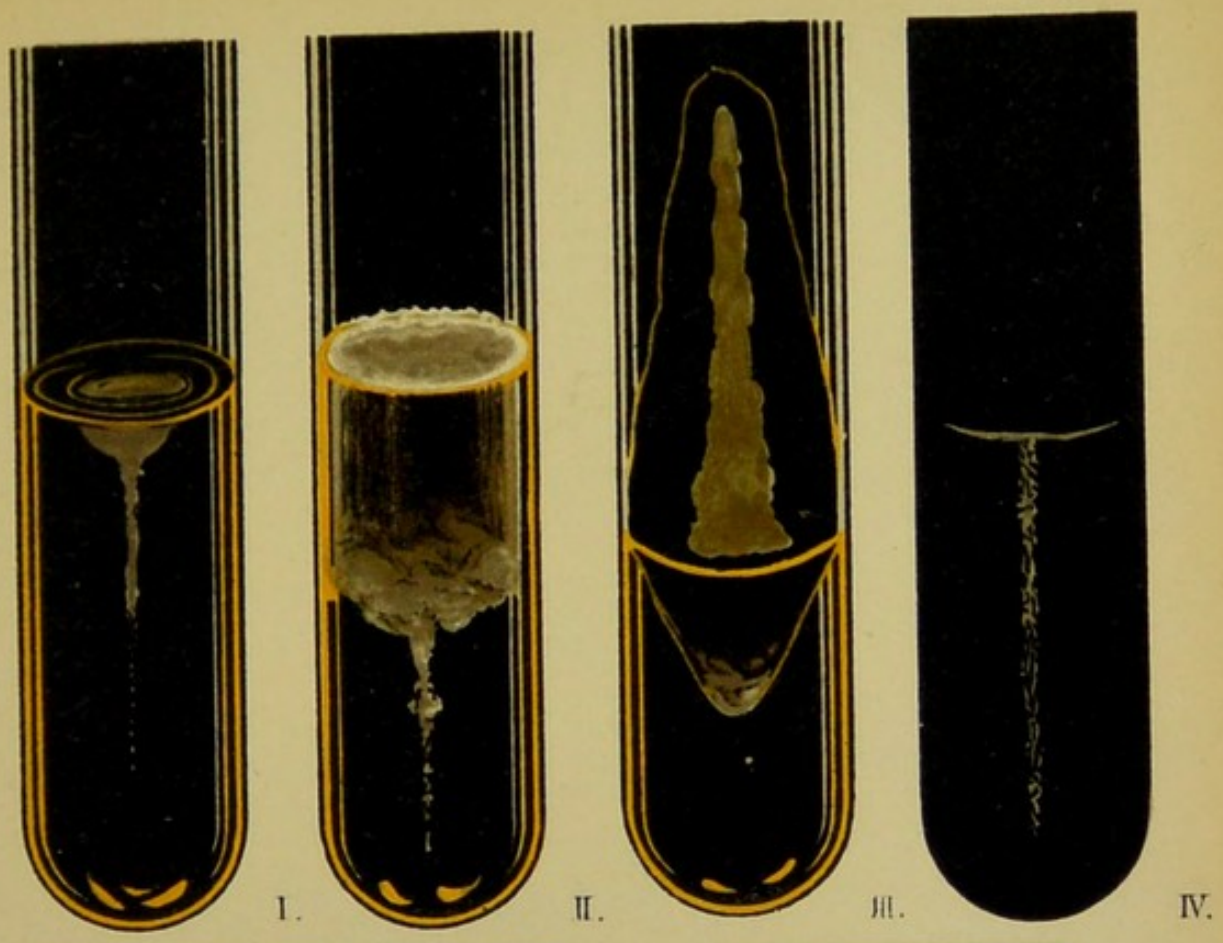
XI

Explanation of Plate 36.

BACILLUS SUBTILIS. F. Cohn.

(Hay bacillus.)

I. Gelatin stick culture, thirty-six hours at 22°.
II. Gelatin stick culture, eight days at 22°.
III. Agar streak culture, two days at 37°.
IV. Agar stick culture, two days at 37°. Puncture canal.
V. Agar stick culture, two days at 37°. Surface.
VI. Agar plate, twelve hours at 37°. ×60. Superficial colony.
VII. Agar plate, twelve hours at 37°, ×60. Deep-seated colony.
VIII. Agar plate, twelve hours at 37°. Natural size.



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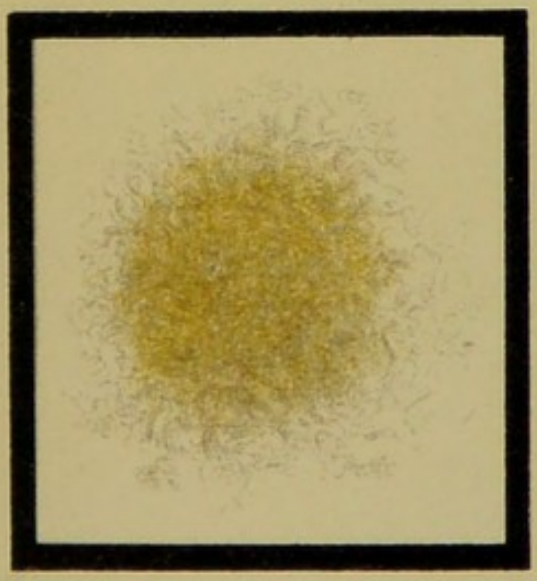

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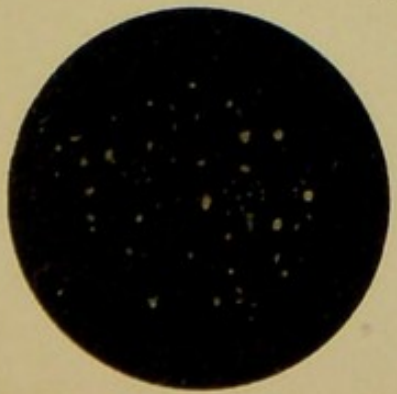
V.



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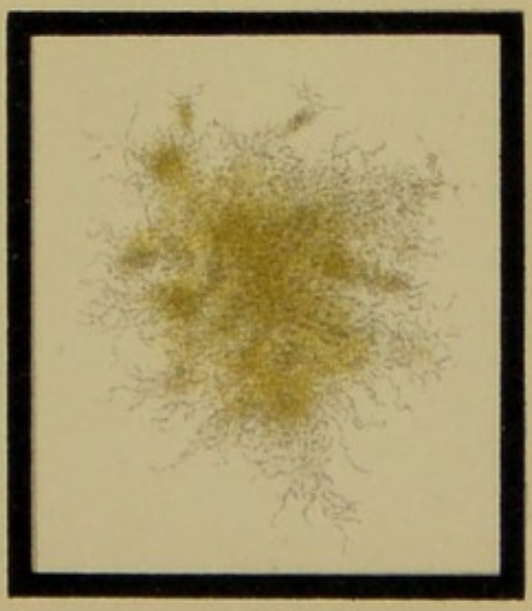

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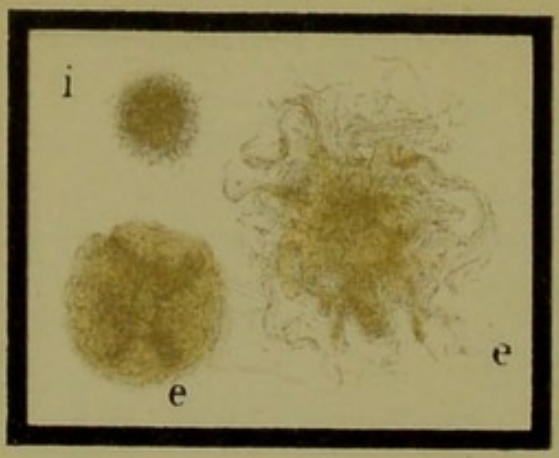

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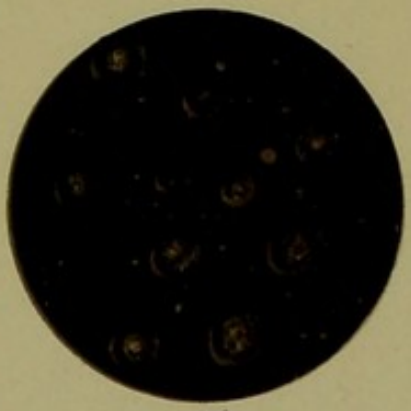
I.



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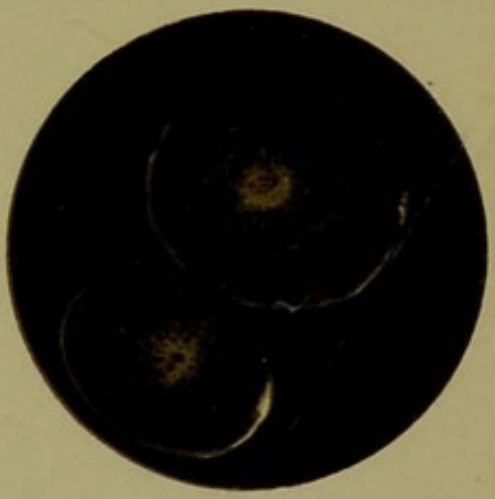

IV.

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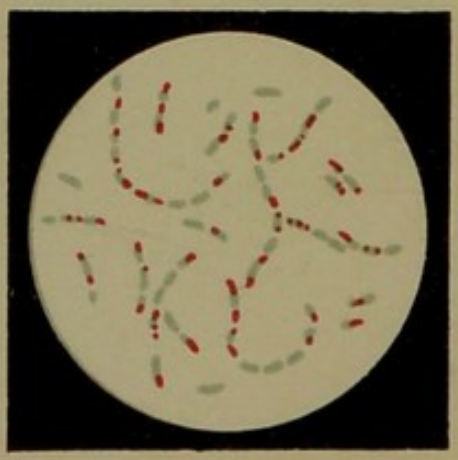


VIII.

Explanation of Plate 37.

BACILLUS SUBTILIS. F. Cohn.

(Hay bacillus.)

I. Potato culture, seven days at 22°.

II. Gelatin plate, two days at 22°. ×60. Above, on the right side, a deep-seated colony. Below it, a colony lies directly at the surface. On the left a superficial colony.

III. Gelatin plate, two days at 22°. Natural size.

IV. Gelatin plate, two days at 22°. ×10.

V. Microscopical preparation (×1,000) from an agar colony three hours old at 37°. Stained with fuchsin.

VI. Microscopical preparation. Bacilli with flagella. (After Fischer.) Very highly magnified.

VII. Microscopical preparation (×1,000) from an agar colony ten days old at 22°. Contains spores. Unstained.

VIII. Microscopical preparation (×700) from an agar colony ten days old at 22°. Double stain with carbolized fuchsin and methyl blue.

IX. Bacilli with numerous flagella. ×1,000. Löffler's stain.

IX.

Explanation of Plate 38.

Bacillus anthracis. F. Cohn and R. Koch.

(Anthrax.)

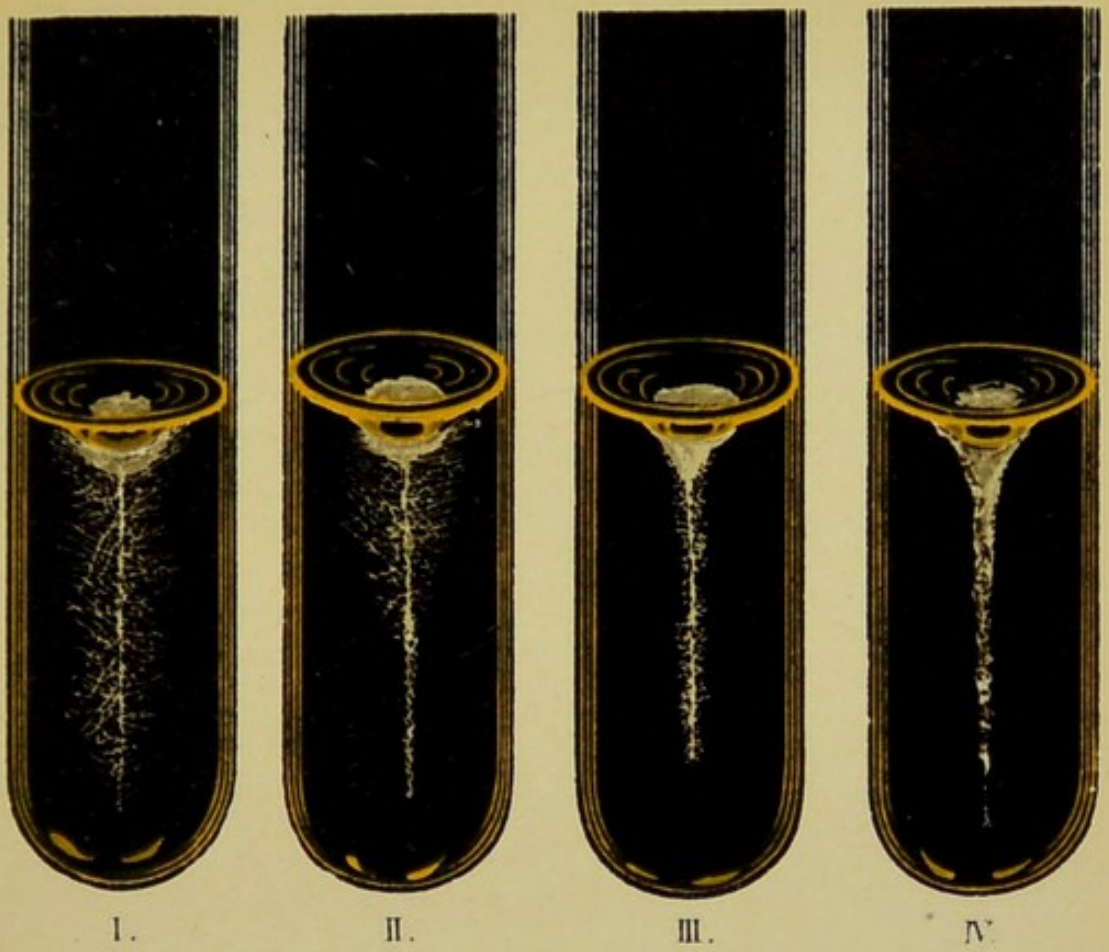
I.–V. Gelatin stick cultures, three days at 22°. Figs. I. and II. typical, the others atypical.

VI. Agar streak culture, two days at 22°.

VII. Agar stick culture, five days at 22°. Puncture canal.

VIII. Agar stick culture, five days at 22°. Surface. Atypical.

IX. Agar stick culture, five days at 22°. Surface. Typical; often has a homogeneous whitish-gray color.



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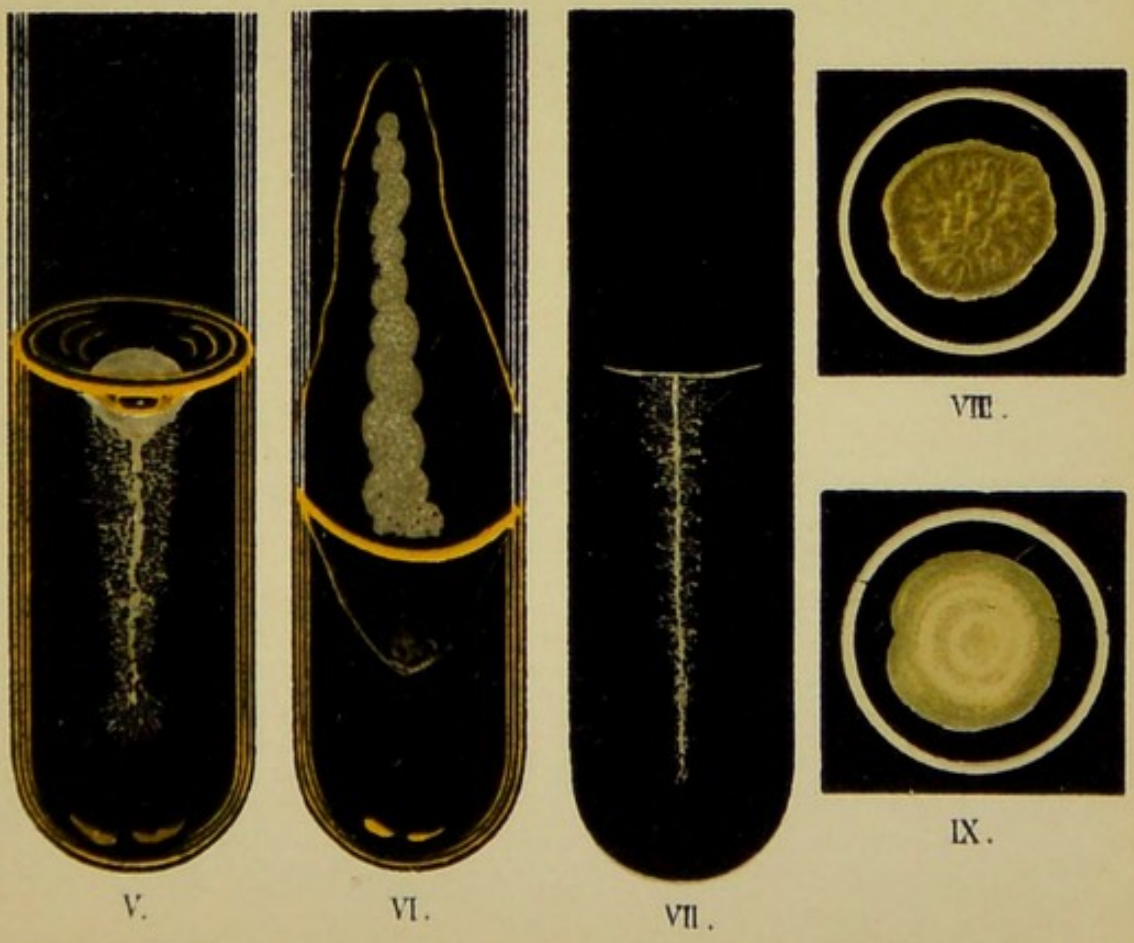
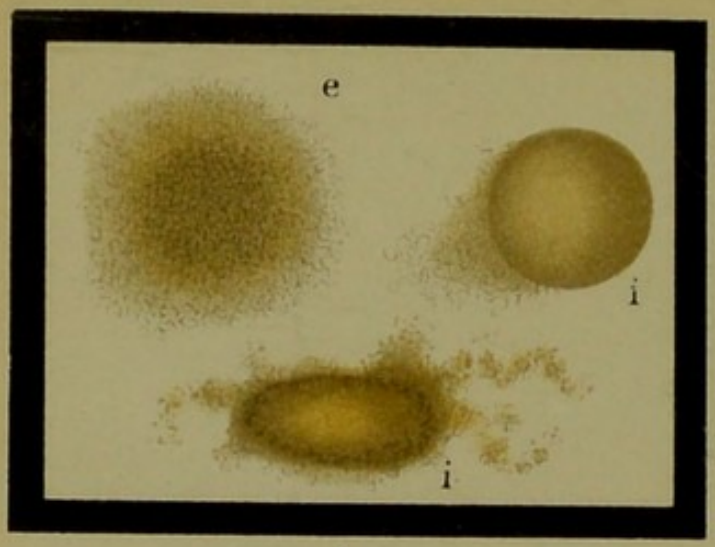


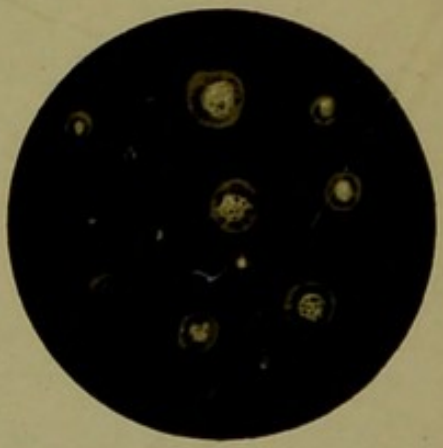
V. VI. VII. VIII. IX.



I.

II.

III.



V.

VI.

IV.

VII.

Explanation of Plate 39.

Bacillus anthracis. F. Cohn and R. Koch.

(Anthrax.)

I. Agar plate, four days at 22°. ×60. On left side, superficial colony; on right side, one lying directly below the surface. Below, a deep-seated colony.

II. Agar plate, four days at 22°. Natural size.

III. Agar plate, thirty-six hours at 37°. ×150. Border of a streak culture. Superficial colony.

IV. Agar plate, thirty-six hours at 37°. ×150. Deep-seated colony.

V. Gelatin plate, three days at 22°. Natural size.

VI. Gelatin plate, three days at 22°. ×60. Superficial colony, about to sink.

VII. Potato culture, six days at 22°. Natural size.

Explanation of Plate 40.

Bacillus anthracis. F. Cohn and R. Koch.

(Anthrax.)

I. Smear preparation from the blood of a mouse's spleen. ×1,000.

II. Impression preparation from agar plate culture, one day at 22°. ×1,000.

III. Unstained preparation in hanging drop from bouillon culture, thirty-six hours at 37°. Spores beginning to drop out. ×1,000.

IV. Anthrax threads from agar, thirty-six hours at 37°. Stained with Ziehl's solution. Spores red, bacilli blue. ×1,000.

V. Involution forms, five weeks old, from agar. Stick culture stained with fuchsin. ×1,000.

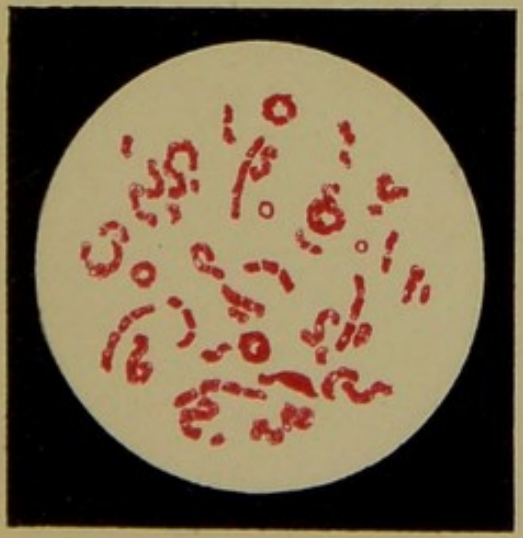
VI. Unstained preparation in hanging drop from bouillon culture, eight hours at 37°. Beginning of sporulation. ×1,000.

I.

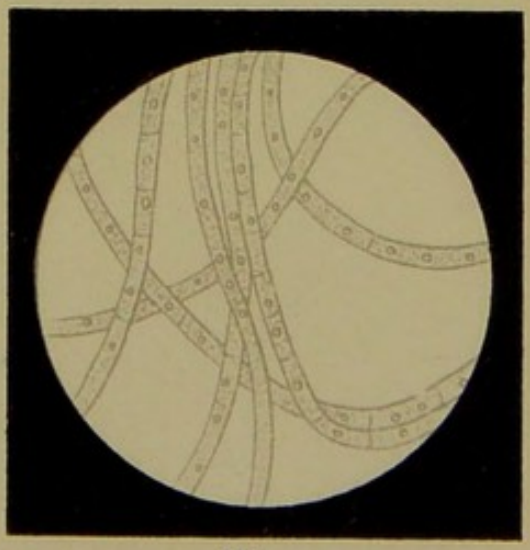
II.

III.

IV.



V.



VI.

I.

II.

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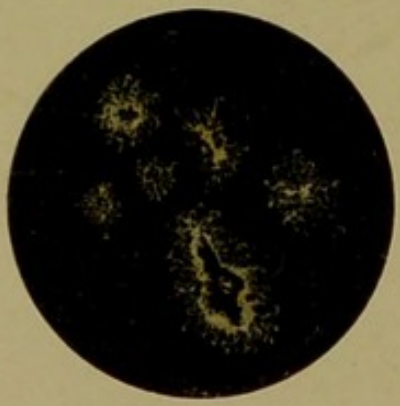
VI.



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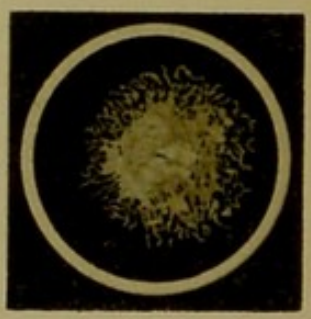


V.

IX.

VIII.

Explanation of Plate 41.

BACILLUS MYCOIDES. Flügge.

(Root bacillus.)

I. Gelatin stick culture, four days at 22°.
II. Gelatin stick culture, fourteen days at 22°.
III. Agar streak culture, two days at 22°.
IV. Agar stick culture, eight days at 22°. Puncture canal.
V. Agar stick culture, eight days at 22°. Surface.
VI. Gelatin plate, one day at 22°. Natural size.
VII. Agar plate, one day at 22°. Natural size.
VIII. Agar plate, four days at 22°. Natural size.
IX. Gelatin plate, four days at 22°. Natural size. The colony is about to sink.

Explanation of Plate 42.

BACILLUS MYCOIDES. Flügge.

(Root bacillus.)

I. Agar plate, one day at 22°. ×20. Superficial and deep colony.
II. Potato culture, seven days at 22°. Natural size.
III. Microscopical preparation. Pure agar culture, twenty-four hours. Fuchsin stain. ×1,000. A few bacilli show spores.
IV. Agar plate, one day at 22°. ×100. Superficial and deep colony.

BACILLUS BUTYRICUS. Hüppe.

(Butyric-acid bacillus.)

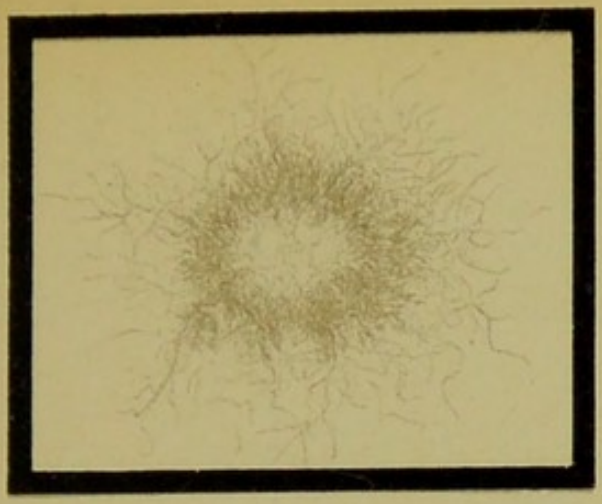
V. Potato culture, three days at 22°.
VI. Gelatin plate, one day at 22°. ×60. Above, superficial; below, deep colony.
VII. Gelatin plate, one and a half days at 22°. ×60. Part of a superficial colony.
VII. *a*. Flagella preparation. ×1,000. Löffler's stain.

BACILLUS VULGATUS. Migula.

(Bacillus mesentericus vulgatus Flügge. Potato bacillus.)

VIII. Potato culture, five days at 22°.
IX. Potato culture, five days at 22°. Natural size. Both forms of growth occur.

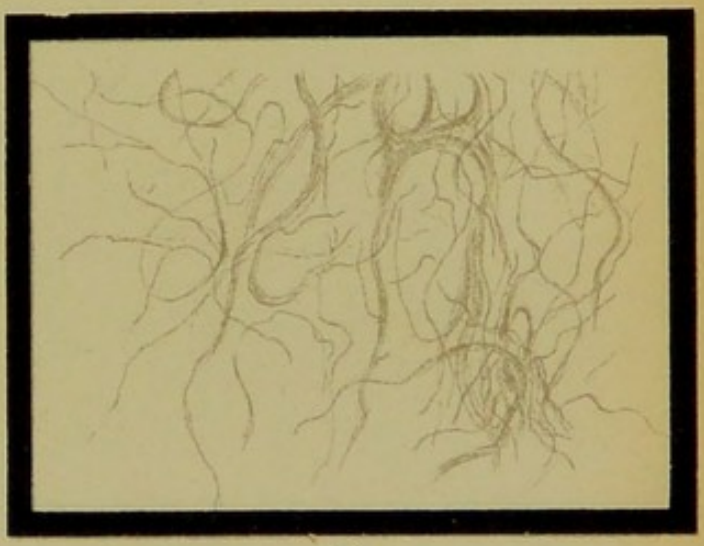
VII. *a*.



I.

II

III.



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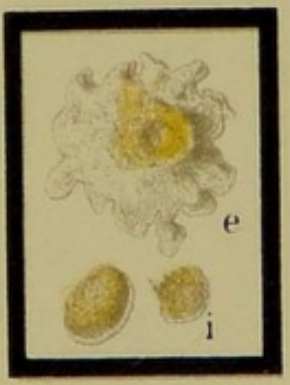
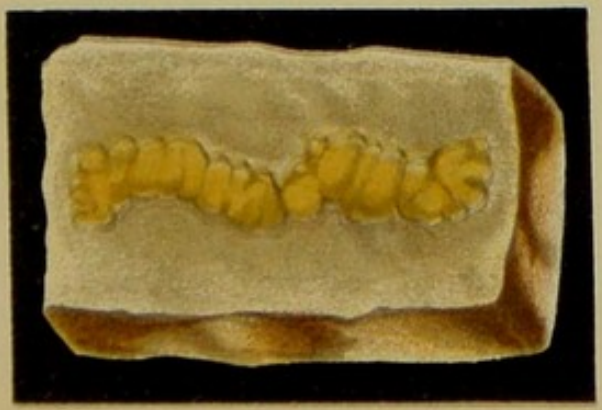
V.



VI.

VII.

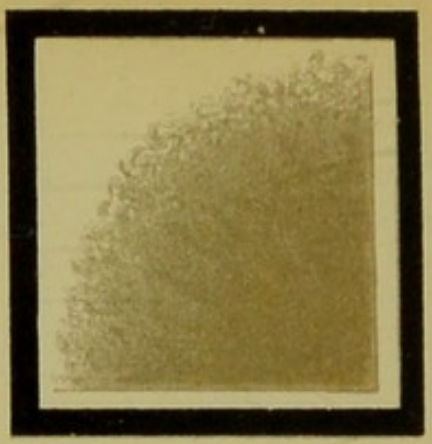


VIII.

IX.

I.

II.



VI.

IV.

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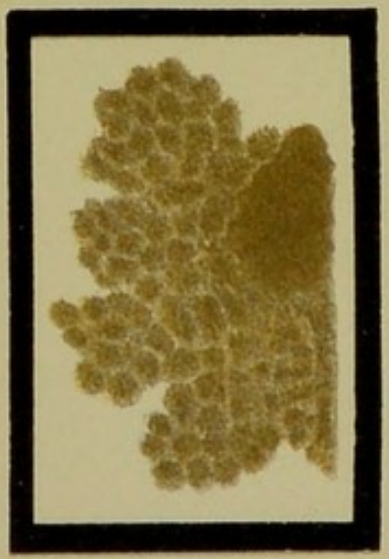
III.



VIII

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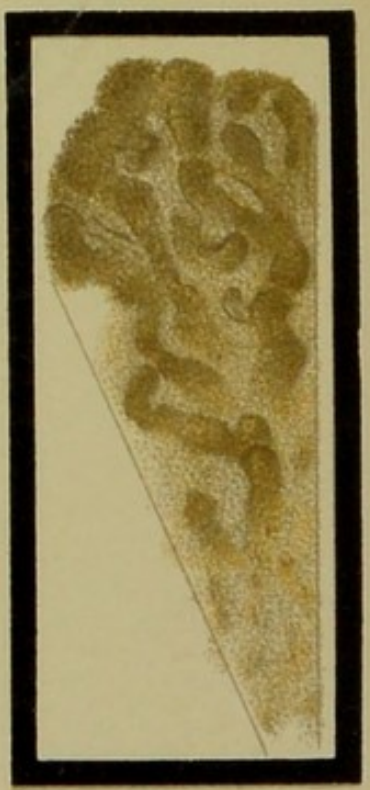

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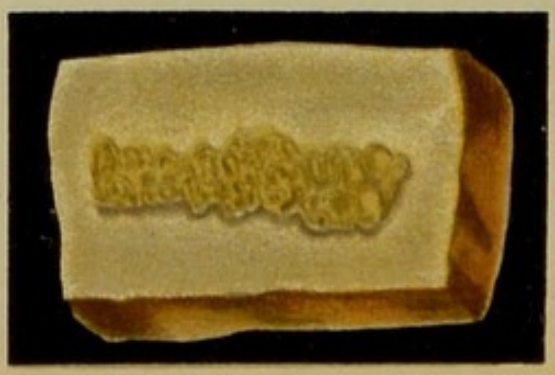


X.

Explanation of Plate 43.

BACILLUS VULGATUS. Migula.

(Bacillus mesentericus vulgatus Flügge. Potato bacillus.)

I. Gelatin stick culture, ten days at 22°.
II. Agar streak culture, ten days at 22°.
III. Agar stick culture, six days at 22°. Surface.
IV. Agar plate, six days at 22°. Natural size.
V. Agar plate, six days at 22°. ×60. Deep colony.
VI. Agar plate, six days at 22°. ×60. Superficial colonies.
VII. Gelatin plate, eight days at 22°. Natural size.
VIII. Gelatin plate eight days at 22°. ×60. Part of a superficial colony.
IX. Gelatin plate, eight days at 22°. ×150. Part of a superficial colony.
X. Potato culture, five days at 22°. Natural size.
XI. Microscopical preparation. Pure culture from agar, one day. ×800. Fuchsin stain.
XII. Bacilli with numerous flagella. ×1,000. Löffler's stain.

XII.

Explanation of Plate 44.

BACILLUS MESENTERICUS. Lehm. and Neum.

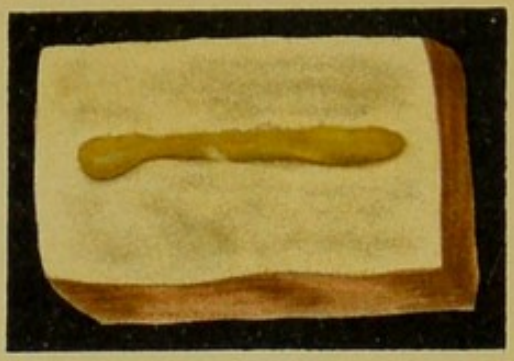
(Bacillus mesentericus fuscus Flügge.)

I. Gelatin stick culture, two days at 22°.
II. Agar streak culture, three days at 22°.
III. Potato culture, one day at 22°. Natural size.
IV. Potato culture, five days at 22°. Natural size.
V. Agar plate, two days at 22°. Natural size.
VI. Agar stick culture, four days at 22°. Surface.
VII. Agar plate, two days at 22°. ×60. Above, superficial colony; below, deep colony.
VIII. Gelatin plate, thirty-six hours at 22°. ×60. Deep colony.
IX. Gelatin plate, thirty-six hours at 22°. ×60. Superficial colony.
X. Gelatin plate, two days at 22°. Natural size.
XI. Gelatin plate, one day at 22°. ×60. Right side, deep colony; left side, superficial.
XII. Microscopical preparation. Pure culture from agar, two days. ×800. Fuchsin stain. A few bacilli with spores.
XIII. Bacilli with numerous flagella. ×1,000. Löffler's stain.

XIII.

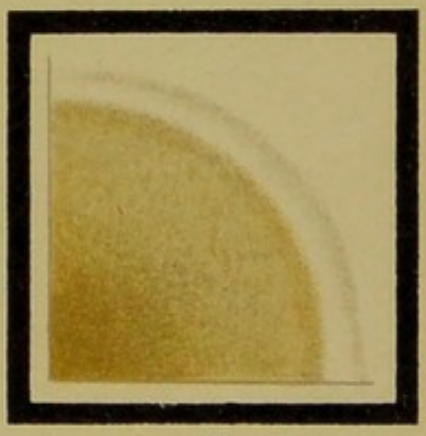
I.

II.



III.

IV.



IX.

VIII.

V.

VI.

X.

VII.

XII.

XI.

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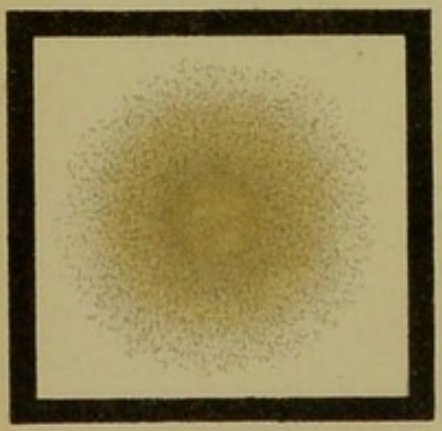
III.

IV.



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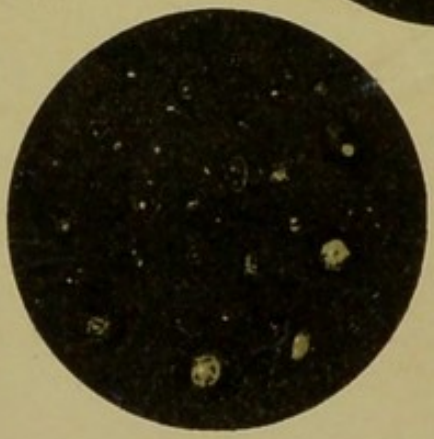

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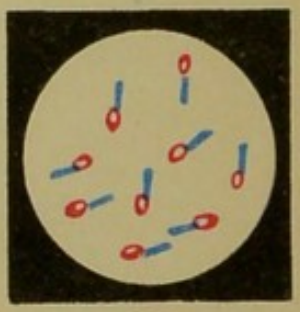


VII.

VIII.

IX.

X

Lith Anst v F Reichhold München

Explanation of Plate 45.

BACILLUS TETANI. Nicolaier.

(Tetanus bacillus.)

I. Sugar-agar stick culture, three days at 37°.

II. Sugar-gelatin stick culture, six days at 22°.

III. Sugar-gelatin plate, four days at 22°. Natural size. Cultivated anaërobic.

IV. Sugar-gelatin plate, four days at 22°. ×60. Superficial and deep colony. Cultivated anaerobic.

V. Sugar-agar plate, four days at 37°. Natural size. Cultivated anaërobic.

VI. Sugar-agar plate, four days at 37°. ×60. Superficial and deep colony. Cultivated anaërobic.

VII. Microscopic preparation. Pure culture from sugar-agar, three days at 37°. ×1,000. Bacilli with spores. Ziehl's double stain.

VIII. Microscopic preparation. Pure culture from sugar-agar, two days at 37°. ×1,000. A few bacilli with spores. Fuchsin stain.

IX. Microscopical preparation. Pure culture from sugar-agar, twenty-four hours at 37°. ×1,000. Extremely long filaments with faintly colored interspaces.

X. Microscopical preparation. Pure culture from sugar-agar, six days at 37°. ×1,000. Fuchsin stain. Long filaments and spore chains with faintly colored interspaces.

Explanation of Plate 46.

BACILLUS CHAUVŒI OF FRENCH WRITERS.

(Symptomatic Anthrax.)

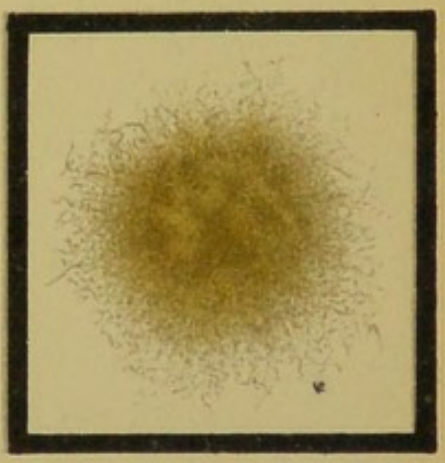
I. Sugar-gelatin stick culture, six days at 22°.
II. Sugar-agar stick culture, three days at 37°.
III. Sugar-agar stick culture, three weeks at 37°.
IV. Sugar-agar plate, four days at 37°. Natural size. Cultivated anaërobic.
V. Sugar-agar plate, four days at 37°. ×60. Superficial and deep colony. Cultivated anaërobic.
VI. Sugar-gelatin plate, four days at 22°. Natural size. Cultivated anaërobic.
VII. Sugar-gelatin plate, four days at 22°. ×60. Deep colony. Cultivated anaërobic.
VIII. Sugar-gelatin plate, two days at 22°. ×150. Part of a superficial colony. Cultivated anaërobic.
IX. Microscopical preparation. Pure culture from sugar-agar, three days at 37°. Bacilli with spores and spores that have fallen out. Fuchsin stain. ×1,000.

I.

II.

III.

IV.



V.

VII.

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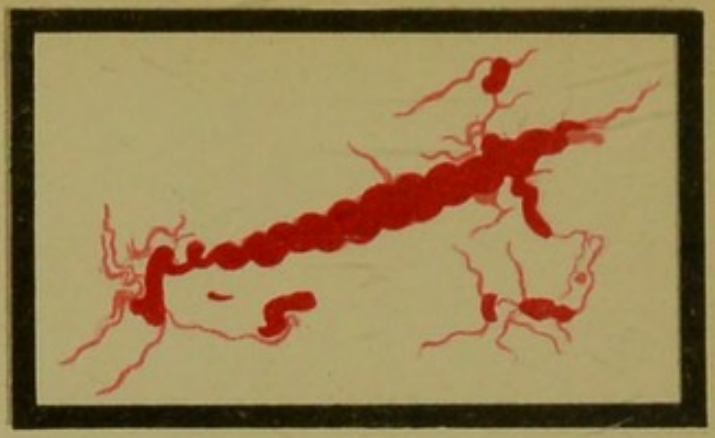
IX.

VIII.

I.



II.

III.

IV.

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VI.

VII.

Explanation of Plate 47.

BACILLUS ŒDEMATIS MALIGNI. Koch.

(Malignant œdema.)

I. Sugar-agar stick culture, eight days at 37°.

II. Microscopical preparation. Flagella plait. ×1,500. (Copied from G. Novy: *Zsch. f. Hygiene,* Vol. XVII., Pl. I., 2.)

III. Microscopical preparation. Bacilli with flagella. Pure culture from agar, twenty-four hours. Löffler's stain. ×1,000.

IV. Sugar-agar plate, four days at 22°. ×60. Part of a superficial colony.

V. Sugar-agar plate, six days at 22°. Natural size.

VI. Microscopical preparation. Pure culture from agar, two days at 37°. Rods with spores. × 1,000. Fuchsin stain.

VII. Microscopical preparation. Tissue juice from guinea-pig. Smear preparation. (Copied from Fraenkel and Pfeiffer: "Mikrophotog. Atlas," Pl. XXIII., 46.)

Explanation of Plate 48.

MYCOBACTERIUM TUBERCULOSIS (Koch). Lehm. and Neum.

(Tubercle bacillus.)

I. Glycerin-agar streak culture, fourteen days at 37°.

II. Glycerin-agar streak culture, forty days at 37°.

III. Potato culture, forty days at 37°.

IV. Colonies of tubercle bacilli in a blood-serum culture. ×700. (Copied from Koch: "Aetiol. d. Tubercul. Mittheil. d. kais. Gesundheitsamt," Vol. II., Pl. IX., 44.)

V. Culture on blood serum from a piece of freshly extirpated scrofulous gland. (Copied as above.)

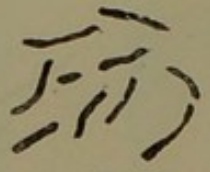
VI. Giant cell with radiating arrangement of the bacilli. From the cheesy bronchial gland of a case of miliary tuberculosis. (Copied as above, Pl. II., 9.)

VII. Microscopical preparation. Pure culture. Stained by Ziehl's method. ×1,000.

VIII. Branching of tubercle bacilli. (Copied from Hayo Bruns: C. B., XVII., No. 23.)

IX. Microscopical preparation. Sputum. Ziehl's stain. ×1,000.

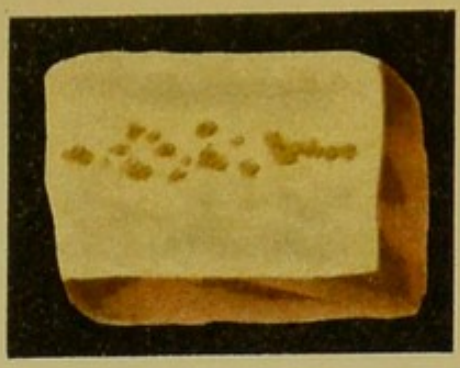
X. Individual bacteria. Highly magnified.



X.

I.

II.



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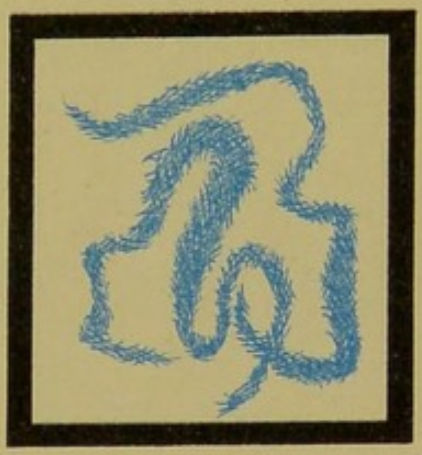
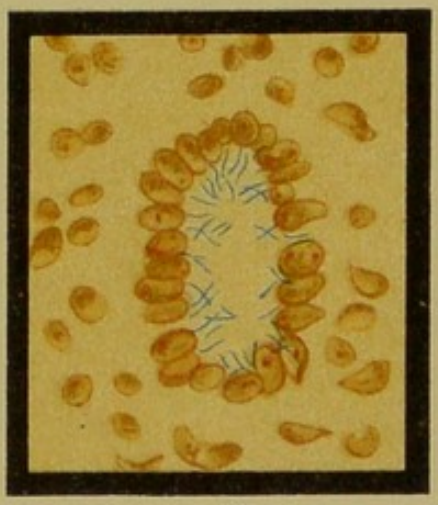


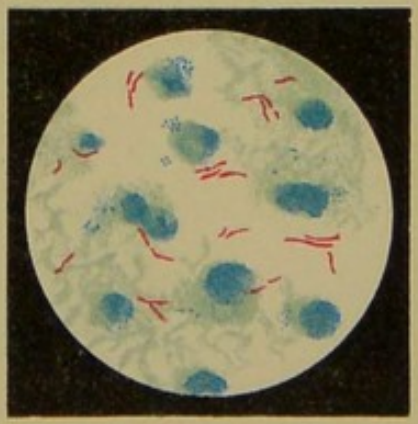
IV.

V.



VI.

VII. VIII.



IX.

I.

II.

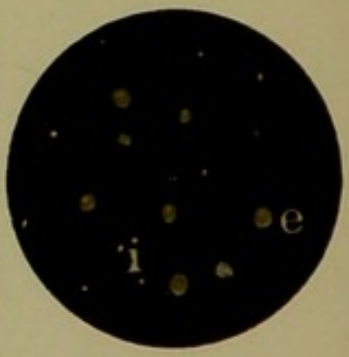
III.

IV.

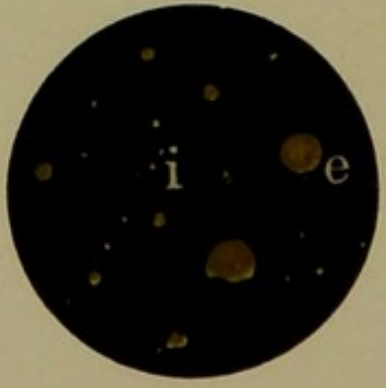
V.

VI.

VII.



VIII.



IX.

Explanation of Plate 49.

VIBRIO CHOLERÆ. (Koch) Buchner.

(Comma bacillus.)

I. Gelatin stick culture, two days at 22°.
II. Gelatin stick culture, seven days at 22°.
III. Gelatin stick culture, eight days at 22°. Culture from a case of Asiatic cholera in Hanover.
IV. Gelatin stick culture, eight days at 22°.
V. Agar streak culture, eleven days at 22°.
VI. Agar stick culture, eight days at 22°. Puncture canal.
VII. Agar stick culture, eight days at 22°. Surface.
VIII. Agar plate, six days at 22°. Natural size.
IX. Agar plate, six days at 22°. Culture from a case of Asiatic cholera in Hanover.

Explanation of Plate 50.

VIBRIO CHOLERÆ. (Koch) Buchner.

(Comma bacillus.)

I. Agar plate, thirty-six hours at 22°. ×60. Left side, superficial; right side, deep colony.

II. Agar plate, two days at 22°. ×60. Left side, superficial; right side, deep colony.

III. Agar plate, three days at 22°. ×60. Left side, superficial; right side, deep colony.

IV. Agar plate, three weeks at 22°. ×60. Left side, superficial; right side, deep colony.

V. Agar plate, five days at 22° (×60), from a case of Asiatic cholera in Hanover. Superficial and deep colony.

VI. Gelatin plate, four days at 22°. Natural size. Much depressed liquefaction funnel.

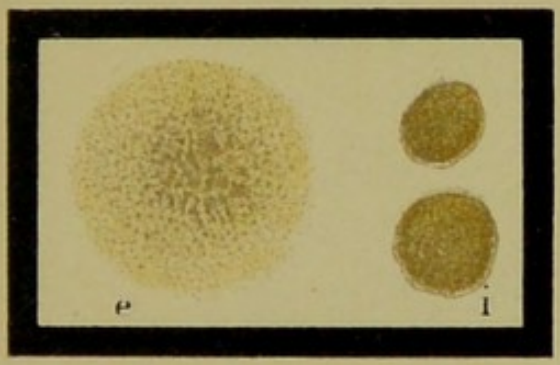
VII. Gelatin plate, fourteen days at 22°. Natural size. Colony with pronounced zonal development.

VIII. Gelatin plate, four days at 22°. Shallow zones of liquefaction.

IX. Gelatin plate, six days at 22°. Shallow sunken colonies with concentric zones of liquefaction.

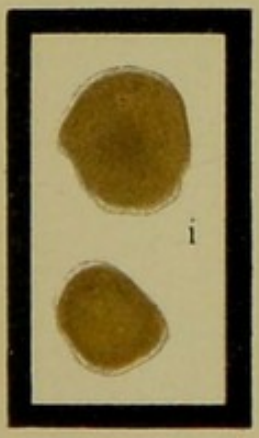
I.

II.



III.

IV.



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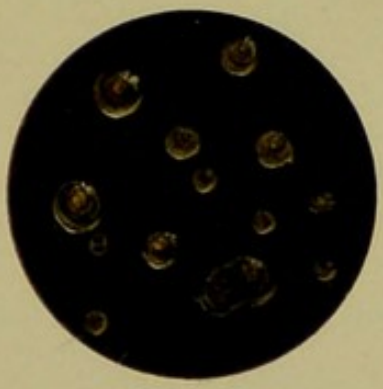

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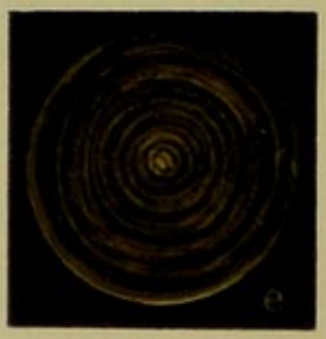

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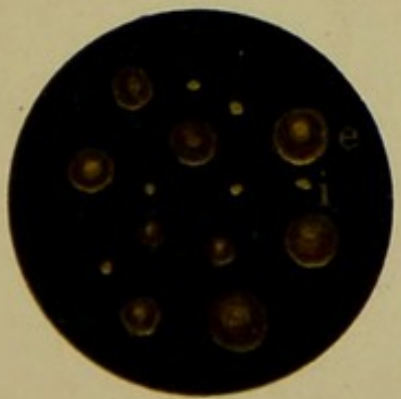
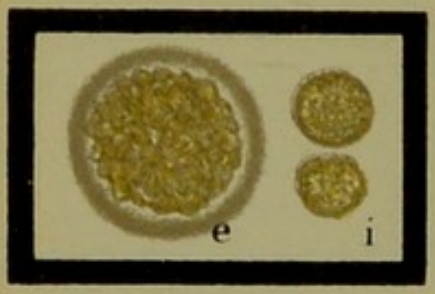


VIII.

IX.

I.



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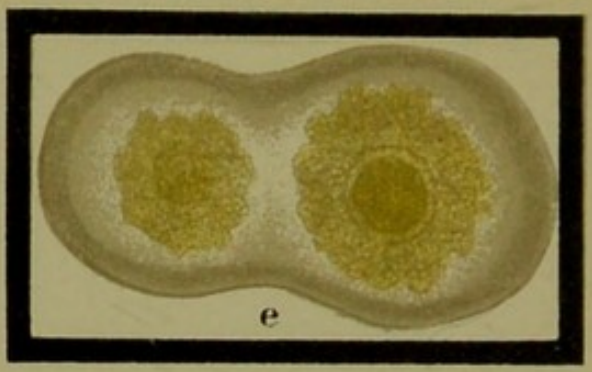

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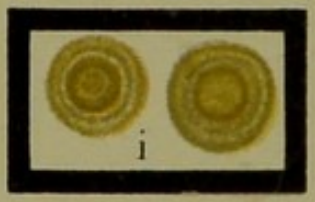

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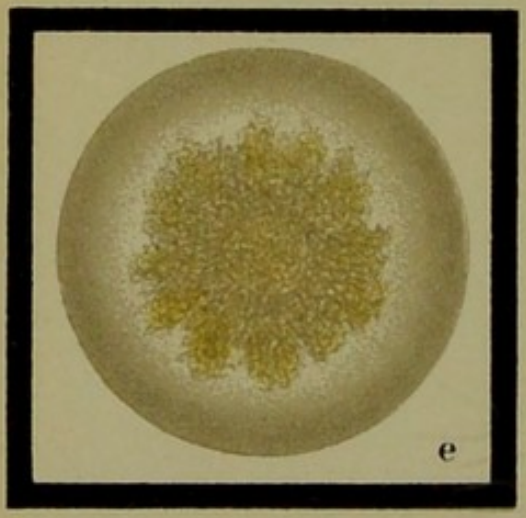


V.

VI.

VII.

VIII.

IX.

Explanation of Plate 51.

VIBBIO CHOLERÆ. (Koch) Buchner.

(Comma bacillus.)

I. Gelatin plate, thirty-six hours at 22°. ×60. Superficial and deep colonies.

II. Gelatin plate, forty-eight hours at 22°. ×60. Left side, superficial; right side, deep colonies.

III. Gelatin plate, three days at 22°. ×60. Superficial colonies with zones of liquefaction.

IV. Gelatin plate, three days at 22°. ×60. Deep colony.

V. Gelatin plate, four days at 22°. ×60. Superficial colony with zone of liquefaction.

VI. Gelatin plate, four days at 22°. ×60. Deep colony.

VII. Gelatin plate, five days at 22°. ×60. Deep colony from a culture from a case in Hanover.

VIII. Gelatin plate, five days at 22°. ×60. Superficial colony. Has undergone complete liquefaction.

IX. Gelatin plate, eight days at 22°. ×60. Superficial colony with zone of liquefaction.

Explanation of Plate 52.

VIBRIO CHOLERÆ. (Koch) Buchner.

(Comma bacillus.)

I. Gelatin plate, five days at 22°. ×60. Abnormal shape of a superficial colony.

II. Gelatin plate, five days at 22°. ×90. Abnormal shape of a superficial colony.

III. Gelatin plate, five days at 22°. ×60. Deeply sunken, superficial colony with strongly reflecting zone of liquefaction.

IV. Gelatin plate, six days at 22°. ×60. Superficial, abnormal colony with compact nucleus. Shallow sinking in, with zone of liquefaction.

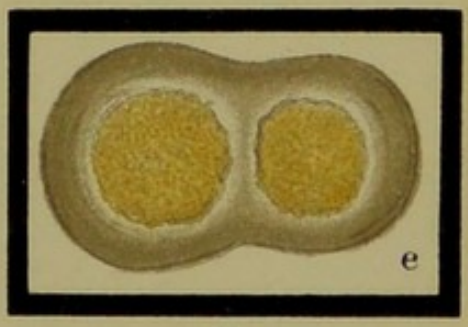
V. Gelatin plate, six days at 22°. ×60. Deep, abnormal colony, dark, with radiating stripes, from the same plate as IV.

VI. Potato culture, two days at 22°. Natural size. Soaked in a solution of soda before inoculation.

VII. Potato culture, five days at 22°. Upon ordinary potato.

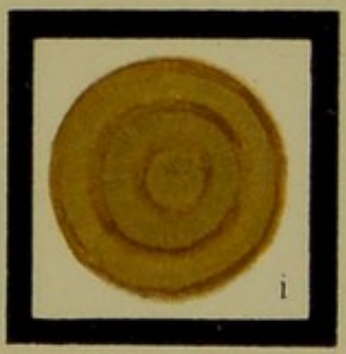
I.

II.

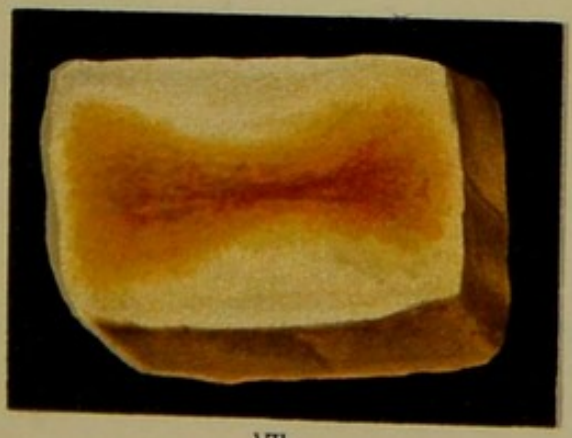


III.

IV.



V.



VII.

VI.

I.

II.

III.

IV.

V.

VI.

Explanation of Plate 53.

VIBRIO CHOLERÆ (Koch) Buchner.

(Comma bacillus.)

I. Pure culture from bouillon, twenty-four hours at 37°. Fuchsin stain. ×1,000.

II. Pure culture from agar, twenty-four hours. × 1,000. Löffler's stain of flagella.

III. Pure culture on gelatin, forty-eight hours. Perfectly fresh preparation from water. (Copied from Fraenkel and Pfeiffer, Fig. 94.)

IV. Pure agar culture, four weeks old. Involution forms stained with fuchsin.

V. Vibrio Metschnikovii Gamaleia. Smear preparation from pigeon's blood. (Copied from Fraenkel and Pfeiffer, Fig. 102.)

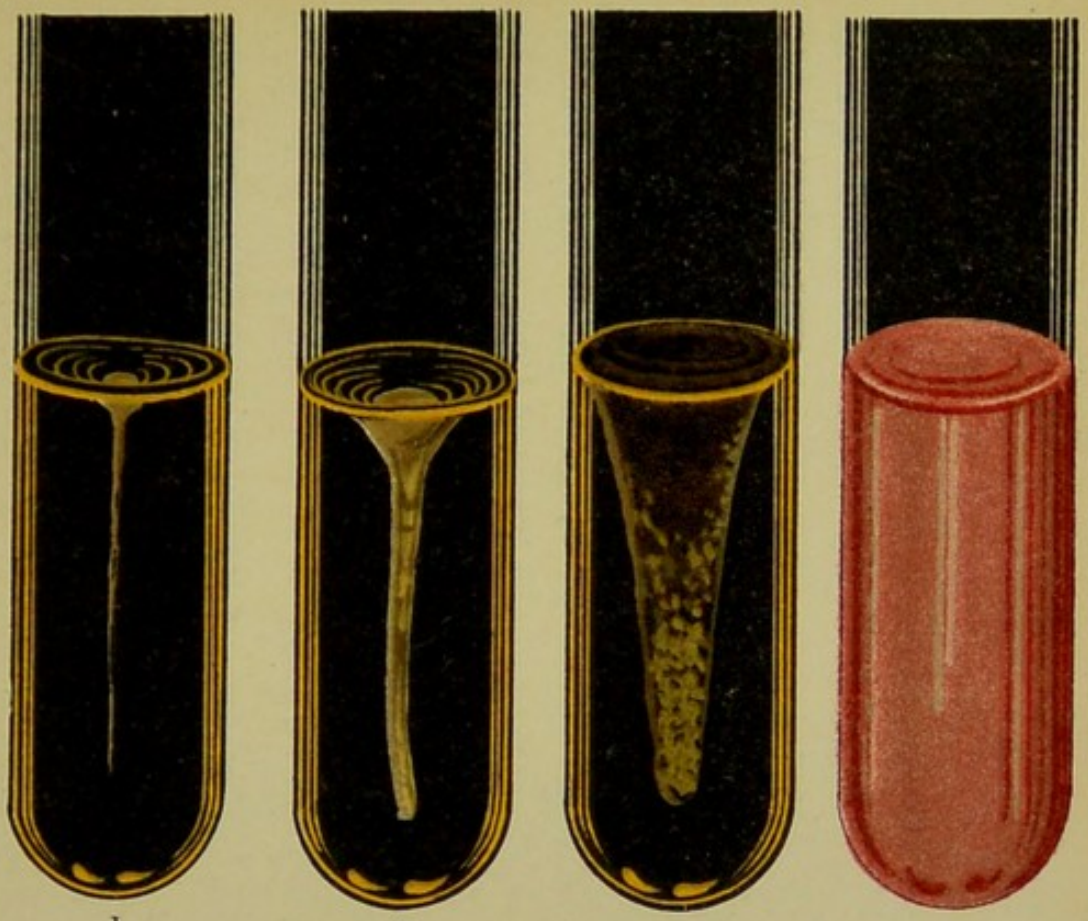
VI. Vibrio proteus Buchner. Pure culture in bouillon, twenty-four hours. Stained with fuchsin.

Explanation of Plate 54.

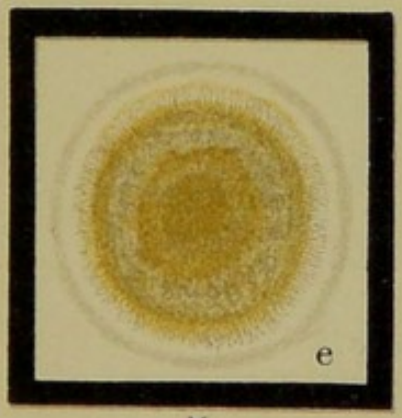
VIBRIO ALBENSIS. Lehm. and Neum.

(Fluorescent Elbe vibrio.)

I. Gelatin stick culture, twenty-four hours at 22°.
II. Gelatin stick culture, four days at 22°.
III. Gelatin stick culture, ten days at 22°.
IV. Indol reaction at end of ten days. Bouillon culture treated with dilute sulphuric acid.
V. Gelatin plate, three days at 22°. ×60. Superficial colony.
VI. Gelatin plate, three days at 22°. ×60. Deep colony.
VII. Gelatin plate, thirty-six hours at 22°. Natural size.
VIII. Microscopical preparation. Pure culture on agar, forty-eight hours. Stained with fuchsin.



I. II. III. IV.



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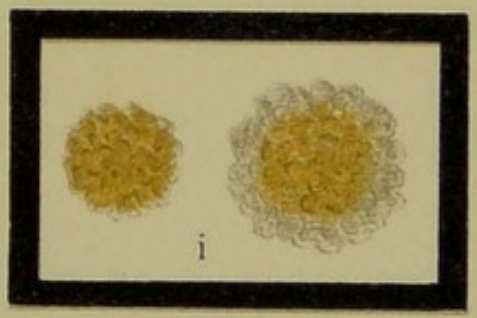
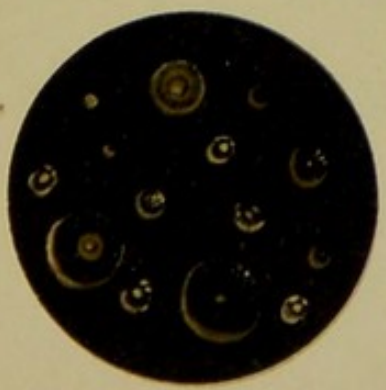
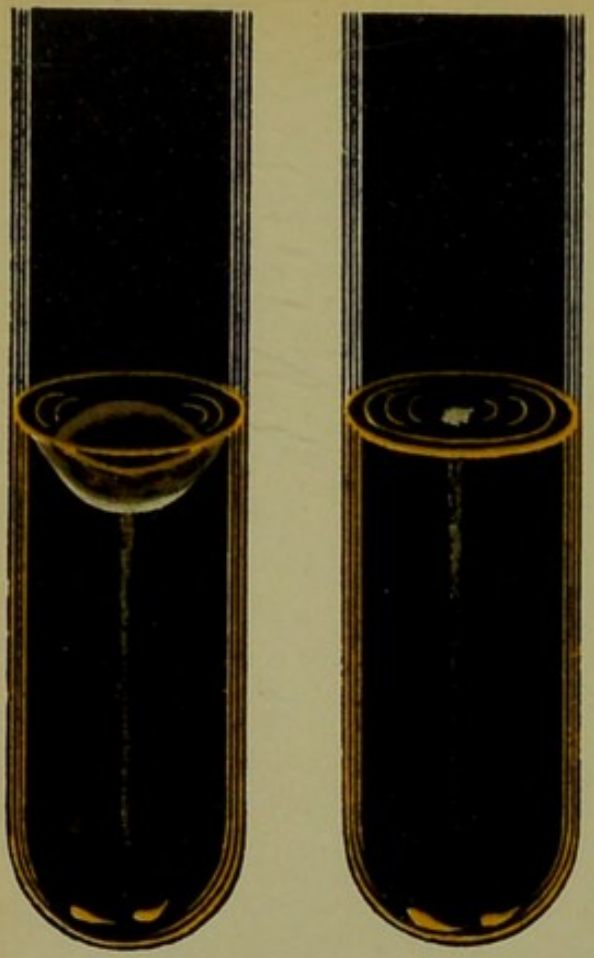


VI.



VII.

VIII.



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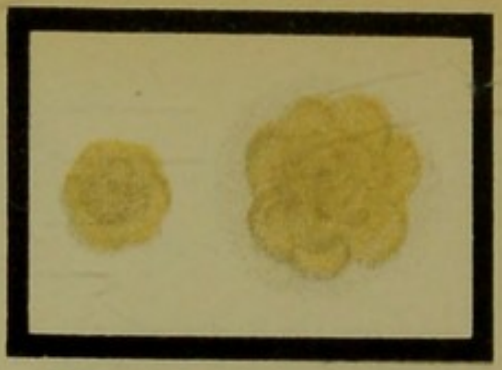
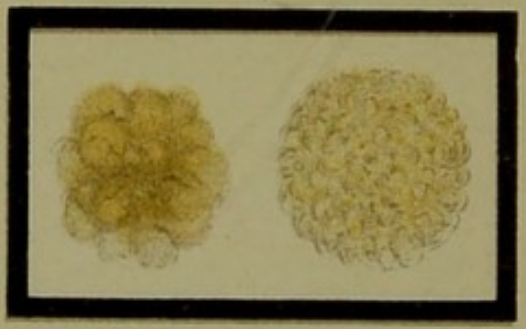


III.

IV.

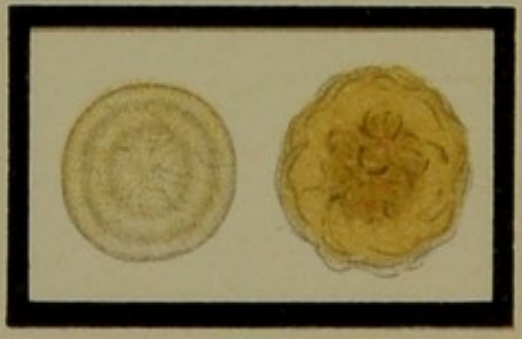


V.

VII.

VI.

VIII.



IX.

Explanation of Plate 55.

VIBRIO DANUBICUS Heider; VIBRIO BEROLINENSIS Rubner; VIBRIOA QUATILIS Günther.

I. Vibrio danubicus. Gelatin stick culture, three days at 22°.

III. Vibrio danubicus. Gelatin plate, three days at 22°. Right side, superficial; left side, deep colony.

IV. Vibrio danubicus. Microscopical preparation. Pure agar culture, twenty-four hours. Stained with fuchsin. ×800.

V. Vibrio berolinensis. Gelatin plate, three days at 22°. ×60. Right side, superficial; left side, deep colony.

VI. Vibrio berolinensis. Microscopical preparation. Pure agar culture, twenty-four hours. Fuchsin stain. ×800.

II. Vibrio aquatilis. Gelatin stick culture, three days at 22°.

VII. Vibrio aquatilis. Gelatin plate, three days at 22°. ×60. Deep-seated secondary colonies, starting from one point.

VIII. Vibrio aquatilis. Microscopical preparation. Pure agar culture, twenty-four hours. Fuchsin stain. ×800.

IX. Vibrio aquatilis. Gelatin plate, three days at 22°. ×60. Right side, superficial; left side, deep colonies.

Explanation of Plate 56.

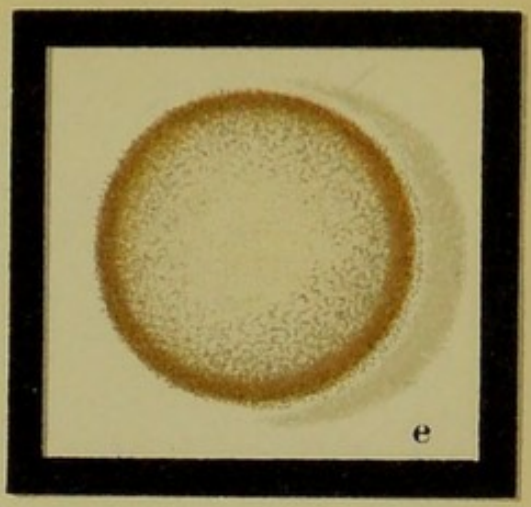
VIBRIO PROTEUS. Buchner.

(Vibrio Finkler.)

I. Gelatin stick culture, one day at 22°.
II. Gelatin stick culture, four days at 22°.
III. Gelatin plate, one day at 22°. Natural size.
IV. Gelatin plate, four days at 22°. ×60. Superficial colony.
V. Gelatin plate, four days at 22°. ×60. Deep colony.
VI. Agar streak culture, six days at 22°.
VII. Agar plate, four days at 22°. ×60. Superficial colony.
VIII. Agar plate, four days at 22°. ×60. Deep colony.
IX. Agar plate, four days at 22°. Natural size.

I.

II.



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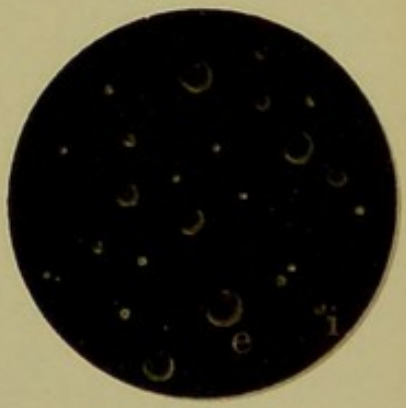

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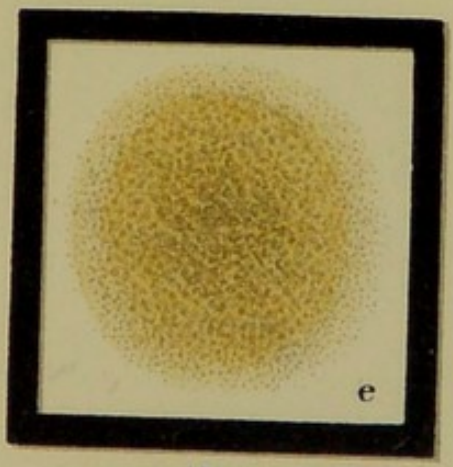

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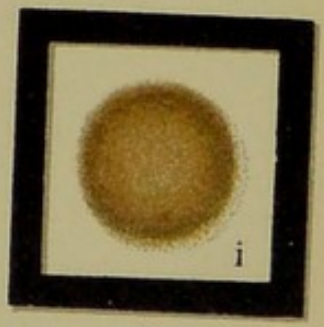

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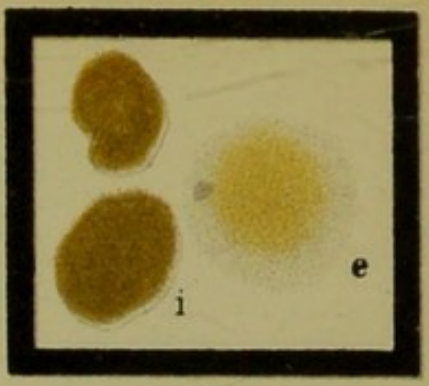

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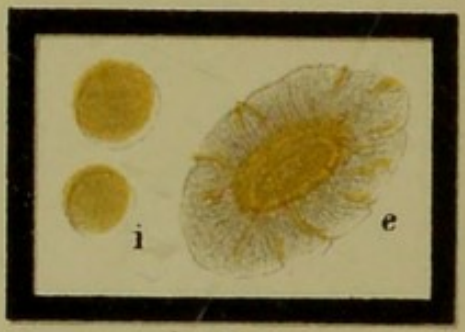

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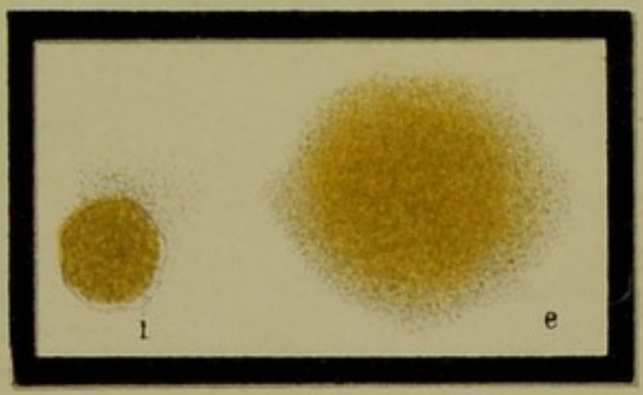

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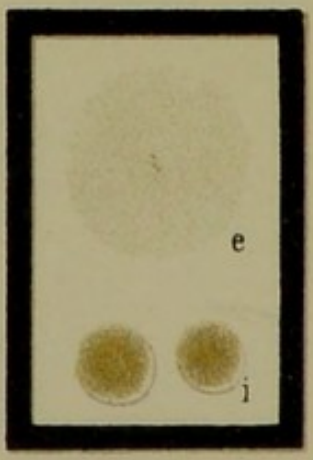


VII.

VIII.

Explanation of Plate 57.

SPIRILLUM RUBRUM. v. Esmarch.

I. Agar stick culture, ten days at 22°.
II. Agar streak culture, twenty days at 22°.
III. Agar plate, five days at 22°. ×60. *e*, Superficial; *i*, deep colony.
IV. Gelatin plate, seven days at 22°. ×60. *e*, Superficial; *i*, deep colony.
V. Microscopical preparation. Pure culture in tenfold diluted bouillon, two days at 37°. × 1,000. Stained with fuchsin.
V. *a*, Flagella preparation of spirillum rubrum. ×1,000. Löffler's stain.

V. *a*

SPIRILLUM CONCENTRICUM. Kitasato.

VI. Agar plate, seven days at 22°. ×60. *e*, Superficial; *i*, deep colony.
VII. Gelatin plate, three days at 22°. ×60. *e*, Superficial; *i*, deep colony.
VIII. Agar plate, seven days at 22°. Natural size.
IX. Microscopical preparation. Pure culture in bouillon, two days at 37°. ×1,000. Fuchsin stain.

Explanation of Plate 58.

SPIRILLA.

I. Spirillum serpens with plasma border which is stained with difficulty. ×1,000. Fuchsin stain. (Copied from Zettnow: C. B., X., Pl. 5.)

II. Spirilla from nasal mucus. Smear preparation. ×1,000. (Copied from Weibel: C. B., II., p. 468, Fig. 1.)

III. Spirilla from nasal mucus. Agar plate, pure culture. ×1,000. (Copied, as above, p. 468, Fig. 2.)

IV. Spirilla from nasal mucus. Gelatin plate, pure culture. ×1,000. (Copied, as above, p. 468, Fig. 3.)

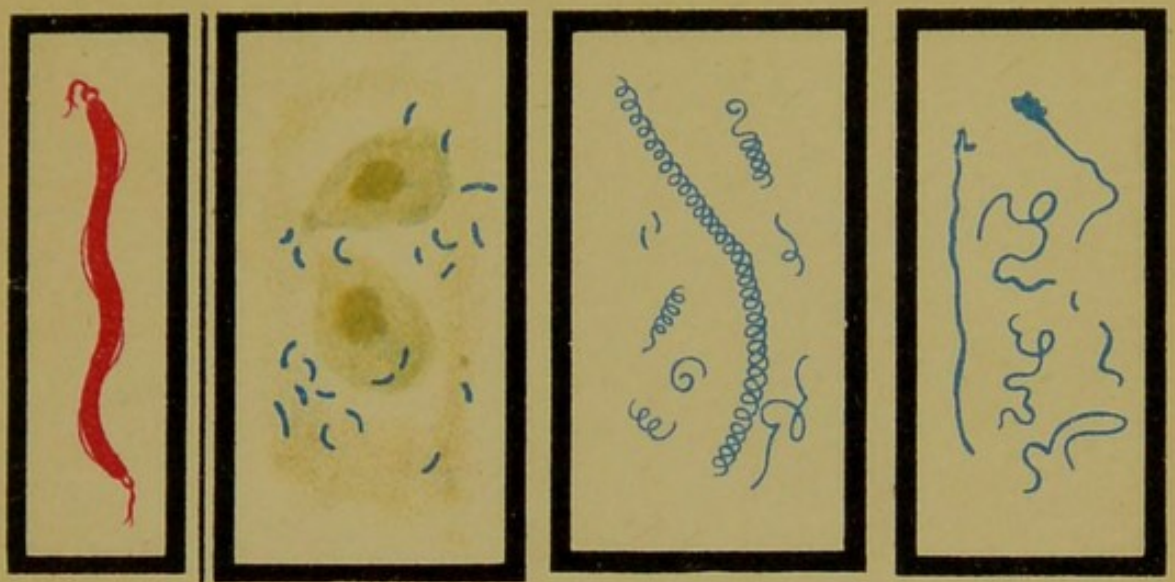
V. Spirillum undula with flagella. ×800. (Copied from Löffler: C. B., VI., Pl. I., Fig. 2.)

VI. Vibrio spermatozoides Löffler. ×1,000. (Copied from Löffler: C. B., VII., Pl. III., Fig. 7.)

VII. Spirochætes from mucus of the gums. (Copied from Löffler: "Bakterien," Pl. I., Fig. 4.)

VIII. Recurrens spirilla. Human blood, smear preparation. (Copied from Fraenkel and Pfeiffer: "Atlas," No. 134.)

IX. Recurrens spirilla. Human blood, spirilla arranged in a stellate shape. (Copied from M. J. Soudakewitsch: *Annales de l'Inst. Pasteur*, Vol. V., 1891, p. 514, Pl. 14, Fig. 1.)



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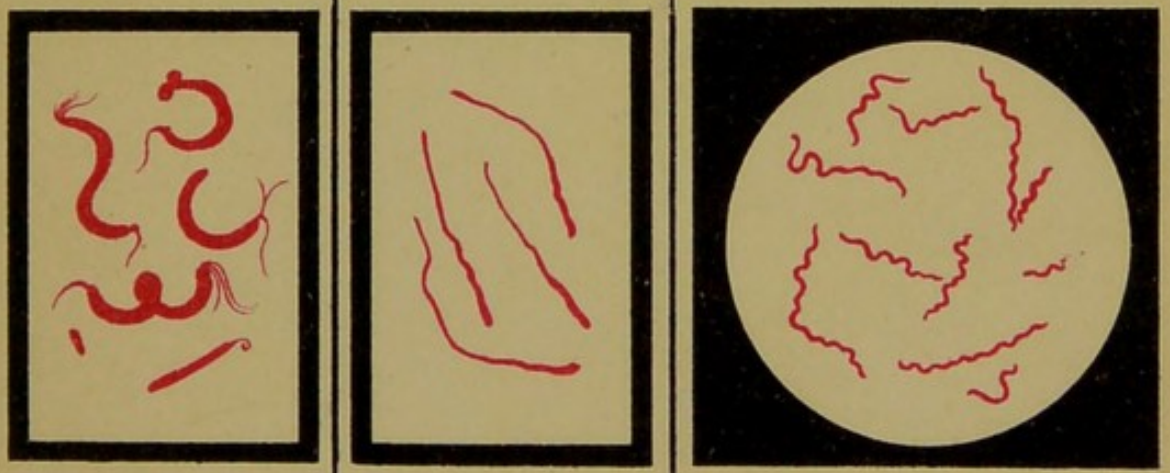

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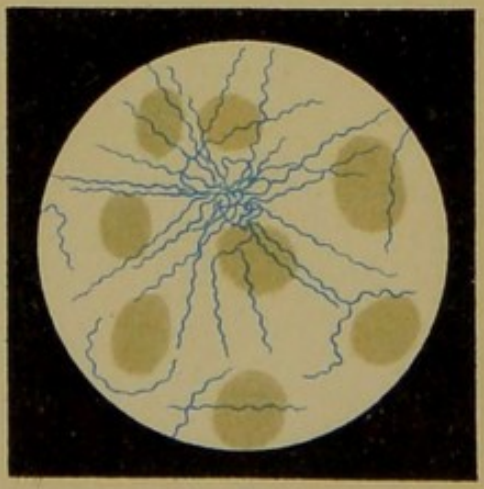
VIII.



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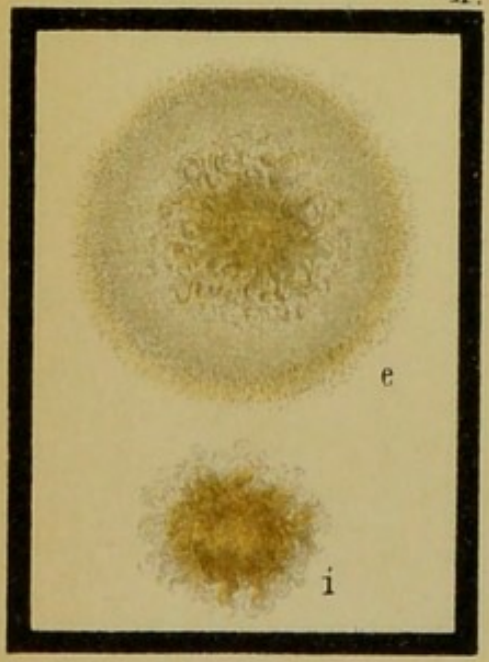

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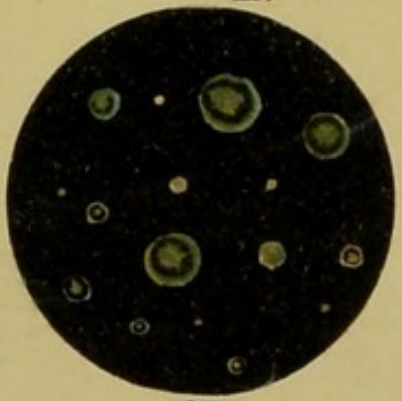

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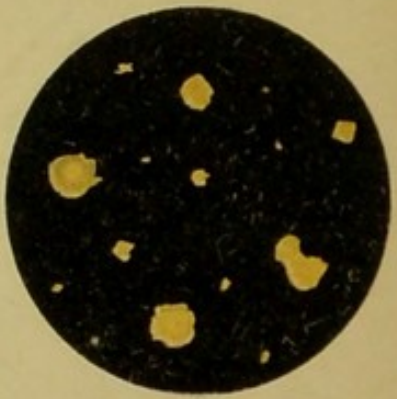

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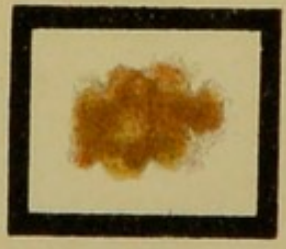

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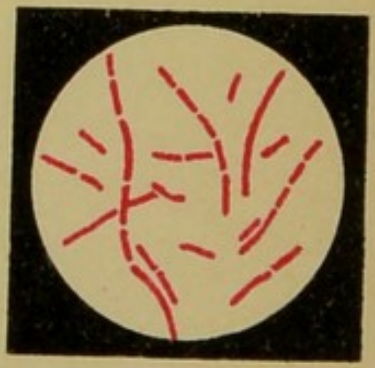

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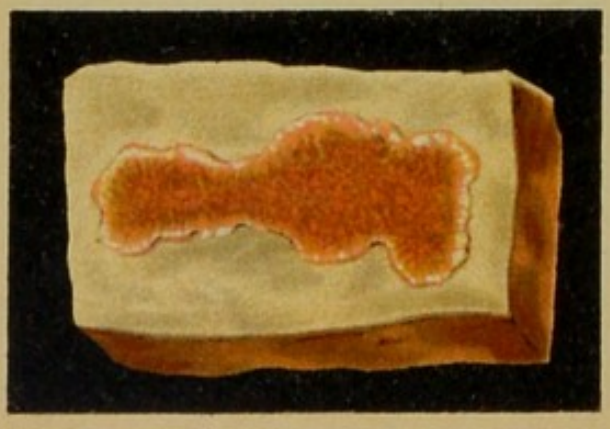

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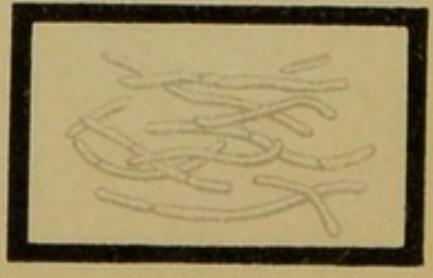

XII.

Explanation of Plate 59.

LEPTOTHRIX EPIDERMIDIS. Biz.

I. Gelatin stick culture, two days at 22°.
II. Agar streak culture, two days at 22°.
III. Agar stick culture, two days at 22°. Puncture canal.
IV. Agar stick culture, two days at 22°. Surface.
V. Agar plate, two days at 22°. Natural size.
VI. Agar plate, two days at 22°. ×90. Part of superficial colony.
VII. Agar plate, two days at 22°. ×90. Deep colony.
VIII. Gelatin plate, two days at 22°. Natural size.
IX. Gelatin plate, one day at 22°. *e*, Superficial; *i*, deep colony.
X. Potato culture, three days at 22°. Natural size.
XI. Microscopical preparation. Pure agar culture, two days at 22°. ×1,000. Fuchsin stain.
XII. Microscopical preparation. Bouillon culture in hanging drop, two days at 22°. ×1,000.

Explanation of Plate 60.

Oöspora farcinica (Noccard) Sauv. and Rad.

I. Agar streak culture, eight days at 22°.
II. Gelatin stick culture, twelve days at 22°.
III. Agar stick cultutre, eight days at 22°. Puncture canal.
IV. Agar stick culture, eight days at 22°. Surface.
V. Gelatin plate, ten days at 22°. Natural size.
VI. Gelatin plate, ten days at 22°. ×60. Superficial (*e*) and deep-seated (*t*) colonies.
VII. Agar plate, six days at 22°. Natural size.
VIII. Agar plate, eight days at 22°. Upper colony superficial; lower one deep.
IX. Potato culture, seven days at 22°. Natural size.
X. Microscopical preparation. Bouillon pure culture, two days. ×800. Stained with fuchsin.

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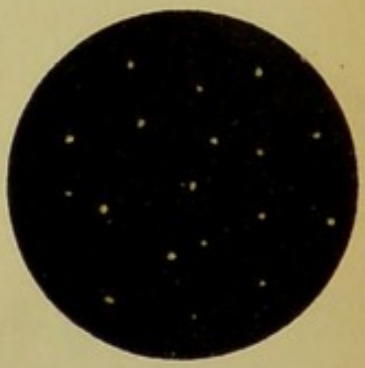

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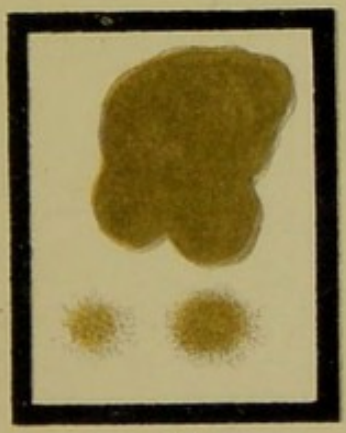

VIII.

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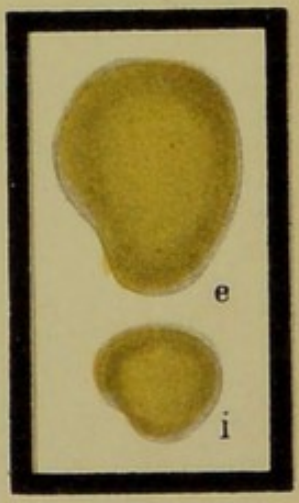

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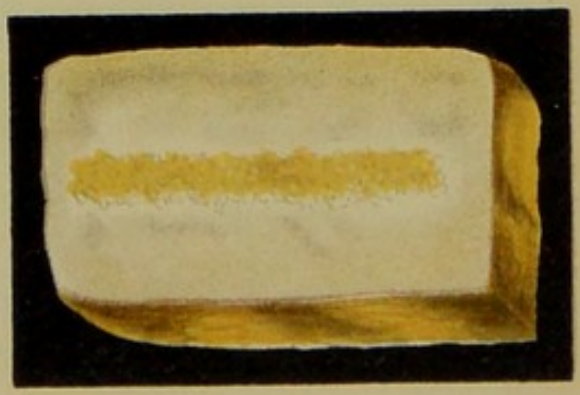

IX.

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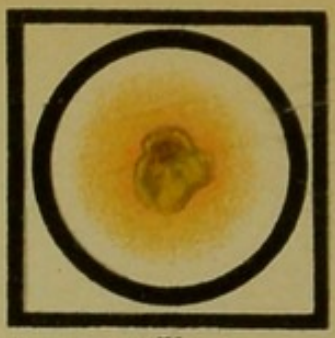

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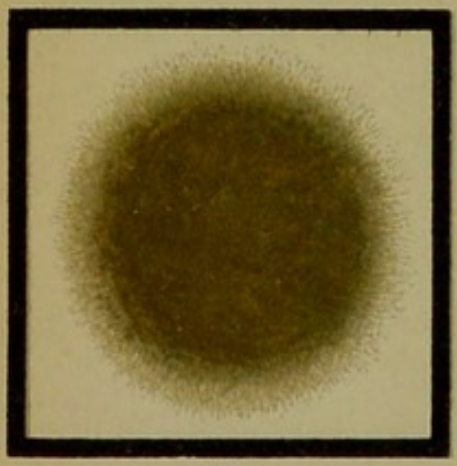

VIII

VII.

VI.

IX.

X.

Explanation of Plate 61.

OÖSPORA CHROMOGENES. Lehm. and Neum.

(Cladothrix dichotoma Autorum non Cohn.)

I. Gelatin stick culture, six days at 22°.
II. Agar streak culture, six days at 22°.
III. Agar stick culture, six days at 22°. Puncture canal.
IV. Agar stick culture, six days at 22°. Surface.
V. Gelatin plate, eight days at 22°. Natural size. View upon a white background.
VI. Gelatin plate, eight days at 22°. Natural size. View upon a black background.
VII. Gelatin plate, eight days at 22°. ×60. Part of a superficial colony.
VIII. Agar plate, four days at 22°. ×60. Superficial and deep colony.
IX. Potato culture, three days at 22°. Natural size.
X. Microscopical preparation. Bouillon pure culture, three days at 22°. ×1,000. Stained with fuchsin.

Explanation of Plate 62.

OÖSPORA BOVIS. (Harz.) Sauv. and Rad.

(Actinomyces.)

I. Agar streak culture, six days at 37°.
II. Agar streak culture, thirty days at 37°.
III. Gelatin stick culture, fourteen days at 22°.
IV. Gelatin plate, six days at 22°. Natural size.
V. Agar plate, six days at 37°. Natural size.
VI. Agar plate, six days at 37°. ×60. Superficial and deep colony.
VII. Gelatin plate, six days at 22°. ×60. Superficial and deep colony.
VIII. Potato culture, ten days at 37°. Natural size.
IX. Microscopical preparation. Bouillon pure culture, three days at 37°. ×1,000. Fuchsin stain.

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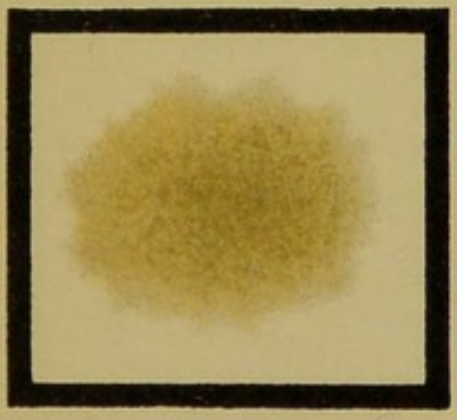

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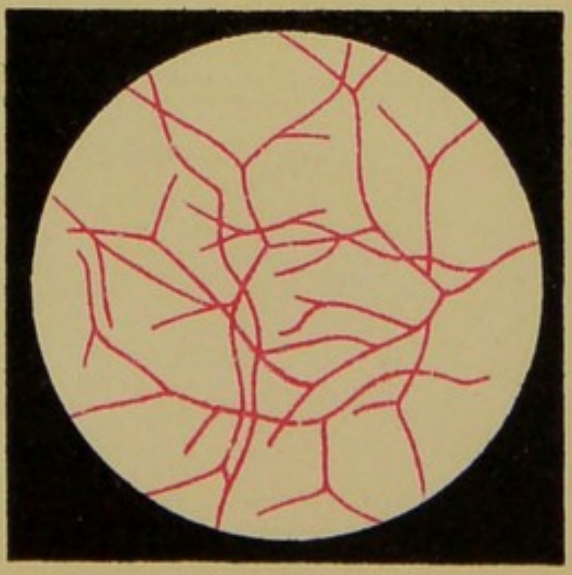

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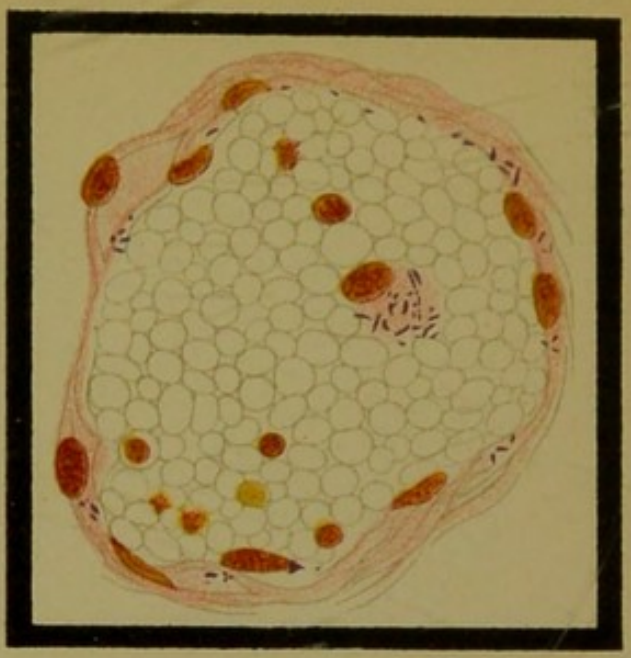
I.



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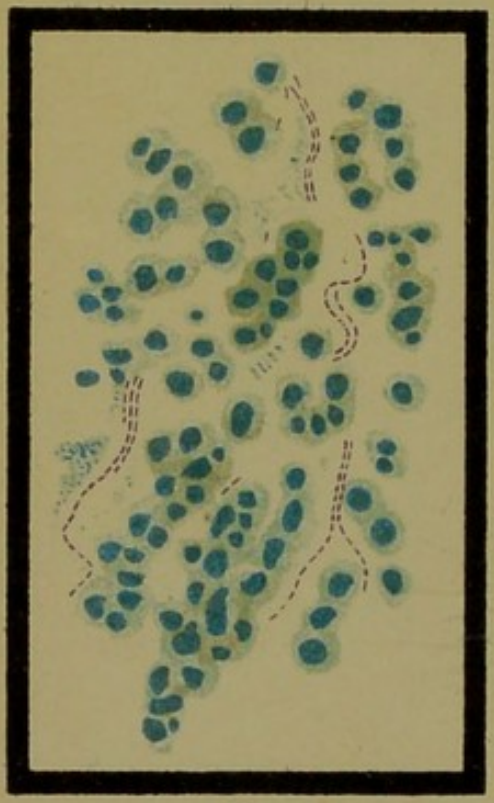

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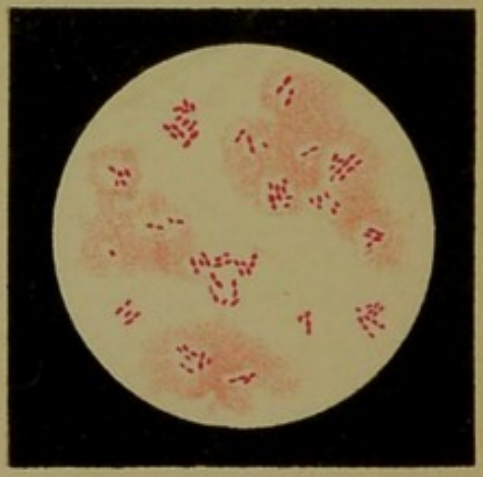

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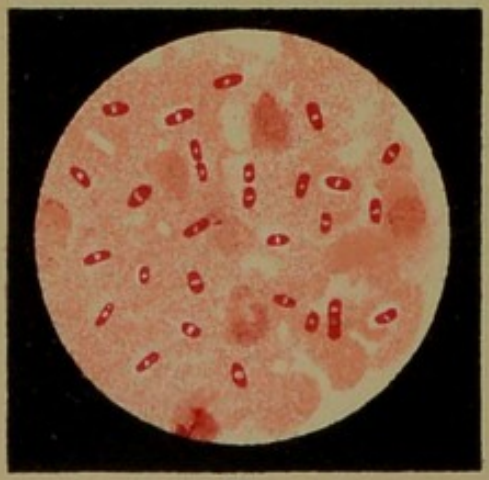


VI.

VII.

Explanation of Plate 63.

MYCOBACTERIUM LEPRÆ (Arm. Hansen) Lehm. and Neum.

BACTERIUM INFLUENZÆ, R. Pfeiffer.

BACTERIUM PESTIS, Lehm. and Neum.

I. Mycobacterium lepræ. Giant cell from leprous ulcer of epiglottis. ×1,000. Stained by Russell's method. (Copied from Seifert and Kahn: "Atlas d. Histopath. d. Nase," 1875, Pl. 38, Fig. 75 *b*.)

II. Mycobacterium lepræ. Transverse section of blood-vessel in a leprous testicle; bacilli in endothelium and in a white blood globule. Stained according to Gram and with Bismarck brown, eosin, bergamot oil. ×1,000.

III. Mycobacterium lepræ. Longitudinal section of ulnar nerve. Staining as above. (Copied from Lie: "Path. Anatomie d. Lepra," *Arch. f. Dermatol. und Syph.*, Vol. XXIX., 1895, Pl. VI.)

IV. Streptobacilli in soft chancre. Section of an untreated chancre, twelve days old. Stained by Unna's method. (Copied from Petersen: "Ueber Bacillenfund bei Ulcus molle," C. B., XIII., Pl. 4.)

V. Bacterium influenzæ. Smear preparation from the nasal secretion. ×1,000. Stained with fuchsin.

VI. Bacterium pestis. Smear preparation from lymphatic gland of a rat which died suddenly. ×1,000. (Copied from Yersin, semi-schematic on account of imperfect photogram, *Annales de l'Institut Pasteur*, 1894, Pl. XII., Vol. 8, Fig. 2.)

VII. Bacterium pestis. Bouillon pure culture. (Copied, as above, Fig. 3.) ×1,000

A. Introduction to the Morphology of Bacteria.

By the term bacteria (schizomycetes of Naegeli) is meant a very large group of the lowest vegetable organisms, which are morphologically very simple and uniform, but biologically are extremely differentiated. They are related to the lowest algæ (phycochromacea) and the lowest fungi by so many intermediate forms that a strict separation by a rigid definition appears difficult. Various bacteria also exhibit great similarity* to the simplest flagellates, which are usually regarded as animals.

A definition is rendered more difficult by the fact that botanical investigations of bacteria are comparatively rare, and that we still possess very imperfect knowledge concerning various details in the structure of bacteria (ramifications, separately stained parts†).

* *Vide* Bütschli in Bronn's "Klassen des Tierreiches," vol. i., part ii., Mastigophora.

† It is to be noted, moreover, that according to Brefeld's mycological investigations (vol. viii., p. 274), forms develop during the process of development of higher fungi which possess a striking resemblance to bacteria during many successive generations. We must therefore concede the possibility that among the varieties of bacteria a number do not deserve the term "species," but belong to the category of other fungi.

The following definition will suffice, perhaps, for the practical requirements of bacteriology:

Small unbranched * cells (almost †) always free from chlorophyll, with a thickness which is hardly ever more than 2 μ, and extremely rarely 3–5 μ; they have a globular, rod, thread, or screw shape, without any

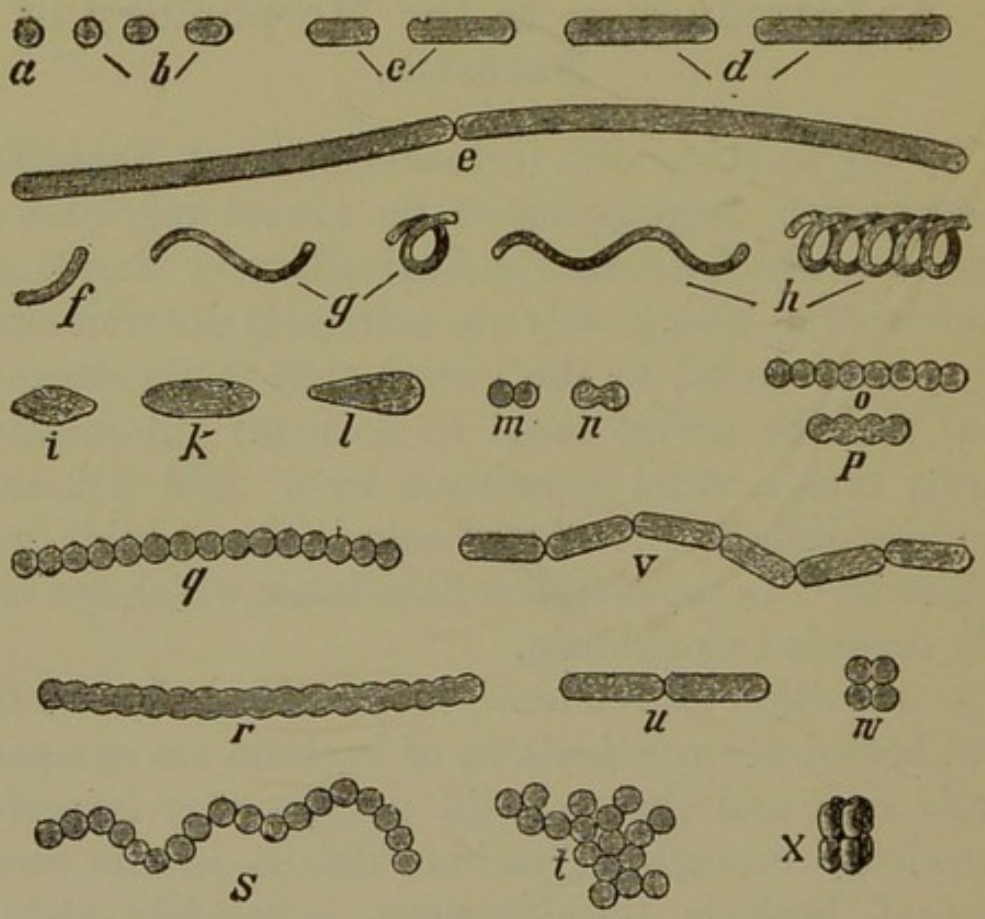


Fig. 1.—The Forms of Bacteria according to Buchner.

other organs than flagella which serve for movement. Vegetative proliferation takes place by transverse

* Concerning our knowledge of branched bacteria, *vide* pp. 67 and 68.

† Practically important bacteria containing chlorophyll are unknown. But J. Frenzel's green tadpole bacillus must probably be classed among the schizomycetes. The position of Dangeard's eubacillus multisporus among the bacteria seems more doubtful. L. Klein described colorless forms with bluish-green spores.

division, very rarely by longitudinal division. One series of forms develop endogenous, round, permanent spores; in others conidia-like constrictions (arthrospores) have been observed or claimed. Other modes of proliferation are unknown at the present time.

So far as we know, the schizomycetes occur only in the forms here delineated, and which were first completely named by H. Buchner.

Forms of Solitary Growth.

Spherical form (*a*), not coccus.

Oval form (*b*), the long diameter at the most twice as large as the transverse diameter.

Short rods (*c*), the long diameter is two to four times the transverse diameter.

Long rods (*d*), the long diameter is four to eight times the transverse diameter.

Filamentous shape (*e*).

Half-screw or comma (*f*), a very short section of a screw, at the most a half winding.

Short screw (*g*), a short screw winding.

Long screw or spiral form (*h*). All screw forms may occur either with steep or flat threads.

Spindle form (*i*).

Oval rods (*k*) are distinguished from the spindle form by the lesser attenuation of the extremities; from the oval form by their greater length (two to four times the transverse diameter).

Club form (*l*).

Growth in Groups.

Double spheres (*m*), with the separation merely indicated; roll shape or biscuit shape (*n*).

Spherical series (*o*), up to eight spheres, with the separation merely indicated; torula shape (*p*).

Spherical threads (*q*), or, if curved, rosary shape (*s*); with the separation merely indicated; filaments free from torula (*r*).

Grape shape (*t*). Double rods (*u*). Filaments of links (*v*).

Tetrads (*w*), a combination upon one plane of four, eight, sixteen, or more cells.

Dice shape (*x*), a combination of eight, thirty-two, etc., cells.

The formation of branches (dichotomy), *i.e.*, the production of a lateral sprout in bacteria, was unknown until recently, and is at all events rare. It has been demonstrated positively in the tubercle, diphtheria, and glanders bacilli (*vide* Plate 48, Fig. VIII.), so that for the present these varieties occupy a position between the bacteriaceæ proper and the hyphomycetes or filamentous fungi.

A different interpretation attaches to pseudodichotomy, which, according to Babès (Z. H., XX., 412), occurs not very rarely in the most typical bacteria. Either the lower link of a filament grows past the upper one and to one side, or, in a coccus series, a division of a coccus parallel to the direction of the filament suddenly initiates the beginning of a second filament.

Much has been written recently concerning the structure of the bacterium cell. We must confine ourselves to a mere abstract.

According to Bütschli ("Ueber den Bau der Bakterien," etc., Heidelberg, Winter, 1890) (Fig. 3), the bacterium cell consists of a membrane; a layer of

plasma, which takes hæmatoxylin stain with difficulty, is often very thin, and indeed often present only at the extremities; and a large central body

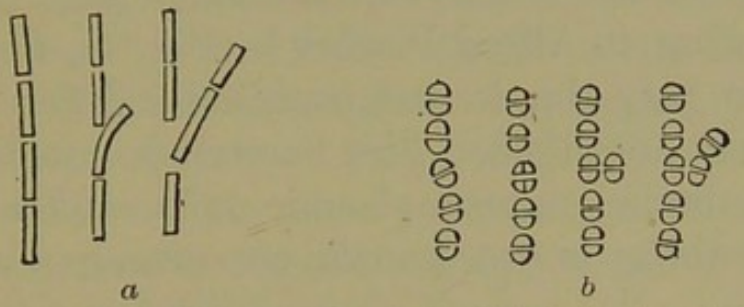


Fig. 2.—Pseudodichotomy. *a*, In bacilli; *b*, in streptococci.

(nucleus), which stains better with hæmatoxylin. The latter shows a distinct, the former not always a distinct honey-combed structure. Among the meshes of the comb, which stain blue with hæmatoxylin, are situated in the central body numerous granules which are stained red by hæmatoxylin.

At an earlier period Schottelius (C. B., IV., 705)

Fig. 3.—Chromatium Okenii Ehrbg. (After Bütschli.)

Fig. 4.—Bacillus oxalaticus Migula. (After Migula.)

expressed a similar opinion of the structure of bacteria. According to him the bacillus anthracis consists of a narrow nuclear filament, which stains a blackish-red with a very dilute watery solution of fuchsin, and

a protoplasmic body, which does not stain so readily. These two structures together constitute the bacillus as ordinarily conceived; they are surrounded by a membrane which stains with difficulty (*vide* page 72).

According to Alfred Fischer* (Fig. 4), the conditions are very simple and essentially different from those just described. The bacterium consists of a cell membrane, a protoplasmic tube, and a central fluid; nothing is yet known concerning a nucleus.

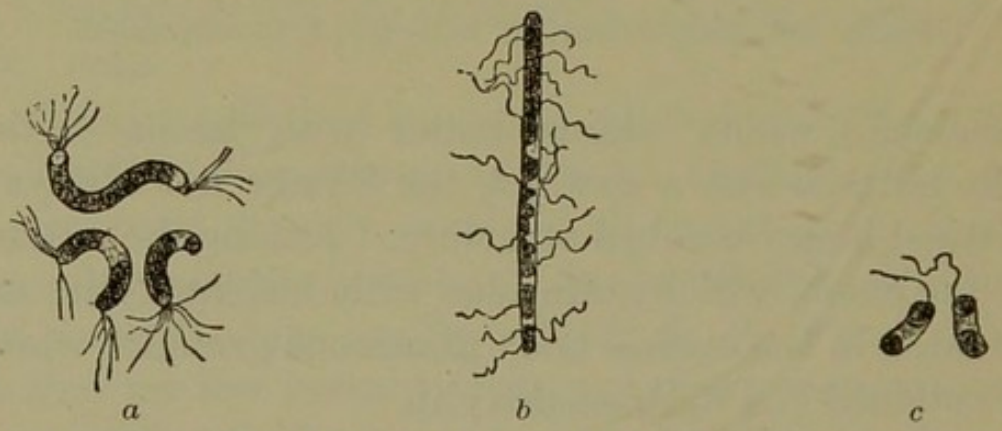


FIG. 4 *a*.—Plasmolysis, according to A. Fischer. *a*, Spirillum undula; *b*, bacterium Solmsii; *c*, vibrio choleræ.

In solutions of salts (sodium chloride, potassium nitrate, etc.)—and the more rapidly the more concentrated the solution—the abstraction of water produces "plasmolysis," *i.e.*, a retraction of the protoplasmic tube with partial detachment from the cell wall.† This explains numerous bright vacuoles which develop in many bacilli on making an ordinary cover-glass preparation, and which were formerly often regarded as spores.

At the same time and independently of A. Fischer,

* "Untersuchungen über Bakterien," 1894, Berlin. Reprint from the Jahrb. f. wiss. Botanik, xxvii., vol. 1.

† Desiccation on the cover-glass often suffices to produce pictures of plasmolysis.

Migula arrived at the same conclusions from a study of the very large bacillus oxalaticus, a sporulating variety related to the hay bacillus. He emphasizes particularly the fact that he has never succeeded in staining the "central body" darker than the protoplasm. In the protoplasmic tube which has been squeezed out of the cell membrane, the central space for the fluid can be made especially distinct by the fact that in media which abstract water it becomes smaller; in water it becomes larger.

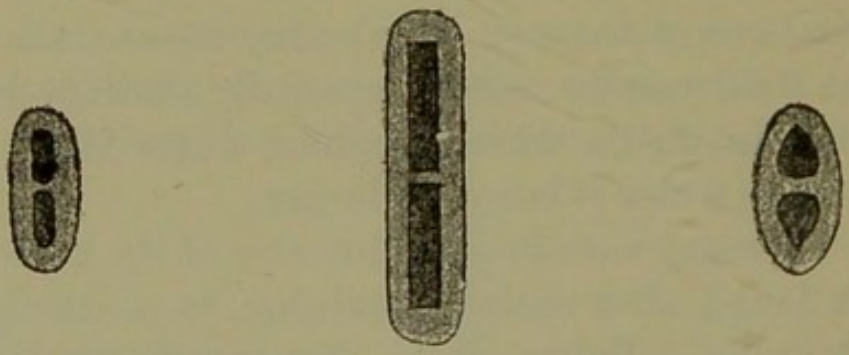
In very many varieties the interior of the bacterium cells is found, after suitable staining, to contain peculiar granules. Babès, their discoverer, applied to them the non-committal term metachromatic granules (*i.e.*, staining differently than the body of the bacterium), while Ernst, their first thorough investigator, terms them nuclei or sporogenous granules.

For the literature, which is rich in controversy, I refer to Babès (Z. H., XX., 412), and here will merely give the very plausible and clear views of R. Bunge, the most recent student of the subject. Bunge (*Fort. d. Med.*, XIII., 1895) distinguishes:

1. Ernst's granules. They are stained by warm Löffler's methyl blue, and are differentiated blackish blue by a solution of Bismarck brown, but they disappear on boiling. These granules are entirely absent in some sporulating varieties (anthrax, megatherium); in others it can be proven that they have no relation to spores—hence they are cell granules of unknown rank.

2. Sporule preliminary stages (Bunge's granules). Small granules, the majority of which are usually

found in the sporulating cells; they are not stained by Ernst's method, but stain in boiling Löffler's solution. After preliminary treatment of the dried preparation with chromic acid, sodium hyperoxide, or hydrogen hyperoxide, they are best shown by the



Bacterium pneumoniæ (Friedländer). Bacillus anthracis (Cohn). Streptococcus lanceolatus (Gamal.).

FIG. 5.—Formation of a Capsule. (Schematic.)

ordinary spore staining (*vide* Technical Appendix). The mature spore is produced by the union of several small preliminary stages.

Bunge explains the controversies by the frequent confusion of the two different varieties of granules.

Concerning the cell membrane, it is to be noted that often it is not sharply defined on the outside and appears somewhat swollen. In some varieties of bacteria ("capsule bacteria" of writers) the thickening of the membrane or of the outer layer of the membrane is so great that the bacterium appears to be surrounded by a veritable mucous envelope or capsule, which is characterized by its slight response to staining with aniline colors. It is an interesting fact that these bacteria form capsules only when they grow in the animal body or upon special nutrient media, such as fluid blood serum, bronchial mucus, and, according to

Paulsen, milk.* The capsules do not form when the cultures are made on gelatin, agar, and potatoes.

Peculiar unilateral thickenings or swellings of the membrane are found in bacterium pediculatum, which is described as a rare cause of the "frog-spawn disease" of sugar factories (Fig. 6).

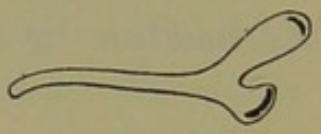
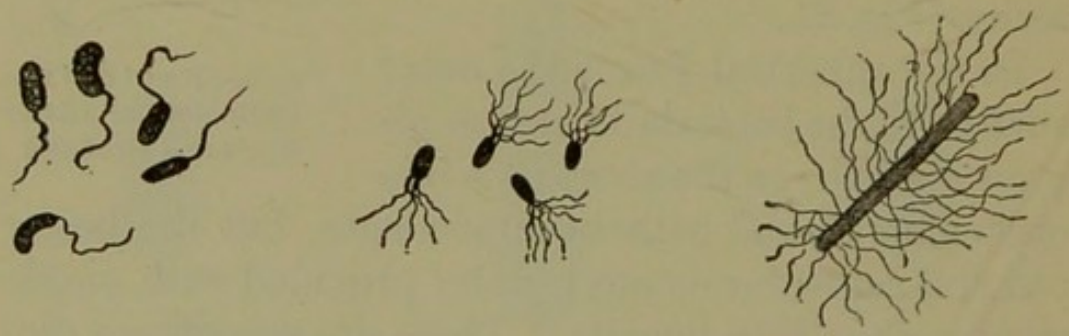


Fig. 6.—Bacterium pediculatum. (After Koch and Hosäus.)

In the spherical forms the outer surface of the bacteria is almost always smooth; in the short rods it is often smooth and without appendages, but the larger rod and screw forms are usually provided with single or numerous thin flagella. These are sometimes distributed over the entire body of the bacterium, sometimes they form a bundle at one pole, sometimes there is only a single polar flagellum. Shortly before division bacteria with polar position of the flagella show one flagellum or a bundle of flagella at each pole. As A. Fischer clearly proved, the flagella are not structures similar to the retractile and extensible pseudopodia, but are true hair-like formations which develop from outgrowth. In order to color the flagella it is necessary to treat the bacteria with unusually powerful staining reagents. Then the mem-

*It is not certain that pronounced capsule formation always takes place in these nutrient fluids. Recently various authors have called attention to the fact that capsule-like formations are observed extensively among bacteria. Johne describes a method by which they are easily made visible in anthrax, and distinct capsules are also seen in this way in bacillus megatherium, bacillus oxalaticus, etc. Babès has depicted capsules in streptococcus pyogenes, and we have occasionally seen similar appearances in many bacteria. Masses of bacteria which are united into mucous clumps by swelling of the capsules are called "zoöglœa."

brane, which usually remains colorless with ordinary stains, is also stained and the bacteria appear to be much thicker. Occasionally broad layers of the membrane remain unstained, and the flagella are then situated upon a narrow annular areola, separated from the bacillus by a colorless zone (Zettnow, von Stöck-



a *b* *c*

FIG. 7.—Types of Flagella. *a*, Vibrio choleræ, a flagellum at one extremity; *b*, bacterium syncyaneum, a bundle of flagella at one extremity, rarely on the side; *c*, bacterium vulgare, flagella arranged round about.

lin, A. Fischer). Unfortunately many of the methods used in staining lead forthwith to exfoliation and degeneration of the flagella, so that their perfect exhibition is often difficult. The above figure gives a schematic representation of the three modes in which bacteria are provided with flagella.

In the cultures of bacteria which are rich in flagella, Löffler first observed the occasional production of peculiar, switch-like bodies, composed of flagella which had fallen off or had been cast off and were plaited into one another (*vide* Plate 47, Fig. II.).

The power to produce flagella may be lost entirely for generations; whether permanently is still unknown. *Vide* Micrococcus agilis, sarcina mobilis (Lehmann and Neumann).

Ordinary vegetative increase of bacteria is effected

by a transverse constriction in the middle of the bacterial cell, which has either been very little (spherical bacteria) or considerably elongated. As a rule, the micro-organisms separate soon after fission, but the opposite event may occur in all groups of bacteria, so that, for example, chains of spheres or rods develop. Under certain nutritive conditions the bacteriaceæ, vibriones, and higher bacteria give rise to the production of long threads, but later these may again be resolved into links. According to all recent investigations, division of the cell starts in the protoplasmic layer upon the wall, the central "nucleus" or "cavity" is divided passively, and the cell membrane takes part secondarily. This is evidently opposed to the interpretation of the central body as a nucleus, because division of the nucleus always precedes division of the cell.

Longitudinal growth with transverse fission is the rule for the mass of bacteria, but in certain forms—for example, sarcina—there is a regular alternation of the fission in the three principal planes. At least occasional division along two planes at right angles to one another has been observed in very different bacteria—for example, in streptococci—and thus cells in four parts may develop, with bifurcation of the chain (*vide* Fig. 2).

Longitudinal division of rod forms has been observed rarely but undoubtedly (Babès: Z. H., XX.). Metschnikoff observed stellate division in a sporulating organism known as "Pasteuria," but this can hardly be classed among the bacteria in the narrower sense.

Ordinary vegetative proliferation must be distin-

guished from that due to the formation of spores. We are acquainted to-day with: (1) Endospores, strongly refracting oval or round bodies developing in the interior of the cell, and which as a rule possess considerable resistance to injurious influences (heat, dryness, chemicals); and (2) arthrospores (De Bary, Hüppe), *i.e.*, sprout-like constriction of one end of the cell. These spores (Fig. 8) are also said to exhibit increased resistance. But as the recent investigations have furnished no absolutely positive proof of the formation of arthrospores which exhibit increased resistance, the difficult question of arthrospores, important as it is, must be regarded as still open.

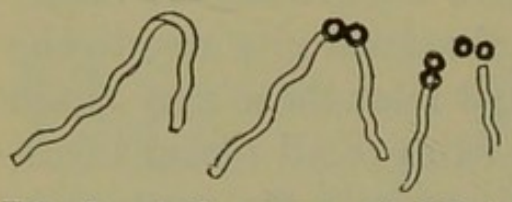


FIG. 8. — Arthrospores of Vibrio choleræ, according to Hüppe.

In the following pages the term spores refers only to endogenous permanent forms.

In the different varieties the development of the endospores runs a similar but not identical course. In examining any definite variety for the development of spores, we resort, as a rule, to agar streak or potato cultures, which are kept at a temperature best adapted to the variety in question. At the end of twelve, eighteen, twenty-four, thirty, thirty-six hours, we examine small tests of the streak culture, first unstained in water and with a narrow angle of aperture. If it is thought that round or oval, strongly refracting spores have been found, the spores are stained according to Neisser's or Hauser's method (*vide* Technical Appendix). For the careful study of the development of spores, it is best to place a few bacilli in a

hanging drop of gelatin or agar, and (if necessary, with the aid of warming apparatus or in a well-heated room) to observe and draw definite individual cells uninterruptedly.

Motile varieties always come to a standstill (according to A. Fischer) before the formation of spores, but they do not cast off their flagella. Certain varieties first grow into long filaments, which at the beginning are unsegmented. This variety includes the bacillus anthracis, whose sporulation will be selected as an example (*vide* Plate 40, Figs. VI. and III.).

The previously homogeneous bacteria first exhibit a delicate, cloudy opacity; then, according to Bunge, the very finest granules are replaced by a smaller number of somewhat coarser granules, which coalesce until small, rounded spores are situated at regular intervals (Plate 40, Fig. VI.), and are converted gradually into the oval, strongly refracting, mature spores (Plate 40, Fig. III.).

When sporulation is complete, we find in the bacterial filament a delicate septum between two spores (Plate 40, Fig. IV.). Not all segments which have begun the development of spores by the formation of spherical preliminary stages mature these spores. Indeed, certain varieties, as the result of various conditions of culture, gradually suffer a permanent loss of the power of producing mature spores and form only preliminary stages, which are physiologically valueless (Roux, K. B. Lehmann).

According to Lud. Klein (C. B., VII., 440), sporulation is entirely different in five usually motile, anaërobic forms of bacilli (bacillus De Baryanus, Solmsii, Peroniella, macrosporus, limosus), which were

discovered and studied by him (but unfortunately not in pure cultures). In these the process ran the following course: Without any cessation in the motion of the bacillus, one extremity becomes somewhat enlarged and acquires a slightly greenish tinge. The

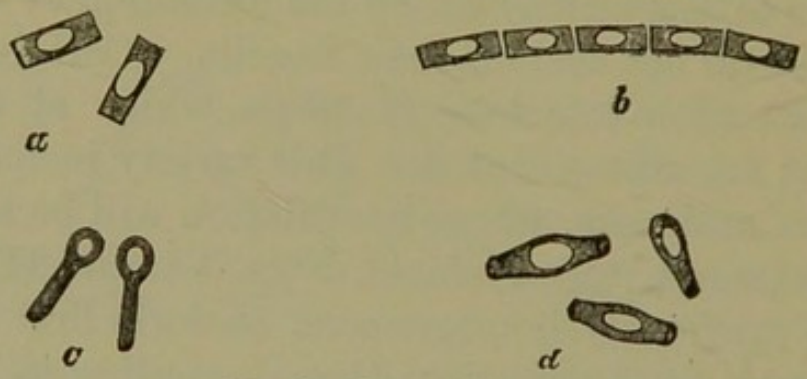


FIG. 9.—Types of Spores.

entire contents of the distended part now contract into a spore of bluish-green color and striking brilliance.

In the most important varieties the mature spores appear as follows (Fig. 9):

1. The spore lies in the interior of a non-distended, short bacterium cell (*a*).

2. The spore lies in the interior of a non-distended, short bacterium cell, which forms merely a link of a long filament (*b*).

3. The spore lies in the interior of a bacterium cell, which has been distended in the middle and has become spindle shaped (*d*).

4. The spore lies at the extremity of a non-distended short bacterium cell, apparently projecting far beyond it (*c*).

The germination of spores has been little studied. They are generally set free before germination by rupture of the filament. An outgrowth of the spores in

the bacillus at right angles to the direction of the filament is rarely observed (*vide* Sorokin, C. B., I., 465).

The following cut shows the germination of a few closely allied varieties which were studied by L. Klein.

The examination is made in a hanging drop of gelatin or agar. This may furnish very valuable mate-

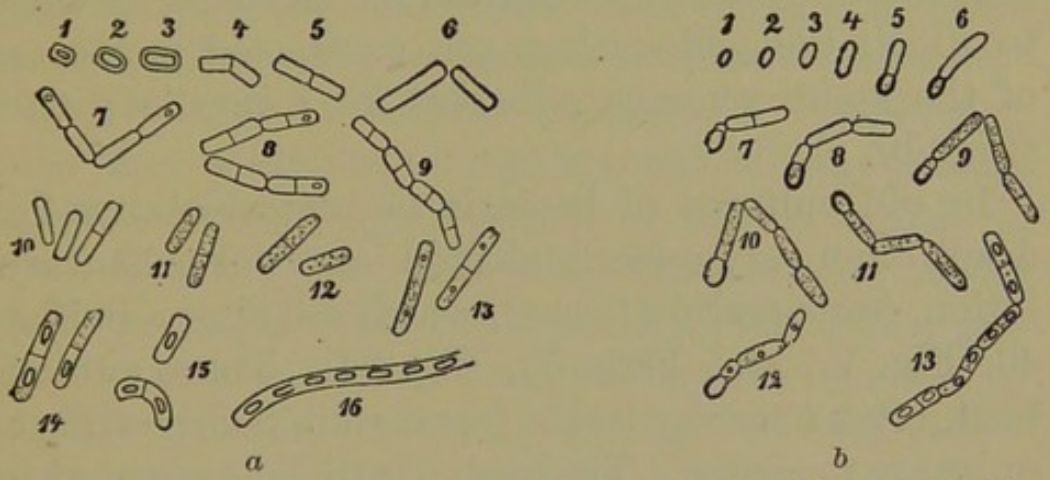


FIG. 10.—Development of Spores, according to L. Klein. *a*, Bacillus leptosporus L. Klein; 1-3, the enlarging spore; 4, the spore is converted into the bacillus without any sharp demarcation; 5-10, further growth; 11-16, development of the spores. *b*, Bacillus sessilis L. Klein; 1-4, the spore swells; 5, the spore sends out a little rod at one pole, and remains behind as an empty envelope; 6-8, further growth; 9-13, development of the spores.

rial in differential diagnosis, as it seems to differ greatly in details.

1. Bacillus anthracis. The spore swells, its refractive power diminishes, its sharply defined membrane becomes indistinct, and without any sharp demarcation the spore becomes a young bacterium cell, which grows further and divides again.

A similar condition obtains in the bacillus leptosporus Klein, described by L. Klein (C. B., VI., 377), which is characterized by narrow, almost quadrangular spores (Fig. 10, *a*).

2. Bacillus subtilis Cohn. The membrane of the growing spore bursts at the equator, the firm-walled membrane of the spore adheres not infrequently to the emerging young rod, even after it has grown into a long filament.

3. Bacillus sessilis Klein. The spore enlarges to a marked degree, then ruptures at one pole, and from the envelope of the spore grows a motionless filament, to which the yellowish-green, contracted membrane of the spore remains adherent for a very long time (Fig. 10, *b*).

In old cultures of bacteria we almost always find dead, often very queerly shaped bacterial cells (involution, degeneration forms), which are shown in Plate 40, Fig. V., and Plate 53, Fig. VI. These swollen, bent, often unrecognizable forms stain poorly with the ordinary reagents. The beginner will often regard involution forms as the result of fouling; the resort to plate cultures will soon show whether we have to deal with one or more forms of bacteria.

B. The Chemical Composition of Bacteria.

The body of bacteria consists in great part of water, salts, and albuminoids;* extractive matters which are soluble in alcohol, and other bodies (triolein, tripalmitin, tristearin, lecithin, cholesterin) which are soluble in ether, are present in smaller amounts.

* Albumin and salts may amount to ninety-eight per cent of the dried bacteria (cholera vibrio), and on the other hand as much as twelve per cent of carbohydrates may be present in the membranes. Hellmich found a globulin in the bacterial albumin (Arch. f. exp. Path. u. Pharm., xxvi., 345).

In no variety of bacteria could E. Cramer discover grape sugar; some varieties (bacillus butyricus, leptothrix forms) contain starch-like masses which are stained blue by iodine. True cellulose was discovered by Dreyfuss in bacillus subtilis and in an organism closely related to bacterium coli, and the bacillus tuberculosis also forms cellulose in the animal body. But no cellulose could be obtained from cultures of bacillus tuberculosis and a "capsule bacillus from water," closely related to bacillus pneumoniæ Friedländer, while they contained a large amount of a gelatinous carbohydrate ($C_6H_{10}O_5$), which is closely allied to hemicellulose (concerning the literature *vide* Nishimura: A. H., XVIII., 318 and XXI., 52). The mucus of leuconostoc mesenterioides was shown by Scheibler (*Chem. Centralbl.*, XI., 181) to be a carbohydrate, $C_6H_{10}O_5$ dextro-rotatory. Kramer obtained a similar substance from the membranes of bacillus viscosus sacchari. Nuclein has not been extracted, but among the nuclein bases xanthin, guanin, and adenin have been found in considerable amounts. One group of bacteria deposits sulphur granules, which are derived from sulphuretted hydrogen (beggiatoa, thiothrix); another variety, which is classed among bacteria by many authors, secretes ferric oxide into its membrane from ferruginous waters (cladothrix, crenothrix).

The methodical investigations of E. Cramer have shed some light upon the quantitative relations, although accurate statements have been obtained hitherto only concerning bacterium prodigiosum, bacillus pneumoniæ, and a few related varieties, and a series of forms of vibrio choleræ (*vide* E. Cramer: A. H.,

XIII., 70; XVI., 150; and XXII., 167). The following statements and figures must suffice for the limits of this work.

The amount of water in a culture which has grown upon a solid nutrient medium, and likewise the amount of ash, depend in a very great measure upon the composition of the nutrient medium.

For example, bacterium prodigiosum contains, when cultivated on potato, 21.49 per cent dry substance, 2.70 per cent ash in the fresh substance; when cultivated on carrots, 12.58 per cent dry substance, 1.31 per cent ash in the fresh substance.

Apart from the concentration of the nutrient medium, higher temperatures and youth of the cultures increase the amount of dry substance and ash.

The amount of dry substance of bacteria also varies in its composition in the same variety, under the influence of the nutrient medium.

For example, the bacterium pneumoniæ Fried., upon a nutrient medium of meat-infusion agar, contained:

	With 1 per cent peptone.	With 5 per cent peptone.
Albumin	71.7	79.8
Ether and alcoholic extract	10.3	11.28
Ash	13.94	10.36

and with one per cent peptone and five per cent grape sugar:

	Per cent.
Albumin	63.6
Ether and alcoholic extract	22.7
Ash	7.88

It is evident that an increase in the amount of peptone in the nutrient medium causes an increased

amount of albumin in the bacillus, while an increased quantity of grape sugar diminishes the amount of albumin.

The differences are much greater as regards the dry substances of cholera vibriones when cultivated upon albuminous soda bouillon and upon the non-albuminous Uschinsky nutrient medium. Cramer found (the figures represent the averages from experiments with five cholera species) that:

	Albumin. Per cent.	Ash. Per cent.
Cholera vibriones on soda bouillon contained.....	65	31
Cholera vibriones on Uschinsky solution contained.	45	11

In the latter case there were evidently very large amounts of non-nitrogenous bodies, which may be regarded in part as hydrocarbons (or fats).

A very important point in classification—although more in a critical negative sense—is the fact discovered by Cramer that closely allied varieties which exhibit analogous, slightly varying composition upon several nutrient media, suddenly act differently upon a new medium. The most interesting illustration was the conduct of five cholera varieties, which in soda bouillon produced vibriones of almost exactly the same constitution, while they differed greatly in Uschinsky solution:

Soda Bouillon.

	Albumin.	Ash.	Total.
Cholera, old.....	65.12	31.55	96.67
Cholera, Hamburg, winter of 1892.	69.25	25.87	95.12
Cholera, Paris	62.25	32.80	95.05
Cholera, Shanghai	64.25	33.87	98.12
Cholera, Hamburg, autumn of 1893	63.94	29.81	93.75

USCHINSKY SOLUTION.

	Albumin.	Ash.	Total.
Cholera, old	48.13	7.14	55.27
Cholera, Hamburg, winter of 1892.	35.75	13.70	49.45
Cholera, Paris	65.63	9.37	70.00
Cholera, Shanghai	47.50	11.64	59.14
Cholera, Hamburg, autumn of 1893	34.37	14.74	49.11

This result again shows how dangerous it is to distinguish two varieties by relying upon a single chemical or biological reaction. Some of these varieties need merely acquire the power of forming thick cell membranes in Uschinsky solution in order to explain these remarkable differences. How easily, for example, could a writer be led, from these figures, to regard the bacilli of the Paris cholera as a distinct species, because they contain almost twice the amount of albumin in Uschinsky solution as those of the Hamburg cholera.

So far as I know the spores of bacteria have not been closely analyzed, but from the analogy to the spores of mould fungi we may expect them to contain a diminished amount of water.

C. The Vital Conditions of Bacteria.

1. NUTRIENT MEDIA.

While a number of schizomycetes have been found hitherto only in the human or animal organism, and therefore appear to be *strict parasites* (for example, spirillum Obermeieri), the majority of parasitic varieties can also be cultivated, either readily (for exam-

ple, bacterium typhi) or with difficulty (for example, micrococcus gonorrhœæ) in artificial nutrient media. Among the inhabitants of the inanimate surroundings of man, the so-called *saprophytes*, the majority can be easily cultivated in the same artificial media as parasites; while in others, for example certain salivary and water bacteria, such cultivation meets with insurmountable obstacles.

All nutrient media must be rich in water, and the presence of salts and of a supply of carbon and nitrogen is also indispensable. The majority of the practically important and all the pathological varieties have a predilection for albuminous and feebly alkaline nutrient media.

The demands of the bacteria upon the composition of the nutrient media vary extremely. As Mead Bolton showed, a number of water bacteria (bacillus aquatilis Flügge and bacillus erythrosporus Flügge) are satisfied with water which has been distilled twice in glass vessels. Here the proliferation of the bacteria must have taken place either at the cost of traces of impurities or of the ammonia and carbonic acid of the atmosphere.

In water which contained ammonium carbonate as the sole source of carbon and nitrogen, and was accordingly free from all organic nutritive material, Heraeus observed abundant proliferation of a variety of fungus—that is, a development of cell substance from the simplest material, such as occurs otherwise only in the higher plants which work with chlorophyll in combination with sunlight. Hüppe and particularly Winogradsky have shown the correctness and importance of this observation as the result of care-

ful studies. The energy necessary to the albumin synthesis seems to be gained by oxidation of ammonia into nitric acid.

Very few practically important bacteria exhibit such simplicity, but very many can dispense at least with albumin in the nutrient and thrive in solutions of very simple composition. Formerly cultures in such fluids were employed very often, and recently Uschinsky has again resorted to simple nutrients. But instead of Uschinsky's somewhat complicated solution:

Water	1,000	Magnesium sulphate	0.2–0.4
Glycerin	30–40	Dikalium phosphate	3–2.5
Sodium chloride	5–7	Ammonium lacticum	6–7
Calcium chloride	0.1	Sodium asparaginicum	3–4

we may choose much simpler solutions; for example, on the recommendation of Voges and C. Fraenkel (*Hyg. Rundschau*, 1894, No. 17) for one litre:

Sodium chloride	5 gm.
Neutral commercial sodium phosphate	2 gm.
Ammonium lactate	6 gm.
Asparagin	4 gm.

In this fluid (although it contains no sulphur) there grow:

VERY WELL.

Bacillus subtilis and mycoides.

Bacterium syncyaneum, pyocyaneum, coli, acidi lactici, pneumoniæ, mallei, vulgare. All vibriones.

FEEBLY.

Micrococcus pyogenes *a* aureus.	Bacterium typhi.
Streptococcus pyogenes.	Bacillus anthracis.

NOT AT ALL.

Bacillus tetani	Bacterium erysipelatos suum.
Bacterium murisepticum.	Bacterium cuniculicida.

The addition of the other substances recommended by Uschinsky did not cause vigorous growth of other varieties (such as diphtheria and tetanus), while on the addition of three to four per cent glycerin the medium becomes very serviceable even for the tubercle bacillus.

Although cultures in the simple nutrient media just described possess great theoretical interest, they are used very little for purposes of differential diagnosis.

Much more frequent use is made of flesh-water peptone gelatin, flesh-water peptone agar, bouillon (with or without the addition of grape sugar or milk sugar), glycerin agar, milk, potato discs.

We must always have these on hand, because no differential diagnosis is possible without them, and no variety can be properly described which has not been tested in regard to its condition in all these nutrient media (with the exception of glycerin agar).

Less use is made of the following nutrient media: potato water, lamb bouillon, blood serum (fluid and firm), serum agar, agar smeared with blood, meat, pieces of bread, mashed potatoes, rice, boiled or raw eggs.

2. REACTION OF THE NUTRIENT MEDIA.

As has been remarked above, the large majority of bacteria, especially the pathogenic forms, have a predilection for neutral or feebly alkaline nutrient

media. Formerly it was recommended that a solution of soda should be added gradually to the nutrient medium until it turns red litmus paper to a faint blue color.

Every chemist knows that there is no sharply defined final reaction for the titration of phosphatic nutrient media with litmus; furthermore, that different litmus papers influence the result, and that, finally, titration is quite impossible by gaslight. For this reason W. K. Schultz, in 1891, recommended phenolphthalein as an indicator in agar tritration, and advised that 8–10 c.c. less of normal soda lye be added to a litre of the nutrient medium than is necessary for complete neutralization with this indicator. In this way a medium is obtained whose reaction is suitable to very many bacteria, but there are some which require complete neutralization (C. B., X., 52).

Without having noticed this suggestion I conceived the same idea, in 1892, during my investigations on bread acids. Since 1894, the neutral gelatin (or agar) used in my laboratory as a nutrient medium has been treated with as much soda lye as is necessary to slight reddening of an addition of phenolphthalein. All the plates in this Atlas have been made according to such cultures, after experiments on five important bacteria had shown that the addition of alkalies and acids to this neutral medium had not improved their growth. Since then, Mr. Winkler, a student of medicine, has systematically tested the power of growth of the large majority of bacteria described in our Atlas. This has been done upon the following nutrient media:

1. Upon agar which, after the use of phenolphthalein, was neutralized with normal soda.

2. On "acid" agar, *i.e.*, neutral agar which has been treated with 10 c.c. of normal sulphuric acid to 1 litre.

3. On three varieties of alkaline agar, *i.e.*, on neutral agar which has received 10, 20, and 30 c.c. of normal alkali to 1 litre.

The results laid down in Table I. show, in brief, that almost all bacteria thrive well upon three of these nutrient media.

At all events the medium made neutral by phenolphthalein may be recommended unreservedly as a universal nutrient; the virulence of the varieties examined by us (anthrax, bacterium coli, mouse septicæmia, chicken cholera) was also well maintained thereon.

This reaction possesses the advantage that it is easily prepared and represents a sharply defined point, viz., that in which all the free acids and the acid salts are converted into neutral salts (monosodium phosphate into disodium phosphate).

If acid media are to be employed, it is best to start with one which has been neutralized with phenolphthalein, to which 10, 20, or 30 c.c. of normal acid per litre may be added. According to Winkler the first degree of acidity is well tolerated by almost all bacteria. According to Schlüter's statements (C. B., XI., 589), which are confirmed by recent publications, many tolerate a much higher degree of acidity; even as much at 100 c.c. of normal acid per litre, according to experiments made in our laboratory.

Apart from yeast and mould fungi, acid nutrient

media should always be employed as auxiliaries when we have to deal with the isolation of a bacterium from an acid medium. In counting the germs in the air, earth, water, milk, etc., the neutral medium should always be used.

3. INJURY TO BACTERIA BY CHEMICAL SUBSTANCES.

In the presence of an excess of acids or alkalies we have just recognized a factor which exerts an inhibitory influence on development, and, in still greater intensity produces death. The most varied chemicals act in a similar manner after a certain degree of concentration. The most effective substances are known as antiseptics or disinfectants.

With Hüppe, we usually distinguish the following degrees of action:

1. The growth is not disturbed but the pathogenic, zymogenic functions are weakened—*attenuation, mitigation.*

2. The organisms can no longer proliferate but are not killed—*asepsis, kolysepsis.*

3. The vegetative conditions of the micro-organisms are destroyed but not the permanent forms—*antisepsis.*

4. The vegetative and spore forms are killed—*sterilization or disinfection.*

Inasmuch as the test of the resisting power to chemicals plays a minor part for diagnostic purposes, this section will be treated very briefly.

The following plan should be adopted in order to determine the minimum concentration of the chemical

poison which will just produce asepsis, *i.e.*, inhibition of development.

For example, a ten-per-cent solution of the disinfectant is prepared, and 1, 0.5, 0.3, 0.1 c.c., etc., are added to 10 c.c. of liquefied gelatin. The tubes then contain 1 per cent, 0.5 per cent, 0.3 per cent, 0.1 per cent of the disinfectant; stick, streak, or plate cultures are then made with the bacterium which is to be tested. We may also inoculate with material which contains only spores (material which has been freed from all bacilli by heating for half an hour to 70° C.) in order to note whether these spores grow into cultures.

Behring makes this test in the following practical form: From the fluid, infected nutrient medium (for example, serum) which is to be tested a drop is taken before the addition of the antiseptic, and, after being placed on the lower surface of a cover-glass, is enclosed, by means of some vaseline, in a hollowed glass slide (*vide* Technical Appendix). Then larger and larger quantities of the disinfectant are added gradually to the serum tube, and after each addition a drop culture is again made. After remaining from twenty-four to forty-eight hours in the incubator, we can convince ourselves with the microscope of the growth in the different drops.

In case of a degree of concentration necessary to antisepsis, the fungus is cultivated in bouillon and 10 c.c. of the bouillon (which is still free from spores and which has been filtered through asbestos in order to get rid of any clumps of bacilli which may be present) are replaced with a corresponding amount of the disinfectant solution. From these tubes we

take, at the end of one minute, five minutes, ten minutes, fifteen minutes, thirty minutes, one hour, etc., a small platinum loop full of material, place the latter in 10 c.c. of lukewarm liquefied gelatin, and form plates. We then obtain statements like the following: x per cent of the disinfectant proves fatal in twenty minutes, y per cent in one minute, etc. If we suspect that the trace of disinfectant, which is conveyed by the loop, may have made the gelatin aseptic and may thus have simulated destruction of the bacteria, we should make a control inoculation of fresh material in gelatin to which a similar trace of the disinfecting fluid has been added.

The disinfectant to be tested should always be dissolved in water. If, on account of the slight solubility in water, the use of alcohol in the production of the original solution is indispensable, special control experiments are necessary in order to show that the action of the alcohol was not injurious.

When using nutrient media which are rich in albumin, we require much larger amounts of the disinfectant, both for the production of asepsis as well as for that of antisepsis, than when using media which are poor in albumin.* Thus in bouillon creolin produces asepsis when present in the proportion of 1:15000–1:5000; in beef serum only in the proportion of 1:150. In bouillon free from peptone or containing one per cent peptone, cholera vibriones are killed in one-half hour on the addition of 0.01 per cent HCl; on the addition of two per cent peptone, only when 0.04 per cent HCl is added. For diagnostic purposes we will usually make the tests in one

*Phenol is said to be an exception.

per cent peptone solutions if we do not wish to use one of the non-albuminous media described on page 86. At all events, the bacteria which are to be compared must be treated in exactly the same way, and, if the results are to be published, the various conditions of the experiment must be described in detail. Of bacteria which are free from spores not much is known concerning their varying resistance according to variety and nutrient medium, but a few statements in this regard have been made concerning staphylococci (Esmarch: Z. H., V., 1889, p. 72).

A combination of disinfectants increases their action. In particular, the addition of acid (hydrochloric or tartaric acid) intensifies the effect of sublimate, and also of solutions of phenol and cresol. Moreover, the effect is more certain upon a few than upon many germs, and greater at a higher than at a lower temperature.

4. DEFICIENCY OF FOOD AND WATER.

If bacteria which require nutritious substances in order to thrive are placed in distilled water, they usually die rapidly (within a few days). In spring water (even when sterilized), the duration of life is usually not more than eight to fourteen days, and proliferation is rare. In a series of cases, however, a much longer duration of life had been observed (*vide* Löffler: "Das Wasser u. d. Mikroorg.," Fischer, 1896). The sensitiveness of bacteria to a deficiency of water varies greatly. Upon drying nutrient media growth soon ceases. On the other hand, the duration of life upon nutrient media (agar, gelatin,

potato) which are drying slowly at the temperature of the room, is often astonishingly long, even when this cannot be attributed to the development of endospores. Even after the lapse of a year it is found occasionally that such a shrivelled remnant of a culture furnishes the most beautiful cultures in bouillon.

The question has often been investigated—and with very contradictory results—as to the length of the time during which bacteria free from spores, which have dried upon pieces of glass or threads of silk, may remain alive. We know now that this is influenced by numerous factors. An idea of their viability is furnished by the following table of Sirena and Alessi (C. B., XI., 484).

In bouillon cultures free from spores or in watery deposits of bacteria silk threads were dipped, and part of them were placed in test tubes one-third full of sulphuric acid or calcium chloride, part were allowed to dry in the open air under various conditions.

On drying.	Period at which Death Occurred (in Days).				
	By sulphuric acid.	By calcium chloride.	In incubating oven at 37 degrees.	In a dry room in the shade.	In a damp room.
Vibrio choleræ asiaticæ.....	1	1	1	1	12
Bacterium choleræ gallinarum	2	1	1	5	59
Bacterium typhi.....	41	1	18	64	68
Bacterium mallei.....	35	44	31		
Bacterium erysipelatos suum	63	53	31	5	59
Streptococcus lanceolatus....	114	31	131	164	192

Cholera vibriones are especially well known for their slight power of resistance to desiccation. Ex-

tended experiments of the authors just mentioned put their duration of life at from one to five hours according to the mode of desiccation (the results are similar to those of R. Koch in his first experiments).

But it is evident from the results obtained by all writers that desiccation experiments must be especially varied and many-sided, if they are to be convincing. The surprising result has recently been obtained in regard to very many varieties which are sensitive to desiccation (this is true particularly of the cholera vibriones) that, under certain circumstances, they may remain alive, when dried, for a much longer time. Thus Koch found the duration of life to be a few hours; Kitasato (Z. H., V., 135) fourteen days; French writers and Berckholz (A. G. A., V., 1) found it one hundred and fifty to two hundred days under specially favorable conditions. According to most writers these favorable conditions include: stay in the desiccator, removal from agar or potato cultures instead of bouillon cultures, the use of silk threads instead of pieces of glass. Special mention must also be made of the fact that in none of these experiments could anything of the nature of spores (arthrospores) be positively recognized.

5. RELATIONS TO OXYGEN AND SOME OTEHR GASES.

In their relations to oxygen bacteria are divided usually into three classes (Flügge and Liborius):

I. Strict Aërobics.—Growth occurs only when the air finds access; any obstruction to the latter interferes with the growth. Free oxygen is particularly necessary to the development of spores.

II. Strict Anaërobics. — Growth and sporulation take place only during complete exclusion of oxygen. This class includes bacillus œdematis maligni, bacillus tetani, bacillus Chauvoei, and a large number of inhabitants of slime and earth. When exposed to the free oxygen of the atmosphere the vegetative forms of these bacteria perish very readily, but the spores exhibit great resistance to oxygen. As the anaërobics are excluded from the main supply of energy which is at the command of aërobic bacteria (oxidation of the absorbed nutritive material by means of free oxygen), they must rely upon nutritive substances which possess great potential energy and which, like grape sugar, for example, set free energy (heat) by separation into two smaller molecules (for example, alcohol and carbonic acid; or acetic and lactic acids). Hence anaërobics are almost always cultivated upon gelatin or agar which contains one to two per cent of grape sugar.

III. Facultative Aërobics and Facultative Anaërobics.—The large majority of the bacteria which, as a rule, are cultivated aërobic (including almost all the pathogenic forms) tolerate a restriction in the supply of oxygen without suffering injury or exhibiting diminished growth. In many cases life in the animal body, for example in the intestinal canal, decidedly involves a diminution or abolition of the supply of oxygen. When oxygen is excluded the formation of pigment is almost always abolished, while virulent products of disassimilation are produced in greater abundance (Hüppe).

It is a very important fact that recent investigations

have shown that aërobic races exist among the anaërobic varieties.

It is observed not very rarely that varieties which on isolation exhibited more or less anaërobic growth (for example, grew chiefly into the depth of the agar stick canal), in time manifest a purely aërobic condition, *i.e.*, distinct growth upon the surface and dwarfed growth in the canal.

These observations show that two varieties cannot be distinguished from one another by simply calling one aërobic, the other anaërobic.

In addition to the strict anaërobics all the facultative anaërobic varieties thrive well in nitrogen and hydrogen, but they tolerate carbonic acid in various ways.

A large number do not flourish at all, but their development is entirely checked until oxygen is again supplied—for example, bacillus anthracis, bacillus subtilis, and allied forms. Of several varieties (anthrax, cholera) it has been ascertained that the majority of individuals are killed very quickly by carbonic acid, while certain ones offer a very vigorous resistance and render complete sterilization by CO_2 impossible. A second group exhibits—especially when the experiment is made at incubating temperature—feeble growth (staphylococci, streptococci), while a third group is not at all injured (bacterium prodigiosum, bacterium acidi lactici, bacterium typhi). These grow as well as they do in the air, and the liquefaction of the gelatin is not interfered with. As a matter of course, pigment is not formed on account of the absence of oxygen. A mixture of twenty-five per cent air with seventy-five per cent carbonic acid exerts no

demonstrable injurious influence upon bacteria which remain absolutely undeveloped in pure carbonic acid (C. Fraenkel: Z. H., V.)

Sulphuretted hydrogen in large amounts is always an active bacterial poison; small amounts kill the bacterium Pflügeri very rapidly (Lehmann and Tollhausen: C. B., V., 785).

6. INFLUENCE OF TEMPERATURE ON THE LIFE OF BACTERIA.

Each variety of bacteria makes certain demands upon the temperature of its nutritive medium. Vegetative bacterial life is possible from 0° to about 70°, but there are some varieties which flourish at the lower range, others at the upper range. In each variety the minimum and maximum of temperature are separated by about 30°, and the following comprehensive classification may be made according to the temperature requirements:

Psychrophilic bacteria: minimum at 0°, best at 15°–20°, maximum at about 30°. These varieties usually live in the water. They include, for example, many phosphorescent bacteria of the ocean (*vide* Forster: C. B., XII., 431).

Mesophilic bacteria: minimum at 10°–15°, best at 37°, maximum at about 45°. These include all the pathogenic varieties, because acclimatization to the bodily temperature is a necessary condition of their pathogenic action.

Bacillus vulgatus,* which still thrives at 50°, furnishes a transition to the following group.

* Bacillus vulgatus thrives at from 15°–50°, and one variety of Globig's ranges from 51°–68°, but such cases are very rare. Glo-

Thermophilic bacteria: minimum at 40°–49°, best at 50°–55°, maximum at 60°–70°. These include many bacteria of the soil, and almost all the sporulating bacilli related to bacillus mesentericus. According to Globig about thirty varieties are still capable of development at 60°, and a few at 70° (Z. H., III., 294). Miquel (*Ann. de Micrograph.*, I., 4; C. B., V., 281) has described a bacillus thermophilus Miqu., which thrives at from 42°–72°, best at 65°–70°, and has its habitat in privies, the intestinal contents, and dirty water. The description is insufficient to distinguish the bacillus.

Recently Lydia Rabinowitsch has described eight thermophilic facultative anaërobic varieties; they were all non-motile sporulating rods, which throve best at 60°–70°, but proliferated slowly even at 34°–44°, best in an anaërobic agar culture (Z. H., XX., 163). These varieties are widely diffused, particularly in the fæces, but Rabinowitsch did not make any comparison with the forms described by previous writers.

By gradually increasing and lowering the temperature Dieudonné (C. B., XVI., 965) succeeded in increasing the temperature interval within which the bacillus anthracis is capable of proliferating. The bacillus could be adapted gradually to a temperature of 42°. According to the assumption of some writers pigeons are tolerably immune to ordinary anthrax on account of their high temperature (42°), but when the bacilli had been adapted to high temperatures the pigeons died more frequently after inoculation.

Still more striking were the results when Dieudonné

big found unusually narrow ranges for many thermophile varieties; for example, one form grew only at between 54°–65°.

gradually acclimatized the bacilli to a temperature of 12° and showed that they could then kill frogs which are kept at 12°.

Temperatures somewhat below the minimum for the variety in question inhibit the development but are not otherwise injurious. Petruschky has recently recommended keeping them in an ice-box (about 4°–6°). He claims that in this way varieties which perish easily can be kept not only alive and capable of proliferation but also virulent, after they have been allowed to grow for two days at a temperature of 20° (streptococci, etc.).

Temperatures below 0° also act very slowly and injure the different varieties with varying rapidity.

If temperatures 5°–10° above the best act upon the culture, the latter is injured in various ways. Races of diminished intensity of growth develop, the virulence and fermentative power diminish, and the capability of sporulation is gradually lost. The injurious influence sometimes predominates in one direction, sometimes in another. If the maximum temperature is exceeded, the culture dies. For the psychrophilic forms about 37°, for the mesophilic forms about 60°, for the thermophilic forms 75°, are quite rapidly fatal temperatures. No bacterium free from spores can tolerate a temperature of 100° even for a few minutes.

7. MECHANICAL AND ELECTRICAL EFFECTS.

Our cultures are made almost exclusively upon nutrient media which are kept quiet (it is only to secure abundant sporulation in fluid media, in the

case of aërobic varieties, that a slight movement of the fluid is usually secured). Hence a theoretical interest alone attaches to the fact that, according to Meltzer's recent investigations, brief or feeble shaking of bacteria cultures in vessels one-third full acts favorably on the development of the bacteria, while constant and vigorous shaking for a number of hours, especially when balls of glass are placed in the fluid, scatters the bacteria into a fine dust and kills them. The various bacteria act in different ways (*Ztschr. f. Biolog.*, XXX., p. 454).

Meltzer makes the very remarkable statement that the feeble tremor which a steam engine running day and night communicated to the floor of a brewery was sufficient to kill, in four days, all the germs of bacillus mycoides and subtilis kept in a bottle of nutrient fluid.

Concerning our scanty knowledge of the influence of the electrical current upon bacteria, *vide* Friedenthal: C. B., Part I., XIX., 319.

The majority of the effects of the electrical currents hitherto observed are readily explained by the action of heat and electrolysis.

8. EFFECT OF LIGHT.

The development of many bacteria, perhaps of the majority, is impeded by the action of diffuse daylight upon the cultures, and still more by the action of direct sunlight. After a time the bacteria lose the power of proliferating freely in the dark and we obtain a generation of feeble organisms; for example, they liquefy imperfectly, form pigment imperfectly,

are less pathogenic, etc. It is only after repeated transference to fresh nutrient media in the dark that they regain their old power. When the action of light is still more prolonged the micro-organisms die. In order to test the sensitiveness to light it is best, according to H. Buchner, to expose to diffuse light or to sunlight densely crowded plates of gelatin or agar, a black paper cross being pasted on the light side. In order to exclude the action of heat the light may first be passed through a layer of water or alum a few centimeters in thickness. After exposure to the light for one-half, one, one and a half, two hours, etc., the plates are placed in the dark and it is noted whether the bacteria develop only at the location of the cross. When all the colonies which were illuminated have perished, we find a sharply defined cross, formed of cultures in a light field.

During March, July, and August bacteria putidum and prodigiosum are killed in one-half hour by direct sunlight. In November, at the end of one and a half hours, their power of producing pigment and trimethylamin is interfered with materially, they grow slowly, and bacterium prodigiosum liquefies poorly. The organisms died in one and a half and two and a half hours.

In diffuse daylight, inhibition of development occurs in the spring and summer in three and a half hours, in winter in four and a half hours; death occurs in from five to six hours. The electric arc light, of 900 candle power, inhibited development in five hours, and killed the germs in eight hours. Bacterium coli, bacterium typhi, and bacillus anthracis reacted in a similar manner.

The ultra violet, violet and blue light have a powerful injurious effect, green light has a feeble effect, and red and yellow have none at all.

The action of light seems to be dependent in part on the oxygen of the air. Strict anaërobic (tetanus) and facultative anaërobic varieties (bacterium coli) tolerate sunlight very well if there is complete exclusion of oxygen.

Richardson and recently Dieudonné have discovered a fact which possesses great importance in regard to the mechanism of the action of light, although it does not explain everything. They found that hydrogen hyperoxide (H_2O_2) develops in a short time (in ten minutes in direct sunlight) upon illuminated agar plates, but only in blue to ultra violet light.* An agar plate, half covered with black paper, is exposed to the light, then a paste containing a small amount of potassium iodide is poured over it and this followed by a weak solution of sulphate of ferric oxide, the illuminated side turns a bluish-black. In gases which contain no oxygen H_2O_2 does not form and light does not give rise to any injury. This also explains the fact that slight "attenuation" of the bacilli is also observed frequently when agar plates which have been standing in the sun † are inoculated. Bacteria which have been previously exposed to the light develop with special difficulty on an illuminated nutrient medium.

* Hours elapse before H_2O_2 can be demonstrated upon gelatin.

† Other decompositions of the nutrient media by sunlight may interfere occasionally with the subsequent growth of bacteria, for example, the development of formic acid from tartaric acid (Duclaux).

9. EFFECT OF OTHER BACTERIA UPON BACTERIAL GROWTH.

Although it is the object of every bacteriologist to obtain only pure cultures, it must not be forgotten that in nature bacteria often appear in mixed cultures. When water, milk, the intestinal contents of sick or healthy individuals, etc., are examined, several varieties will always be found at the same time. Although this admixture usually appears to be purely accidental, it is found on closer investigation that, in the domain of bacteriology, there are synergetic (favoring the growth of one another) and antagonistic (injuring one another) varieties. Nencki speaks of symbiosis and enantobiosis.

Garré demonstrated the antagonism experimentally by making streak cultures of various bacteria upon gelatin plates, in the shape of parallel or intersecting lines. It was then found that certain varieties thrive very poorly or not at all when another variety is growing in their immediate neighborhood. In very many cases the antagonism is one-sided. For example, bacterium putidum grows very well when inoculated between closely approximated, well-developed streaks of staphylococci. On the other hand, micrococcus pyogenes does not grow when inoculated between luxuriantly developing cultures of bacterium putidum, and the former remains very meagre when both varieties are applied in streak cultures at the same time (Garré: *Corresp. f. Schweizer Aerzte*, 1887).

Or we make plates of gelatin or agar (for liquefying varieties) which have been infected, in the melted

condition, with an equal number of individuals of two different varieties of bacteria. In many cases only one variety will undergo development (Lewek: C. B., VII., 107).

The following is the third method of making the experiment. The same fluid nutrient medium is inoculated with two varieties and later we ascertain the victor in the struggle, either with the microscope or macroscopically upon thin plates. To this category belongs the frequent experience that fermentation-producers, when present in large numbers in a suitable medium, prevail over contaminating bacteria. The latter sometimes disappear entirely.

The following practical inference may be drawn from these experiences. In counting bacteria very dense plates may not be regarded as decisive, and in the isolation of certain varieties thin plates may also be necessary. For example, in isolating bacterium Pflügeri from an abundance of bacterium putidum; no bacteria Pflügeri grow within a circle of several millimetres around each culture of bacterium putidum (K. B. Lehmann).

Finally, bacteria may antagonize one another within the animal body. As Emmerich showed, animals infected with anthrax may be saved by subsequent inoculation with streptococcus pyogenes. It is impossible to enter into the mechanism of this process within the limits of this work.

Greater practical importance attaches to the symbiosis of bacteria, as is shown by the following examples.

1. A series of bacteria thrive better in company with others than alone. Certain anaërobics even

thrive on the admission of air, if other aërobic varieties are present (*vide* bacillus tetani).

2. Certain chemical actions, for example, the decomposition of nitrate into gaseous nitrogen cannot be effected by some bacteria alone, while it can be done by two forms in combination. This experience is to be remembered in looking for the producers of certain decompositions. When the isolated varieties do not act singly or act incompletely, combinations must be examined.

3. In a similar way it has been observed, for example, that among a series of soil bacteria each single variety is not pathogenic, while certain combinations, when introduced into the animal, make the latter sick. This experience also merits special attention in the search for the producers of a new or obscure disease.

Some writers also assume the production of cholera by two germs (diblastic theory).

4. Feeble pathogenic varieties (for example, attenuated tetanus bacilli) are said to gain in virulence when cultivated with bacterium vulgare.

D. The Conditions of Formation and Germination of Spores.

Biological Characters of Spores.

The extent of the formation of endogenous spores appears to be imperfectly known at the present time. Apart from a large group of bacilli which are related to bacillus anthracis and bacillus tetani, undoubted endogenous spores are known only in sarcina

pulmonum and the peculiar spirillum endoparagocicum.

As H. Buchner (C. B., VIII., 1) showed, the formation of spores takes place in suitable varieties when the nutrient medium is beginning to be exhausted, *i.e.*, it is most rapid in very poor media.

On the other hand, a good nutrient medium not alone facilitates the development of the bacilli but also that of the spores, in so far as the vigorously growing bacilli also sporulate luxuriantly and constantly. The crop of spores is disproportionately large. Whether the quality (power of resistance) of the spores, which grow upon different nutrient media, also differs, does not seem to have been investigated methodically.

The temperature must sometimes (always ?) be higher for sporulation than for vegetative growth. For example, the bacillus anthracis flourishes at 13°–14°, but does not form spores under 18°.

All aërobic bacteria require the entrance of oxygen particularly for sporulation. The mode in which facultative anaërobic varieties act has not been ascertained.

Strict anaërobics produce spores only on the exclusion of oxygen or on the admission of oxygen in mixed cultures or when synergetic bacteria have perished.

Spores never germinate in the exhausted nutrient medium in which they have been formed, or which has been affected injuriously by the products of disassimilation. It is only after removal to a new nutrient medium that germination takes place (the morphological details have been described on page 79).

Spores are much more resistant than vegetative forms to all injurious influences. They require no nourishment or water in order to remain capable of germination for years and decades,* they are much more indifferent to gases than bacilli, and the spores of anaërobic varieties usually tolerate free oxygen well.†

The power of resistance of the spores to dry and moist heat is very considerable. Dry heat is tolerated relatively very well, and many spores resist a temperature of 100°. In the moist condition a temperature of 70° kills the anthrax bacillus in one minute, while the spores resist this temperature for hours, and in water or steam at 100° they live from two to five minutes, occasionally even from seven to twelve minutes. The varying resistance of different anthrax spores (v. Esmarch: Z. H., V., p. 67) seems to be partly a race peculiarity. It is very probable, moreover, that the nutrient medium, the temperature at the formation of the spores, the degree of maturity, etc., also exert an influence upon the resistance. Careful investigations on this subject are almost entirely lacking, but Percy Frankland has shown that spores formed at 20° are more resistant to light than those formed at incubation temperature (C. B., XV., p. 110).

*According to an observation of v. Esmarch the virulence of anthrax spores seems to be lost, in the course of time, before their power of germination.

†Dry garden earth containing the spores of malignant œdema preserved the latter excellently in my laboratory for four years. On the other hand, tetanus spores which were dried on threads and kept in the room had perished at the end of three days; they were still alive on the second day.

The resistance is tested by simply hanging in the steam chamber little tulle bags containing fragments or bits of glass upon which anthrax spores have been dried. From minute to minute a bag is removed and the bits of glass placed upon an agar plate which is kept at incubating temperature. Anthrax spores are obtained by careful removal of sporulating agar streak cultures, and warming the emulsion, prepared with little water, to 70° for five minutes.

The varying resistance of apparently identical anthrax spores possesses great practical importance: (1) in disinfection tests which may be made only with spores of known resistance; (2) in differential diagnosis, because it shows that we must be on our guard against creating two species based on a difference in resistance.

Various forms which occur in hay and soil possess remarkable resistance.

Christen found (C. B., XVII., p. 498), for example, that in steam under pressure the resisting spores of the soil required for their destruction: At 100°, more than sixteen hours; 105°–110°, two to four hours; 115°, thirty to sixty minutes; 125°–130°, five minutes or more; 135°, one to five minutes; 140°, one minute. The apparatus raised objects very rapidly to the desired temperature.

Spores are also very resistant to chemical agents. Thus, anthrax spores require, according to their origin (v. Esmarch: *l. c.*) a five-per-cent solution of carbolic acid at least two days, in some cases even forty days. A one-per-cent aqueous solution of corrosive sublimate is withstood by very resistant anthrax spores as much as three days, although their virulence was lost

in twenty hours. These tests are made best with thin deposits of the spores in water, to which the disinfectant is added, as we have indicated above in regard to the tests of antiseptic action against bacilli.

In order to test the resistance of spores to gases it is best to dry them upon pieces of glass; the gases are allowed to act first in a dry chamber, then in one saturated with water.

Spores are also less damaged by light than bacilli are; as in the case of bacilli an oxygenated atmosphere is necessary in order to produce injury by light. Anthrax spores on agar plates were found by Dieudonné to be killed by direct sunlight in three and a half hours (bacilli in one and a half hours); when oxygen was excluded they were not injured by exposure for nine hours.

E. The Effects of Bacteria, Especially in Regard to Their Employment for Diagnostic Purposes.

The effects of bacteria* *in vitro* may be classified as (1) mechanical; (2) thermal; (3) optic; and (4) chemical. They will be discussed in this order and a fifth section will deal with the effects of bacteria upon the living animal body and will explain the guiding principles necessary to the comprehension of

*It goes without saying that a classification of bacteria into zymogenous, saprogenous, chromogenous, and pathogenic, is no longer admissible. For example, bacterium coli produces fermentation in solutions of sugar, indol and sulphuretted hydrogen in albuminous media, brownish-yellow foci upon potatoes, and is pathogenic to guinea-pigs, *i.e.*, it combines the characteristics of all four groups.

their pathogenic influence, the struggle between the bacteria and the tissue cells.

All the effects of bacteria depend: (1) upon the present condition of the bacteria; (2) upon the nutrient medium; (3) upon the entrance of air; (4) upon the temperature; and (5) upon the illumination. A large number of other circumstances—in part less important, in part imperfectly known—also appear to play a part.

As the most important points in reference to temperature and illumination have already been given, I will discuss chiefly the influence of the nutrient medium and the entrance of air on the one hand, and the composition of the terminal culture on the other hand. Emphasis must be constantly laid upon the latter point in order to show as clearly as possible how much the effects of bacteria vary according as they are examined in a fully virulent zymogenic, chromogenic, or pathogenic condition, or in an attenuated condition.

1. MECHANICAL EFFECTS.

Under the microscope it is readily seen that many bacteria exhibit a pronounced active movement, and the study of flagella proves that almost all the motile varieties* present flagella and move by means of these appendages. The movement varies greatly in character; for example, creeping (bacillus megatherium), waddling (bacillus subtilis), sinuous (vibri-

*In the actively motile spirochæte Obermeieri and the slowly creeping beggiatoa flagella have not been demonstrated, so that the motion is supposed to be due to an undulating narrow membrane which encloses the organism.

ones). It is sometimes very slow, sometimes so rapid that observations in detail are hardly possible (bacterium typhi).

In some cases it is difficult to decide whether there is a real active movement or whether the micro-organisms do not exhibit an unusual degree of the so-called Brownian molecular movement—*i.e.*, the dancing and trembling which are also found in finely divided, non-organized particles. In such cases, apart from the attempt to render the flagella visible, it is well to examine the organism in a drop of five-per-cent carbolic acid or one-per-cent corrosive sublimate. If the movements then continue, we have had to deal only with molecular movements. Some varieties do not always exhibit movements of their own, but they may be absent in certain nutrient media. According to A. Fischer the vital movements may be lacking, although the flagella are perfectly developed—for example, in bacillus subtilis upon a nutrient medium containing two to four per cent ammonium chloride. In two different cultures of micrococcus agilis Ali-Cohen, drawn from a good source, we saw neither vital movements nor flagella, and reached the conclusion that the same variety may occur with or without flagella.

Certain chemical substances attract bacteria (positive chemotaxis), others repel them (negative chemotaxis). Oxygen in particular attracts aërobic, and repels anaërobic bacteria. As Beyerinck showed, very beautiful chemotaxic or aërotaxic figures can be obtained in the following way: In a test tube filled three-quarters full with sterilized water is placed an unsterilized bean, pea, or the like. By diffusion the

bean gives off nutritive substances, which slowly extend upward. In this feeble nutrient solution certain bacteria which have been introduced with the bean develop in sharply defined horizontal planes, which slowly ascend. Certain varieties form several planes above one another. I have had these interesting statements investigated by Mr. Miodowski, who corroborated them in great measure. But instead of the non-sporulating bacillus perlibratus Bey., which usually formed the planes in Beyerinck's experiments, we found chiefly an organism allied to bacillus mesentericus and bacillus subtilis (*vide* Beyerinck: C. B., XIV., 827, and Miodowski: Diss., Würzburg, 1896).

Schenk has observed a positive thermotropism. If a hanging drop containing bacteria is warmed at one point with a warm wire (temperature difference 8°–10°) the bacteria congregate in that spot (C. B., XIV.).

2. OPTICAL EFFECTS.

Phosphorescent bacteria are distributed quite widely, especially in and near salty media (the ocean, rivers, salted fish), and a considerable number—mainly bacilli and vibriones—have been carefully studied. Phosphorescence is a vital symptom and does not depend upon the oxidation of a photogenic substance secreted by the bacteria (K. B. Lehmann and Tollhausen: C. B., V., 785). It is destroyed by all factors which injure the life of the bacteria; cold produces rigidity of the organisms and interrupts the phosphorescence as long as it lasts. High temperatures, acids, chloroform, etc., interfere temporarily with the

phosphorescence. Living bacteria can always be obtained from phosphorescent cultures, and a filtered culture free from germs is always devoid of phosphorescence. But although the organism cannot give light without life, it may live without giving light—for example, in an atmosphere of carbonic acid. In like manner the muscles cannot contract without life, but they may be alive without contracting.

According to Beyerinck (C. B., VIII., pp. 716 and 651), who includes all phosphorescent bacteria in one (physiological) genus, photobacterium, they require peptone and oxygen in order to produce light. Four of his six varieties also require, in addition to peptone, a supply of carbon which may also contain nitrogen. Small amounts of sugar (dextrose, levulose, galactose, maltose), glycerin, and asparagin act in this way. In some varieties a higher percentage of sugar causes cessation of the phosphorescence, after the formation of acids and marked fermentation.

When the phosphorescence is to be maintained, we would recommend a gelatin nutrient medium, made by cooking fish in sea water, to which one per cent peptone, one per cent glycerin, and one-half per cent asparagin have been added. But even in this medium phosphorescence is soon lost if inoculations are infrequent, so that in the majority of laboratories the phosphorescent bacilli do not emit light. By repeated rapid transfers to a suitable nutrient medium we can often succeed in restoring the photogenic power. I recommend that two salt herrings be cooked in one litre of water, and ten per cent gelatin added to the filtrate without neutralization.

3. THERMIC EFFECTS.

The development of heat during the metabolism of bacteria is not noticeable in our ordinary cultures on account of its slight amount. Even luxuriantly growing, fermenting fluid cultures do not reveal to the hand any noticeable production of heat.

But there is no doubt, on the other hand, that the heat given out by moist decomposing organic matters, such as beds of tobacco, hay, manure, etc., depends, at least in part, on bacterial activity. In view of the high temperature produced, it is very probable, according to Lydia Rabinowitsch, that the thermophilic bacteria take part in the process. Careful investigations concerning the producers of these high temperatures are still wanting (*vide* Rabinowitsch: Z. H., XX., 163).

4. CHEMICAL EFFECTS.

The chemical actions of bacteria, which are accompanied in part by the production of light, and always by the production of heat, are known only in their main outlines, despite the extremely numerous and successful investigations of the last twenty-five years. In many cases we know only the final products, and have no accurate information concerning the mechanism of their development, the intermediate products, and the substances which appear in small quantities.

We may distinguish the following three principal varieties of chemical effects:

1. The bacteria store up their cell substance.

2. The bacteria excrete ferments, designed to make the surrounding nutrient medium more suitable for assimilation. The products which develop at this time in the vicinity of the bacteria may be called transformation products.

3. The bacteria assimilate some substances and excrete others—true products of disassimilation. A separation of fermentative products and disassimilative products, such as is still attempted at times, is incorrect because the substances are only fermented when they have previously entered the bacterium cell. Hence fermentation products are products of disassimilation under the influence of special nutrition (*vide* page 124).

I. Bacterial Ferments and the Changes Produced by Them.

Under the term ferments in the narrower sense (enzymes) we refer to chemical bodies which, in minimum amounts and without being used up, are able to separate large amounts of complicated organic molecules into simple, smaller, more soluble and diffusible molecules.*

Ferments may be regarded as chemical only when we can prove:

1. That the fermentation continues in the presence of substances (for example phenol, three per cent; thymol, .01 per cent; chloroform, ether) which kill bacteria but do not endanger ferments; or

2. That the germless filtrate of the bacterial culture

* This definition does not hold good for a single ferment, the milk ferment, which coagulates the milk (*vide* page 123).

through a clay or porcelain cylinder possesses the power of fermentation; or

3. That this power inheres in a sterile preparation of the ferment, made in the shape of a powder.

Of the extremely numerous details which we have learned from Fermi's methodical and thorough investigations, we can here give only the most important. All ferments dialyze as little as ordinary albuminoids through good parchment paper.

Proteolytic—*i.e.*, albumin-dissolving enzymes—are widely distributed. The liquefaction of the gelatin, which is chemically allied to albumin, in our nutrient media is sure evidence of the presence of a proteolytic ferment. As the reaction at which the gelatin is dissolved is always or may be alkaline, the bacteria cultures do not contain pepsin (which is effective only with acid reaction) but trypsin. The different bacterio-trypsins vary greatly in their resistance to heat (in a moist condition they tolerate a temperature of from 55°–70° for one hour), their sensitiveness to different acids, etc. Some are efficient even when a considerable amount of acid has been added, but they never act better than in an alkaline reaction.

The action on fibrin is much weaker than that on gelatin, and hence Fermi has recommended the following method as the most convenient and certain demonstration of the presence of even a trace of proteolytic ferment. A non-neutralized solution is made of about seven per cent gelatin in one per cent carbolic acid and equal amounts are placed in test tubes of the same size. The solution to be tested for the ferment is then placed on the solid gelatin, after receiving two per cent carbolic acid. We can then read

off on a millimetre scale, at the temperature of the room, the rate at which the liquefaction of the gelatin proceeds for days and weeks. Qualitative tests may be simply made by using 1 c.c. of a liquefied gelatin culture which has been sterilized with carbolic acid.* This material also suffices in testing the influence of the nutrient medium upon the formation of the ferment. By this method we may also compare the action of different degrees of concentration of different bacterio-trypsins. The less the percentage of gelatin and the nearer the temperature to incubating temperature, the more certainly do we obtain the action of even traces of ferment. In such critical cases the experiment is continued for two weeks and we then note whether the test tubes in the refrigerator, provided with the ferment, remain fluid, while the control tubes remain rigid.

In order to demonstrate the production of a true peptone, we proceed in the following way:

The variety of bacteria in question is cultivated upon a fluid albuminous nutrient medium free from peptone (blood serum, milk serum, milk). If the culture grows well, all the albuminoids, with the exception of the peptone, are precipitated by the addition of solid ammonium sulphate (about 30 gm. to 20 c.c.). Milk and milk serum may be warmed to 60°–80°, blood serum to about 40°. The precipitate is then filtered, the filtrate cooled; a part is made strongly alkaline by the addition of potash, and one-per-cent solution of copper sulphate is then added

* As a matter of course we must never fail to make a control test with two-per-cent solution of carbolic acid in water (free from germs).

drop by drop. The appearance of a rose color indicates the presence of peptone.*

The formation of proteolytic ferments varies in many, perhaps in all, species to a much greater extent than we would imagine from the ordinary descriptions. In the case of two phosphorescent vibriones Beyerinck found that one which at first liquefied gelatin very slowly, did so more rapidly after longer culture, while the other variety acted in the opposite way. Katz made a similar observation in experiments on Australian phosphorescent bacteria. Max Gruber and Firtsch have watched very closely the development of feebly liquefying races in vibrio proteus (A. H., VIII., 369), and similar statements have been made concerning cholera vibrio, bacterium vulgare, and micrococcus pyogenes. Indeed, some observers have even seen a liquefying streptococcus pyogenes.

We have also observed in many varieties that on thin plates the individual distinctly visible, superficial colonies exhibit very different degrees of liquefaction. In fact a beginner would be convinced that he had to deal with several varieties.

It is to be regretted that, as a result of these observations, one of the most convenient diagnostic aids, viz., the liquefaction of gelatin, has lost considerably in value.

The causes of the increase and decrease of liquefaction with prolonged culture are looked for in our artificial nutrient media, or in the influence of the

* Recent investigations have shown, however, that in addition to peptone a few albumoses remain unprecipitated in part by ammonium sulphate.

products of disassimilation of the micro-organism, but we are unable to give any positive data.

Concerning the influence of the nutrient media upon the formation of trypsin in a culture or the liquefaction of the gelatin, the following facts are known:

1. The majority of circumstances which impair the growth of a variety of bacteria upon a nutrient medium also interfere with liquefaction—for example, the addition of phenol, or a large percentage of glycerin. Wood found that the impaired power of liquefying gelatin, which was produced by phenol, was transmitted during several generations upon a good nutrient medium (C. B., VIII., 266).

2. The liquefying facultative anaërobics do not liquefy gelatin * in hydrogen and nitrogen, but they do in carbonic acid, if they are able to grow in the latter medium. As the gases, according to Fermi, have no effect upon the action of the ferment, they must influence the formation of the ferment. Strict anaërobics, on the other hand, produce the most pronounced liquefaction of gelatin.

3. In many bacteria the addition of sugar interferes not with their growth, but with the liquefaction of gelatin—for example, in bacterium vulgare (proteus vulgaris) but not in bacillus subtilis (Kuhn: A. H., XIII., 70). This is explained, perhaps, by the fact that bacterium vulgare produces an acid from sugar, and the vulgare trypsin is very sensitive to acids. Upon 10 c.c. of a one-per-cent grape-sugar gelatin, in five days bacterium vulgare produced 3.7 c.c. of one-tenth normal acid, vibrio proteus 2.1 c.c., bacillus

* With the single exception of bacterium prodigiosum, but this also ceases to liquefy on the addition of grape sugar.

subtilis 1.7 c.c., bacillus anthracis 0.9 c.c.; bacterium vulgare was the only one which did not produce liquefaction.

4. In fluid, non-albuminous, glycerin-containing (free from sugar) nutrient media, very few bacteria produce proteolytic ferments—for example, bacterium prodigiosum and bacterium pyocyaneum. The production of ferment also appears to be less on peptone bouillon than on peptone-bouillon gelatin (Fermi).

Upon albuminous nutrient media the liquefying bacteria produce bitter products of disassimilation —for example, this is done in milk by very many varieties (Hüppe). An enumeration of the trypsin-forming varieties is unnecessary because they are characterized as trypsin-producers by their liquefaction of gelatin.

The other ferments have been studied less carefully.

Diastatic ferments convert starch into sugar. They are demonstrated in the following manner: A thin starch paste containing one per cent thymol is combined with a culture to which one to two per cent thymol has been added, and is kept six to eight hours in the incubating chamber. A little Fehling's solution is then added and sugar is recognized by the reduction of copper (reddish-yellow precipitate) on boiling. We can also make a direct examination of mashed potato cultures of the bacteria by boiling the cultures and testing the extract.

According to Fermi about one-third of the varieties examined—only upon albuminous nutrient media —possess the power of forming such a ferment (A. H.,

X., and C. B., XII., p. 713) viz., the bacilli of the subtilis group (anthrax, megatherium, Fitzianus, etc.), the vibriones related to the cholera vibrio, also micrococcus tetragenus, micrococcus mastitidis, bacterium violaceum, bacterium mallei, bacterium pyogenes fœtidum, bacterium phosphorescens, bacterium pneumoniæ, bacterium synxanthum, bacterium aceticum; the others are not active or are doubtful. In addition all the actinomyces and oöspora varieties (with the exception of oöspora carnea). The majority of the varieties mentioned subsequently convert the sugar into acid but some do not, for example, bacillus subtilis.

Inverting ferments (*i.e.*, those which convert cane sugar into grape sugar) are rare, according to Fermi and Montesano. They are demonstrated in the following way: A one to two per cent solution of cane sugar containing one per cent of carbolic acid is added to a culture containing one per cent of carbolic acid. After a few hours we test whether the fluid reduces Fehling's solution; as is well known, cane sugar does not produce this reaction. Control tests with a solution of cane sugar alone are always necessary. Bacteria invertin tolerates (always?) a temperature of 100° for more than an hour, and also develops upon a non-albuminous nutrient medium if glycerin is present. The above-named writers mention only the following forms as producers of inverting ferments; bacillus megatherium, bacillus kiliense, bacillus fluorescens liquefaciens, bacterium vulgare, vibrio choleræ and Metschnikovii.

The attempts to find a ferment similar to emulsin have been unsuccessful. Micrococcus pyogenes

tenuis transforms amygdalin into benzaldehyd, but this function cannot be separated from cell life.

Rennet ferments—*i.e.*, bodies which coagulate milk of a neutral (or amphoteric) reaction and independently of the action of acids—are not wanting among the bacteria. It can be demonstrated, for example, in not too old cultures of bacterium prodigiosum which, sterilized at 55°-60°, can easily coagulate sterilized milk solid in one or more days (Gorini: C. B., XII., 666).

So far as I know, thorough investigations concerning the distribution of this ferment are still lacking. We may suspect it in all varieties which coagulate milk without possessing the power of forming lactic acid out of milk sugar.

II. The Chemical Actions of Bacterial Metabolism.

Like the production of ferments, the majority of the other chemical actions of bacteria depend, in great measure, on the nutrient medium. This is most striking when the growth of many forms of bacteria is observed upon an albuminous nutrient medium, which at one time is free from sugar, at another time contains sugar. In the former event, apart from pigment substances and perhaps some badly smelling substances, hardly any perceptible metabolic products are formed, but in the latter event there is often a very striking change, characterized by the development of gas and active production of acid. The organism produces fermentation in the sugar-containing medium, in the other it does not.

On account of the practical (and diagnostic) importance of the fermenting power we must here give a precise definition of this process. The term fermentation is used in literature in various senses.

1. Some writers call every typical decomposition produced by bacteria a fermentation, and speak, for example, of the putrid fermentation of albuminoids.

2. Others confine the term fermentation to processes which are attended with the visible development of bubbles of gas. According to this definition the conversion of nitric acid into nitrogen is a fermentation as well as the fermentation of milk sugar by bacterium acidi lactici.

3. Still others use the term only in cases of decomposition of hydrocarbons with or without the formation of gas.

It seems to me that the term fermentation is always in place when it can be shown that an organism, in addition to or instead of its other metabolic products, forms one or a few special metabolic products in an unusual amount—metabolic products which are almost always derived from the merely superficial splitting up of a bacterial nutrient which is easily split up. Oxidative fermentation is rarer. A necessary condition of fermentation is the presence of a definite nutrient matter which the bacteria attack with special ease, often discarding substances which are less accessible but which they decompose in the absence of the fermenting substance.

Every fermentation is intended to carry a supply of energy to the fermenting organism. In the fermentation which splits up organic material, this is due to the fact that the complicated, fermentible

molecule in the bacterial cell is decomposed into smaller particles, during which process heat is given off. I will illustrate this by the ordinary form of fermentation of sugar in which the process is very simple.

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$$

1 grape sugar = 2 alcohol + 2 carbonic acid.

Or,

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

1 grape sugar = 2 lactic acid.

Or,

$$C_6H_{12}O_6 = 3C_2H_4O_2$$

1 grape sugar = 3 acetic acid.

The organism requires such a source of energy, particularly when it grows in the absence of oxygen, and there is a failure of the source of energy at the command of the aërobic varieties and which consists in the oxidation of absorbed substances by the oxygen which has been taken up. Hence all anaërobic varieties are provided with great power of fermentation of sugar, and some facultative anaërobics only give rise to fermentation of a saccharine nutrient when oxygen is excluded.

In contradistinction to fermentation by the splitting-up process is the much rarer oxidative fermentation, the best example of which is the production of acetic acid from alcohol. Here we find a one-sided metabolic activity of the acetic acid bacteria. These obtain a considerable supply of energy, not by splitting up, but by oxidation of the absorbed alcohol. The gain in energy occurs simply from a one-sided intensification of the ordinary nutritive processes of bacteria.

It is evident from these remarks that products of

fermentation are products of metabolism like all the other products of the bacterial cell, and hence a division of fermentations in principle is not warranted. But it will be advisable to discuss the individual bacterial products according to their development upon a saccharine or non-saccharine nutrient medium, and then to add some functions of the bacteria which are manifested by decomposition of salts of the fatty acids, alcohols, etc.

A. Functions upon which the Amount of Sugar in the Nutrient Medium Exerts no Great Influence.

1. Formation of Pigment.

The chemistry of the pigment matters has been very little studied, but in recent times a preliminary survey has been made by some of Migula's pupils. In regard to the fluorescent pigments I follow the statements of K. Thumm ("Arbeiten d. bact. Instituts Karlsruhe," published by Klein and Migula, Vol. I., Pt. 3, p. 291) and those of Paul Schneider (*eod. loco*, Vol. I., Pt. 2, p. 201) in regard to the other pigments.

1. Red and Yellow Pigments. According to Schneider the twenty-seven yellow and red bacteria furnish, in almost all cases,* pigments which are soluble in alcohol, insoluble in water,† and are also

* The coloring matter of micrococcus cereus flavus Passet was soluble only in dilute caustic potash.

† A striking contrast to these results is furnished by M. Freund (C. f. B., xvi., 640). In examining four newly discovered red and yellow bacteria he found the pigment always soluble in water, and insoluble in alcohol and ether.

soluble in ether, carbon bisulphide, benzol, and chloroform.

The large majority,* in the dry condition, are colored bluish-green by concentrated sulphuric acid and red or orange by caustic potash, or they retain these colors when so treated. But the various pigments show various chemical differences and quite a different reaction in the spectrum. The majority may be placed unhesitatingly in the large group of lipochromata which are widely distributed in the animal and vegetable kingdoms, and to which belong the coloring matter of fat, yolk of the egg, the carotin of carrots, and many others.

Entirely different from these substances are the pigments of bacterium prodigiosum and bacterium kiliense. These take a brownish-red color with concentrated sulphuric acid, and a yellowish-brown and yellowish-red color with caustic potash. They are allied to one another but still quite distinct.† It has often been assumed, especially on account of the golden shimmer of the prodigiosum culture, that we have to deal here with a coloring matter resembling fuchsin, but on careful examination the resemblance is found to be very superficial.

Violet Pigments. Bacterium violaceum and bac-

* Thirteen red and fourteen yellow bacteria were examined, and the only exceptions were bacterium prodigiosum and bacterium kiliense. Schneider furnishes full tabulated statements concerning the reactions of the alcoholic solution and of the dry coloring matter with various agents, and also concerning the spectrum reactions.

† The fact that this coloring matter or one of its derivatives is not entirely insoluble in water is evident from the fact that in old agar cultures garnet-red pigment is diffused in the agar.

terium janthinum were found to contain a violet coloring matter, which was insoluble in water, readily soluble in alcohol, but insoluble in ether, benzol, and chloroform. In the dry state it is turned yellow by concentrated sulphuric acid and emerald green by caustic potash. In alcoholic solution it assumes a greenish to bluish-green color on the addition of strong acids and ammonia. The pigment loses its color on the addition of zinc and sulphuric acid.

Claessen and Schneider examined, in a very imperfect manner, the beautiful blue coloring matter of bacterium indigonaceum Claessen. This pigment is insoluble in the ordinary solvents; in hydrochloric acid it gives at first a blue, then a yellowish-brown solution. Other acids dissolve it but cause decomposition. Caustic potash gives a bluish-green color.

Distinct from these blue coloring matters is the blue pigment formed by bacterium syncyaneum (blue milk) in addition to bacterio-fluorescein (*vide* below) and for which I propose the term syncyanin. Thumm describes the pigment as very unstable. Acids color it steel blue; in slight acidity it is bluish-black, neutral black, and alkaline brownish-black.

According to the recent investigations of Thumm the fluorescent pigments, which are found in numerous cultures, are all identical. The coloring matter, for which I propose the term bacterio-fluorescein, is lemon yellow and amorphous in the dry state, soluble in water and dilute alcohol, and insoluble in strong alcohol, ether, and carbon bisulphide. The watery solution, when concentrated, has an orange color, when diluted, a pale yellow color; with acid reaction it shows no fluorescence, with neutral reaction a

bluish, with alkaline a green fluorescence. The fluorescence of the cultures is at first blue, later green, on account of the increase of the ammonia formed by the bacteria. The pigment is not sensitive to oxidizing substances. Colorless preliminary stages have not been observed. Phosphoric acid and magnesium are necessary to the development of bacterio-fluorescein.

The variations in the chromogenic functions have been the subject of numerous investigations. All possible factors which have an unfavorable influence on the growth of the bacteria also diminish the development of pigment. After continued culture upon unsuitable nutrient media or at improper temperatures, etc., the formation of pigment by later generations may remain permanently diminished.

For example, there are races of bacterium syncyaneum which form no trace of coloring matter in agar or milk, but on potato give a dark color even to the parts around the culture. The development of pigment appears to have been lost here simply on account of the rare inoculation of the agar cultures.

At 37° bacterium prodigiosum forms no pigment, and if the cultures are kept up at this temperature for a long time, the production of pigment will be lost for many generations even under favorable conditions (Schottelius).

Very interesting communications are scattered throughout the literature on pigment-forming races among otherwise colorless varieties. For example, Fawitzky reports yellow to rusty red colonies of streptococcus lanceolatus; Kruse and Pasquale observed colored races of streptococcus pyogenes

(Ziegler's "Beiträge," XII.). Kutscher has recently published the experience that a pseudo-glanders bacillus, taken from the animal, had a bright orange-red color only in the first culture upon serum, but this color changed to white after a few inoculations. Perhaps still greater importance attaches to the often made observation that, as the result of internal causes, colored and uncolored colonies of one variety, for example, bacterium kiliense, occasionally develop upon plate cultures.

2. *The Formation of Alkaline Metabolic Products and Urea Fermentation.*

According to v. Sommaruga (Z. H., XII., 273) aërobic bacteria, when growing in a non-saccharine nutrient medium, always produce an alkali from the albuminoids.

When sugar is present the majority of varieties form acid out of the sugar, in addition to the production of alkali, and the originally neutral or feebly acid reaction of many young bacterial cultures is explained simply by a slight percentage of sugar in the bouillon (derived from the meat). When the sugar is used up, the production of alkali becomes more pronounced (Th. Smith).

So far as we know at present, the alkaline bodies produced are ammonia (occasionally perceptible to the sense of smell), amine and ammonia bases. In order to determine the degree of production of the alkali, we titrate tubes which contain 10 c.c. peptone bouillon, uninoculated, and one to eight days after inoculation with one-tenth normal acid and phenol-

phthalein as indicator. The difference in the titrations gives the increase of alkali.

The following will serve as an illustration of the production of alkali by bacteria which in the presence of sugar form acid actively (5–7 c.c. normal acid to 100 c.c.). One hundred cubic centimetres of a nutrient medium containing traces of meat sugar and rendered neutral by phenolphthalëin used up:

When Inoculated with Bacterium Coli.

At the end of five days	0.1 normal sodium.
At the end of ten days.....	0.1 normal sodium.
At the end of fifteen days	0.25 normal acid.

A special form of alkali production by bacteria is the conversion of urea into ammonium carbonate: $CO(NH_2)_2 + 2H_2O = CO_3(NH_4)_2$.

Leube (Virch. *Arch.*, 100, p. 540) first isolated a few organisms from decomposing urine which produced ammonia from urea: micrococcus ureæ Leube, bacillus ureæ Leube. This is also done by sarcina pulmonum and a few other unnamed varieties. Flügge has described a micrococcus ureæ liquefaciens.

We have cultivated a large number of white liquefying and non-liquefying cocci and rods from decomposing urine. None of them possessed in any striking degree the power of setting free ammonia from diluted urine or a nutrient medium treated with urea, although they flourished in these solutions. It cannot be denied that natural urea fermentation depends partly on symbiosis.

The ability to decompose urea does not seem to be very widespread. Among twenty-four organisms examined Warington found that two alone (micrococcus

ureæ and bacillus fluorescens) produced ammoniacal decomposition of urine.

Among sixty varieties only three (bacterium vulgare, bacterium prodigiosum, and bacterium kiliense) developed a distinct ammoniacal odor in sterilized human urine.

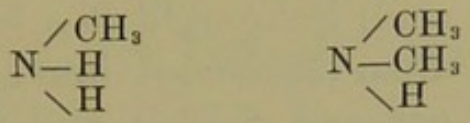
Leube employed Jacksch's nutrient solution: In 1 litre 0.125 acid potassium phosphate, 0.062 magnesium sulphate, 5 gm. Seignette salts, which were sterilized by boiling. To the sterile solution he added 3 gm. urea which had been sterilized in a dry state at 106° (boiling of urea solutions is to be avoided because a part of the urea is thus converted into ammonium carbonate). In order to demonstrate the presence of the ammonia Leube employed Nessler's reagent, a very sensitive test. For the study of the quantitative relations *vide* Brodmeier (C. B., XVIII., p. 380). Urea is not decomposed upon a nutrient medium which contains sugar. Burri, Herfeldt, and Stutzer (C. B., Pt. II., Vol. I., 284) recently described three rods which decompose urea very vigorously.

In addition to ammonia Brieger's investigations have disclosed a large number of basic crystalline nitrogenous bodies as products of bacterial metabolism. These bodies are now usually called ptomains (πτῶμα, putrefaction) or putrefaction alkaloids, when they are not poisonous, and toxins* when they are poisonous.

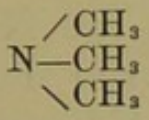
* With the growth of our knowledge of bacterial poisons the conception of toxins has been enlarged, so that now the majority of writers call all bacterial poisons toxins, irrespective of their chemical constitution.

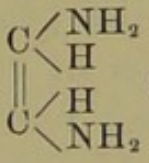
So far as they have been closely examined, the majority belong to the following groups:

1. Amins. Methylamin, dimethylamin, and tri-

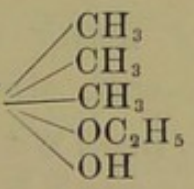


methylamin, similar to ethylamin, diethylamin, and



triethylamin. Ethylendiamin  and its homologues, dimethylethylendiamin-putrescin, with which sepsin is isomeric; pentamethylendiamin is called cadaverin. The most virulent one is ethylendiamin.

2. Ammonium Bases. The best known is cholin-

bilineurin = 

Muscarin ($C_5H_{15}NO_3$) is closely allied, and likewise vinylcholin ($C_5H_{13}NO$) and neuridin ($C_5H_{14}N_2$).

3. Pyridin derivatives. Derived from pyridin C_6H_5N; the principal ones that have been found are collidin $C_8H_{11}N$ and parvolin $C_9H_{13}N$.

4. Indol (C_8H_7N) and skatol (C_9H_9N), *vide* page 142.

In addition, amido acids (leucin, tyrosin, etc.), substances allied to guanidin ($C(NH)(NH_2)_2$) and numerous other imperfectly characterized bodies have been discovered. It would be useless to mention them here as the poisonous ones are no longer regarded as the true viruses of the disease (*vide* page 135).

The isolation of these bodies can only be hinted at. According to Brieger's method, which is usually employed, the culture of a feebly acid reaction (hydrochloric acid) is brought to a boil for a short time, the filtrate then condensed into a syrup, dissolved in ninety-six-per-cent alcohol, and then freed from impurities (especially traces of albumin) by alcoholic lead acetate. The lead is then removed, the filtrate concentrated, and from this the mercurial binary compound of the ptomains are precipitated with alcoholic solution of corrosive sublimate. When the alcohol has been removed by heat and the mercury by sulphuretted hydrogen, the characteristic gold and platinum binary compounds are produced, or we attempt directly to obtain the crystalline chlorhydrates and, by the aid of caustic soda, the free, often fluid, bases.

Some ptomains, like very many vegetable alkaloids, can be easily obtained with ether in a watery solution as soon as they have been set free by potash lye. But Brieger's method is much better because it secures many substances which do not dissolve in ether.

3. *Formation of Complicated "Albumin-like" Toxic Metabolic Products.*

("Toxalbumins," Toxins.)

In connection with the discussion of the relatively simple, basic, more or less poisonous metabolic products of bacteria, we may make a few brief remarks on other bacterial poisons. In the present state of our knowledge they may be divided into two classes.

1. Bacterial Proteins (Buchner).—This term refers to certain albuminoid substances which produce fever (pyogenic) and inflammation (phlogogenic). They are obtained by boiling, for several hours, potato cultures together with one-half-per-cent potash lye (about fifty volumes potash to one volume bacterial substance). The clear fluid, filtered through paper, allows the precipitation of the proteins after careful feeble acidulation. The proteins may then be washed, dried, and, before using, dissolved in a weak solution of soda.

The best-known protein is Koch's tuberculin. Mallein also belongs to this category. According to Buchner and Roemer all bacterial proteins have essentially the same action. According to Mathes deuteroalbumose, which is obtained by the action of pepsin on albumin and has no connection whatever with bacteria, produces the same effects on tuberculous guinea-pigs.

2. "*Toxalbumins.*"—C. Fraenkel and Brieger (*Deut. med. Wschr.*, 1890, 4 and 5) confirmed in great measure the statements of earlier observers (Christmas, Roux and Yersin, Hankin) that measures which precipitate albumin will also precipitate from the bouillon cultures of many bacteria amorphous poisons which exert an intense and usually specific (similar to the living culture) toxic action. They called these poisons toxalbumins and considered them analogous to the toxic albuminoid bodies in many plants (ricin in ricinus communis, abrin in abrus precatorius, etc.). The majority of investigators regarded—and some still regard—these poisons as "labile" albuminoids, which are derived from the bacterial cell.

They are also regarded as analogous to snake poisons and to the enzymes. With these bodies they share a great sensitiveness to heat, reagents, light, etc.

The toxalbumins are obtained as a raw product by precipitating, with absolute alcohol or ammonium sulphate, old bouillon cultures of the bacteria which have been concentrated in a vacuum, and which have been freed from living germs by passing through a porcelain filter. If the ammonia salt has been used, this is removed from the filtered precipitate by dialysis with flowing water in a parchment coil and, after renewed concentration in a vacuum, precipitation of the bodies with absolute alcohol. It has recently been discovered that zinc chloride precipitates these bodies quantitatively, and the toxins can be separated from the precipitate by the aid of sodium phosphate (Brieger and Boer: Z. H., XXI., 268).

From the beginning, however, doubts were expressed whether these toxalbumins were not merely carried down by the precipitated albumin and perhaps had no connection with the albumin.

In the case of tetanus poison, Brieger and Cohn (Z. H., XV., 1) succeeded in obtaining from the raw product, by means of lead acetate and ammonia, a pure virus which showed a faint violet color with copper sulphate and soda lye but gave no albumin reaction; it is free from phosphorus and almost entirely from sulphur. It thus seems to be proven that the tetanus virus is not an albuminoid.

The statements of Uschinsky that he obtained an albuminoid tetanus virus and diphtheria virus upon a non-albuminous nutrient medium have not been tested hitherto because German observers did not

succeed in securing a sufficient growth of these organisms upon a non-albuminous medium. Brieger and Cohn found that cholera vibriones formed a non-albuminous virus upon the Uschinsky nutrient medium. The diphtheria virus is also recognized now as free from albumin (Brieger and Boer: *l. c.*).

It is becoming more and more customary to call the bacterial poisons simply toxins and to ignore entirely the existence of the above-described crystallizable toxins of simple constitution.

Concerning the other characteristics of these toxins I will make a few remarks, taking the tetanus virus as an illustration (Brieger and Cohn: *l. c.*). Watery solutions are not coagulated by heat but lose their poisonous properties in time. The addition of small amounts of acid or alkali to produce solution, and prolonged transmission of carbonic acid and sulphuretted hydrogen impair the toxicity very materially. In the dry state the virus tolerates a temperature of 70° very well for a long time, higher temperatures decompose it rapidly. When dried and protected from light, air, and moisture, it is converted slowly into an inert substance. It is better preserved when covered with absolute alcohol, anhydrous ether, and the like.

The virulence of the purest tetanus virus is almost inconceivable. A mouse weighing 15 gm. is killed by 0.00005 mgm.; a man weighing 70 kgm., with the same susceptibility, would be killed by 0.23 mgm. Thirty to one hundred milligrams of strychnine are required to kill a man.

4. *Sulphuretted Hydrogen.*

Sulphuretted hydrogen is a very widely distributed bacterial product. It is easily demonstrated by fastening, by means of the cotton plug, a moist strip of lead acetate paper in the neck of the culture tube and closing it with a rubber cap (made of black rubber free from sulphur). Frequent observations of the originally brownish, later black, often very feeble discoloration of the paper is necessary, because sometimes the color fades away at a later period. Tests which are apparently negative should not be terminated too soon. The literature consists mainly of articles by Petri and Maassen (A. G. A., VIII., 318 and 490), Rubner, Stagnitta-Balistreri, and Niemann (A. H., XVI.).

Sulphuretted hydrogen may be formed from:

1. Albuminoid bodies. (It is well known that mere boiling eliminates H_2S from egg albumin). According to Petri and Maassen this power inheres in all the bacteria examined upon a fluid nutrient medium which is rich in peptone (five to ten per cent) and free from sugar; in bouillon free from peptone very few varieties form H_2S (for example, bacterium vulgare); in bouillon containing one per cent peptone, about fifty per cent of the bacteria (Stagnitta-Balistreri).

2. Powdered sulphur. In nutrient media to which pure powdered sulphur has been added all bacteria produce much larger amounts of sulphuretted hydrogen than without this addition. Petri and Maassen regard this production of sulphuretted hydrogen

as a function of the nascent hydrogen which the bacteria produce, *i.e.*, they regard the formation of H_2S as a proof of the formation of nascent hydrogen.

3. Thiosulphate and thiosulphite. This has been studied especially in yeast but has also been demonstrated in the case of some bacteria (by Petri and Maassen).

4. Sulphates. Beyerinck in particular has demonstrated this practically important function for his (morphologically poorly characterized) motile, strict anaërobic spirillum desulphuricans. It is rarely found developed among other bacteria (C. B., Part II., Vol. I., 1).

Rubner has shown that in bacterium vulgare the decomposed organic sulphur always suffices for the production of sulphuretted hydrogen.

The presence of sugar in the nutrient media rarely prevents or diminishes the production of sulphuretted hydrogen, even when the bacteria are able to decompose (ferment) sugar vigorously. The decomposition of hydrocarbons does not protect the albuminoids from decomposition. The presence of saltpetre is a disturbing factor and under these circumstances very little H_2S but an abundance of nitrite is formed (Petri and Maassen). The exclusion of oxygen favors the production of sulphuretted hydrogen. On passing air through the cultures of facultative anaërobic producers of sulphuretted hydrogen the amount of H_2S produced diminishes considerably, and in its place sulphates are formed.

Many producers of sulphuretted hydrogen also produce stinking mercaptan (CH_3-SH), demonstrable by the green color which it gives to the yellow-

ish-red isatin sulphate. Upon the culture glass is placed a tube open on both sides; this is filled with glass beads which are moistened with a one and a half per cent solution of isatin in concentrated sulphuric acid. The presence of sugar in the nutrient media diminishes or prevents the formation of mercaptan.

5. *Reduction Processes.*

(Reduction of Coloring Matters, Nitrates, etc.)

We have seen that aërobic bacteria in general possess the power of converting powdered sulphur into sulphuretted hydrogen and that nascent hydrogen is necessary thereto.

Similar processes, and probably also due in part to nascent hydrogen, are the following:

1. Reduction of blue litmus coloring matter, methyl blue, and indigo when added to colorless leuco-products. The upper layer in contact with the air often shows no reduction, only the deeper layers. On shaking in the air the color is restored, but occasionally the litmus coloring matter is restored with a red color on account of the coincident production of acid. The mode of experiment goes without saying; bouillon serves as the nutrient medium. According to Cahen the reduction of litmus is effected by all liquefying bacteria. It is observed very beautifully, for example, in bacillus fluorescens liquefaciens, but there are also non-liquefying varieties (for example, bacterium coli) which exhibit this characteristic.

2. Reduction of nitrates to nitrites and ammonia. The former power seems to belong to very many bac-

teria. At least Petri and Maassen found that, among six varieties cultivated in bouillon containing 2.5–5 per cent peptone and 0.5 per cent saltpetre, there was almost always a pronounced production of nitrites; in one case, indeed, only ammonia was found. Rubner (A. H., XVI., 62) found the production of nitrites absent only in isolated cases. Among twenty-five varieties Warington found that eighteen produced nitrites. In our experiments with bacterium coli, typhi, vulgare, bacillus anthracis, subtilis, vibrio choleræ, the addition of sugar was not a disturbing factor. At the end of three days the nitrite reaction was equally pronounced, with or without the presence of one per cent grape sugar, in one per cent peptone bouillon containing one-half per cent saltpetre.

Nitrites are demonstrated in the following way: After the tubes have remained for a few days in the incubating chamber, some potassium iodide starch solution (thin starch paste with one-half per cent KI) and a few drops of sulphuric acid are added to the nitrate bouillon (also to two uninoculated control tests). The control tubes remain colorless or at the most gradually acquire a very faint blue color, but if nitrites are present, a dark blue to dark brownish-red color develops. Small amounts of nitrite are demonstrated by metaphenylendiamin and somewhat diluted sulphuric acid (yellowish-brown color) or (most clearly) by a mixture of sulphanilic acid and naphthylamin (red color). (*Vide* Dieudonné, A. G. A., XI., 508).

The demonstration of ammonia by the addition of Nessler's reagent is permitted only upon inorganic non-saccharine nutrient media. In bouillon Ness-

ler's reagent is reduced almost immediately to black mercurial oxide. A strip of paper which has been dipped in the reagent may be hung over bouillon cultures, or the latter may be distilled after addition of MgO and the distillate treated with Nessler's reagent. A yellow to reddish-brown color indicates the presence of ammonia. Control tests must be made.

6. Aromatic Metabolic Products.

It is evident that albumin gives rise, under the influence of very many varieties of bacteria, to aromatic bodies of which indol, skatol, phenol, and tyrosin are the best known. Methodical investigations have been made only in regard to indol and phenol, as these bodies are easily recognized.

Demonstration of indol: To the bouillon culture—which should not be less than a week old and made without any addition of sugar—about half its volume of ten-per-cent sulphuric acid is added. If a rose to bluish-red color appears forthwith on warming to about 80°, then indol and nitrite are both present, as this nitrosoindol reaction requires the presence of both bodies. The test is generally successful in cholera and other vibriones and occasionally in diphtheria (red cholera reaction). But as a general thing the addition of sulphuric acid does not suffice, and it is necessary to add a little nitrite. This may be done later, after the culture has been heated without nitrite, and no reaction or a very doubtful one has been obtained. Of the solution containing about 0.05 per cent sodium nitrite we add 1 to 2 c.c. until the maximum of the reaction is secured. The addition of strong nitrite solutions gives the acid fluid a

brownish-yellow color and prevents entirely the demonstration of indol.

Demonstration of phenol: The culture, which is made in non-saccharine bouillon, receives about one-fifth its volume of hydrochloric acid and is then distilled. The distillate deposits flocculi with bromine water, or assumes a violet color on the addition of calcium carbonate and cautiously neutralizing, or of neutral very dilute ferric chloride.

Among sixty varieties examined we found indol formed twenty-three times, and our findings agree with those of Levandovsky (*Deutsch. med. Wschr.*, 1890, No. 51). The chief indol producers are the coli group in the widest sense—glanders, diphtheria, proteus, and the majority of vibriones. According to Levandovsky the indol producers just mentioned, with the exception of the vibriones, also form phenol. We have tested the production of phenol only in bacterium coli and vulgare and found mere traces in five-day cultures.

7. *Decomposition of Fats.*

Pure melted butter is not a nutrient medium for bacteria. The rancidity of butter is due to: (1) a purely chemical decomposition of the butter by the oxygen of the air, aided by sunlight (Duclaux, Ritsert); (2) a lactic-acid fermentation of the milk sugar which has been left over in the butter (*vide* v. Klecki, C. B., XV., 354). Finally fat is attacked by bacteria and acid is formed, if it is mixed with gelatin as a nutrient medium (*vide* v. Sommaruga, Z. H., XVII., 441).

8. *Putrefaction* (*Appendix to 1–7*).

Putrefaction, in the language of the laity, means every decomposition which is produced by bacteria and is attended by the formation of foul-smelling substances.

On scientific investigation it is found that the albuminoids and their allies are the substratum of putrefaction; at first they are often peptonized, then they are split up still further.

Typical putrefaction occurs only when the supply of oxygen is wanting or scanty. The vigorous passage of air through a putrefaction bacteria culture—a process which does not occur in natural putrefaction—modifies the process in the most marked manner. In the first place because the anaërobic putrefaction bacteria are killed or their growth is inhibited, and secondly by the action of the oxygen upon the products or intermediate products of the aërobic and facultative anaërobic bacteria. Finally, it seems conceivable that the same bacteria (anaërobic and aërobic) may from the start furnish different products of putrefaction.

Among the putrefaction products we find the bodies * described in preceding chapters: peptone, ammonia and amins, leucin, tyrosin and other amido bodies, oxyfatty acids, indol, skatol, phenol, finally

* It is often said that in every putrefaction the albuminoid bodies are first peptonized, but inasmuch as bacterium vulgare β Zenkeri, and bacterium putidum are generally recognized as producers of putrefaction, and as they do not even liquefy gelatin, we cannot always speak of peptonization of albumin as constant in putrefaction.

sulphuretted hydrogen, mercaptan, carbonic acid, hydrogen, marsh gas.

But inasmuch as, in putrefaction of different nutrient media by different bacteria, the metabolic products just mentioned are found, as a rule, only in part and in extremely varying combinations, putrefaction can hardly be defined more accurately with chemical aids than is possible with the senses. Hence I believe it is best to employ the term putrefaction only in the general lay signification of every foul-smelling decomposition of albuminoids (*vide* Kuhn: A. H., XIII., 1).

9. Nitrification.

The formation of small amounts of nitrous and nitric acids is widely diffused among bacteria. Heraeus (Z. H., 1, 193), who first investigated the subject with pure cultures, found that in sterilized urine which had been diluted fourfold very many of the well-known bacteria form small amounts of nitrite from urea or ammonium carbonate. These include micrococcus pyogenes citreus, bacterium prodigiosum, typhi, coli, bacillus mycoides, anthracis, vibrio pyogenes, and vibrio proteus. Various soil bacteria also furnish nitrites. The addition of sugar interferes with the production of nitrite from NH_3 until it is destroyed. The formation of nitrate was not studied by Heraeus. Warington failed to find nitrates in a study of twenty-four varieties in pure cultures in nutrient solutions which formed nitrate distinctly when infected by means of the soil (C. B., VI., 498).

According to more recent investigations nitrifica-

tion is particularly the function of a small, special group of bacteria which are cultivated with difficulty and do not thrive upon our ordinary nutrient media.

According to Winogradsky, who has done the most work in this department, the facts of the case are as follows: The soil of Europe contains, widely distributed, two micro-organisms, one of which (nitrosomonas) converts ammonia into nitrite, the other (called nitromonas, later nitrobacter) converts nitrite into nitrate. Both varieties are obtained mixed when bits of earth in flasks are dissolved in boiling water (Winogradsky took the water of a fresh-water lake) containing 1 gm. ammonium sulphate and 1 gm. potassium phosphate to 1 litre. About 1.0 gm. basic magnesium carbonate is added to each flask containing 100 c.c. Considerable development of nitrites takes place, and gradually nitrates are also formed. By inoculation of new flasks the nitrifying organisms are obtained gradually in a purer state, and silicic-acid plates finally permit, with difficulty, a pure culture. Burri and Stutzer have recently cultivated upon the ordinary nutrient media a vigorous nitrate producer (from nitrite), but it forms nitrates only upon inorganic nutrient solutions (C. B., Vol. I., Part II., 731).

P. F. Richter (C. B., XVIII., Part I., p. 129) observed on several occasions a pronounced nitrite reaction in fresh urine evacuated with the catheter. From one specimen he isolated a coccus of medium size, which in twenty minutes produced a very intense nitrite reaction in fresh urine. In addition it reduced nitrate to nitrite.

10. Conversion of Nitrous and Nitric Acids into Free Nitrogen.

This process is carried on by an entire series of bacteria. Burri and Stutzer (C. B., Part II., Vol. I., No. 7 *et seq.*) were the first to describe special nitrate fermenters in such an accurate manner that they could again be recognized. They first isolated from horse manure two bacteria, of which each alone was unable to produce nitrogen from nitrate, but did this vigorously when combined, and when the supply of oxygen was abundant or scanty but never when it was absent. These two synergetic bacteria are: (1) Bacterium coli (this may be replaced by bacterium typhi), and (2) a short rod described as bacillus denitrificans I. Later these writers found a bacillus denitrificans II., which alone effected the entire decomposition of nitrate into nitrogen. We found that bacterium pyocyaneum also converts saltpetre into nitrogen.

The practical importance of these organisms lies in the fact that through their agency considerable amounts of nitrates in the soil, but particularly in manures, may be lost for the nourishment of plants on account of their conversion into nitrogen.

11. Assimilation of Nitrogen.

According to our present knowledge no other vegetable family is able to assimilate the nitrogen of the air, but this power does inhere in one form of bacteria, the bacillus radicicola Beyerinck. This bacterium is found in the small root knobs of various

leguminosæ and may be cultivated from them. From the different forms of leguminosæ we obtain different races of the bacteria, each one being especially adapted to one form of leguminosæ; not every race is able to produce the knobs in every form of the vegetable. There are also "neutral" bacteria, found free in the soil, which are not specially adapted to any form of the leguminosæ and which are able to produce knobs in very different forms of the vegetable.

With the aid of these root knobs, which are due to the immigration of the root bacteria, the leguminosæ are able to furnish crops which are rich in nitrogen from a relatively sterile soil which is very poor in nitrogen. The manner in which the absorption of nitrogen takes place is still entirely unknown. It is claimed that the swollen zoöglœa form of bacteria (bacteroids*), almost always observed in the knobs, is alone able to absorb nitrogen. Recently it seems to have been proven that even without the aid of leguminosæ knob bacteria living free in the soil are able to absorb elementary nitrogen (for a detailed *résumé* of the present status of the question, see Stutzer: C. B., Part II., Vol. I., p. 8).

12. Production of Acids from Carbohydrates.

As Theobald Smith showed (C. B., XVIII., No. 1), the formation of free acid is only possible on a saccharine nutrient medium. Its production upon ordinary bouillon takes place only when the latter

* These bacteroids assume the most bizarre shapes, networks, forks, stars.

contains grape sugar (derived from the meat).* According to Smith all strict or facultative anaërobics form acids out of sugar, the aërobics either do not or they do it so slowly that the formation of the acid is concealed by the parallel production of alkali. Prior to a knowledge of this work we had found that all tested varieties of bacteria (about sixty), which are shown in the Atlas, formed more or less free fixed acid in one per cent grape sugar peptone bouillon (*vide* Table I). The formation of acid may or may not be attended with visible development of gas. Intense production of acid may kill the cultures (for example, bacterium coli, vulgare, etc.).

In many varieties the formation of acid or decomposition of sugar is intense and rapid, so that this metabolism, which is effected chiefly at the expense of the carbohydrates, is called fermentation. Inasmuch as this is attended not infrequently by the development of gas in large quantity, this term also seems justifiable to the laity.

If, after the sugar is used up, the amount of acid produced is insufficient to kill the bacteria, the metabolism which ensues is that common to the non-saccharine nutrient medium, the acid is gradually neutralized, and finally an increasing alkaline reaction sets in.

Among the acids produced (apart from carbonic acid, which will be considered under the heading of "Production of Gas") the most important and widely distributed is lactic acid. In addition we almost

* According to Th. Smith, seventy-five per cent of commercial beef contains distinct amounts of sugar (up to 0.3 per cent).

always find, at least in traces, formic acid, acetic acid, proprionic acid, butyric acid, and not infrequently some ethyl alcohol, aldehyde, or acetone. In rarer cases the lactic acid is wanting and only the other acids are formed.

In order to obtain and separate the acids we employ the following method: In 1 litre flasks are placed ½ litre peptone bouillon with two to five per cent grape sugar or milk sugar and perhaps 10 gm. calcium carbonate. The acids formed combine with the calcium carbonate into a soluble lime salt and carbonic acid escapes; the reaction of the fluid—and that is the main thing—remains neutral. A strongly acid reaction would interfere prematurely with the further growth of the bacteria.

When the growth has ceased (in eight to fourteen days) the undissolved carbonate is filtered off, and the reaction being neutral, the alcohol, aldehyde, acetone, etc., are distilled; the fluid is boiled down considerably during this process. The three substances just mentioned are detected in common by Lieben's iodoform reaction. To the slightly warmed fluid in a test tube are added five to six drops of pure ten-per-cent potash lye, then a weak iodine-potassium iodide solution is added drop by drop until a brown color is produced, and the latter is made to disappear by a drop of potash. The characteristic iodoform odor and the precipitation of microscopic small six-angled iodoform plates are convincing evidence. For the differentiation of alcohol, aldehyde, and acetone, *vide* Vortmann, "Analyse organ. Stoffe," 1891.

A strong acid reaction is now secured with phosphoric acid and the volatile acids are distilled off with the aid of a current of steam. The distillation must be prolonged because the complete removal of the volatile acids is difficult. The lactic acid is left in the distillate, is obtained by shaking repeatedly with pure ether, and the ether is then distilled off.

The lactic acid obtained is always ethylidenlactic acid $\begin{matrix} CH_3 \\ | \\ CHOH \\ | \\ COOH \end{matrix}$, which occurs in two stereoisomeric forms:

(1), dextro-rotatory with lævo-rotatory zinc salt, and (2) lævo-rotatory with dextro-rotatory zinc salt. If, as happens very frequently, exactly the same number of molecules of left and right lactic acids are present, then the combination is optically inactive and forms the so-called "fermentation lactic acid." I assume that both lactic acids often develop from sugar, but that some varieties of bacteria thrive mainly on one, some on the other form, so that sometimes there is a uniform combination, sometimes one form predominates or alone remains.

Since Schardinger (*Mitt. f. Chem.*, XI., 545) discovered that the previously unknown left lactic acid was the product of a short rod bacillus in water, the pupils of Nencki and Rubner have made numerous investigations on the lactic acids formed by the different varieties, in the hope of utilizing the results for purposes of differential diagnosis.

For the method of determining the form of lactic acid, *vide* Nencki (C. B., IX., 305) and Gosio (A. H., XXI., 115).

The most important results of the investigations are:

	Inactive lactic acid.	Right lactic acid = paralactic acid.	Left lactic acid.
Bacterium coli		+	
Bacterium Bischleri	+		
Bacterium typhi			+
Micrococcus acidi paralactici		+	
Vibrio choleræ (Calcutta)			
Vibrio choleræ (Massaua)			
Vibrio Metschnikovi			
Vibrio danubicus			+
Vibrio "Wernicke" I., II., I.–I.			
Vibrio "Dunbar"			
Vibrio proteus			
Vibrio Weibel			
Vibrio Bonhoff *b*			
Vibrio berolinensis	+		
Vibrio aquatilis			
Vibrio tyrogenes			
Vibrio Bonhoff *a*		+	

Although these results are not yet of much importance, a continuance of these theoretically interesting studies is desirable.

Various bacteria—which have in great part been imperfectly studied morphologically and biologically—are able to produce butyric acid, butyl alcohol, or both from carbohydrates.

A review of these varieties is found in an article by Baier (C. f. B., Part II., Vol. I., p. 17). Here we will only mention: bacillus butyricus Hüppe (apparently also other allied varieties), the imperfectly described granulobacter polymyxa Beyerinck, and several anaërobic varieties (clostridium butyricum of the authors).

In connection with the fermentation of sugar we

may mention the splitting up of cellulose by various bacteria, which are found particularly in the gastric and intestinal contents of herbivora, and in muck, and which form marsh gas as a striking product.

Unfortunately the decomposition of cellulose by bacteria has been imperfectly studied. It appears to be certain, however, that at least one anaërobic variety decomposes cellulose into marsh gas and carbonic acid. But the most recent investigator of this question, Van Senus, maintains that the anaërobic bacillus amylobacter isolated by him will attack cellulose only in symbiosis with another small bacillus (*vide* the *résumé* by Herzfeld: C. B., Part I., Vol. II., p. 114).

13. Formation of Gas from Carbohydrates and other Fermentible Fatty Bodies.

The only gas which develops in visible amounts upon a non-saccharine nutrient medium is nitrogen. If sugar is vigorously attacked by bacteria, the development of gas may be lacking inasmuch as pure lactic or acetic acid is produced (for example, typhus bacillus on grape sugar); but very often there is a notable development of gas, especially when the air is excluded. About one-third of the varieties which form acid vigorously also produce an abundance of gas. This consists of carbonic acid which, according to Smith (C. B., XVIII., 1) is always combined with hydrogen. Marsh gas appears to be formed rarely (apart from the bacteria which decompose cellulose). Last year Mr. Conrad isolated in my laboratory, a bacterium allied to bacterium coli, which gives rise to the fermentation of sauerkraut and

always, even when the nutrient medium is free from cellulose, forms some marsh gas in addition to carbonic acid and hydrogen.

In order to determine whether gas is formed, we should use the agitation culture on one-per-cent grape

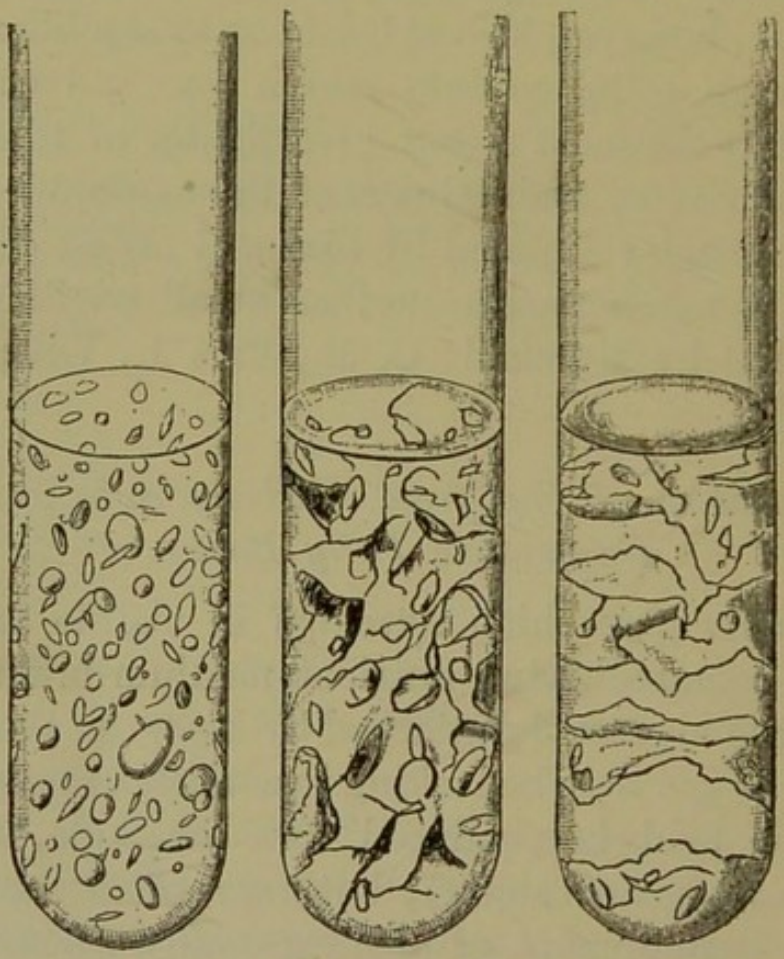


FIG. 11.—Bacterium Coli upon Sugar Agar at the end of Twelve, Twenty-four, and Forty-eight Hours.

sugar agar. At the end of twenty-four hours (often at the end of eight to twelve hours when incubating temperature may be employed) the agar is infiltrated with bubbles of gas or even split up by numerous deep rifts and fissures. If the gas is to be measured or analyzed, it is best, according to Th. Smith, to receive it in the fermentation flask which has been used

for such a long time in physiological and pathological chemistry.

The tubes, which should have the shape shown in Fig. 12, are filled with one-per-cent grape sugar peptone bouillon and sterilized in the steam chamber. After inoculation with a platinum loop in the incubating chamber the following facts develop:

Fig. 12.—Fermentation Flask.

1. If the opacity is produced only in the open spherical part of the flask, we have to deal with an aërobic variety; if produced only in the closed tube and the globe remains clear, we have to deal with an anaërobic variety.

2. The daily amount of gas produced is marked with ink; if the calibre of the tube is known, we are able to state, after the formation of gas has ceased on the fourth to sixth day, what percentage of gas was produced on each day.

3. A rough analysis of the gas should be made. After the amount of gas has been noted, we fill the open sphere completely with ten-per-cent soda lye, close it firmly with the thumb, and shake it for a while. At the end of two minutes all the gas is allowed to pass into the closed tube, and after the thumb is removed the new volume of gas is read off. The part which has disappeared is carbonic acid, the rest is nitrogen, hydrogen, and marsh gas. The quantitative analysis of these gases is best done by means of Hempel's gas pipettes (*vide* Winkler: "Lehrb. d. techn. Gasanalyse," Freiburg, 1892). The method

is based on the fact that hydrogen, when mixed with oxygen and passed over glowing palladium asbestos, is converted into water and accordingly disappears; carburetted hydrogen is changed into carbonic acid in a glowing platinum capillary tube, and is measured as such, and the remainder is nitrogen. With some practice the examination is easy and accurate.

14. Production of Acids from Alcohols and other Organic Acids.

It has long been known that bacterium aceti or its nearest allies convert weak solutions of ethyl alcohol into acetic acid, at the same time using up a large amount of oxygen:

$$\begin{array}{l} CH_3 \\ | \\ CH_2OH \end{array} + O_2 = \begin{array}{l} CH_3 \\ | \\ COOH \end{array} + H_2O$$

Higher alcohols, such as glycerin, dulcite, and mannite, are also converted into acids; glycerin as generally as sugar (v. Sommaruga: Z. H., XV., 291).

Finally, numerous observations have been made on the conversion, by bacteria, of acids of the fatty series (or their salts) into other fatty acids, but unfortunately the majority were not made with pure cultures which meet the modern requirements. Lactate, mallate, citrate, and glycerate of lime were usually employed as the material and almost always acid mixtures were obtained as the result of the bacterial activity. Among these butyric, propionic, valerianic, and acetic acids play the principal part; succinic acid and ethyl alcohol are often found; formic acid is rarer. Among the gases carbonic acid and hydrogen are especially prominent.

Such experiments were formerly made chiefly by Fitz, and recently have been performed with pure cultures and interesting results by P. Frankland. A couple of illustrations will suffice. Pasteur found that anaërobic bacteria convert lactate of lime into butyrate of lime.

$$\underset{\text{Lactate of lime.}}{2(CH_3-CHOH-COO)_2Ca} = CO_3Ca + 3\,CO_2 + 4\,H_2 + \underset{\text{Butyrate of lime.}}{(CH_3-CH_2-CH_2-COO)Ca}$$

According to P. Frankland the bacillus ethaceticus Fitz converts glycerate of lime $(CH_2OH-CHOH-COO)_2Ca$ into ethyl alcohol, acetic acid, carbonic acid, and hydrogen.

III. The Pathogenic Effects of Bacteria.

(Pathogenesis, Predisposition, Resistance, Immunity.)

Whenever we are able to recognize the nature of the pathogenic action of bacteria, they are found to act by means of the chemical substances which they form in the animal body or which are formed from them. But hitherto we have learned to comprehend only the action of those bacteria which produce toxic substances in cultures, and by means of which we can reproduce the characteristic symptomatology in a more or less accurate manner.

The bacteria of this category include particularly the bacillus tetani, bacillus diphtheriæ, streptococcus pyogenes, micrococcus pyogenes, vibrio choleræ, etc. On page 134 we have given a brief sketch of our chemical knowledge of these toxic substances.

In an entire series of important infectious diseases, on the other hand, we are almost entirely unable to explain them on a chemical basis. These include anthrax, rabbit septicæmia, hog erysipelas. Filtrates through porcelain of the most virulent cultures are inert; the cultures, which are cautiously killed by briefly warming them or by a short exposure to chloroform, produce only the general protein action (fever) when injected. Yet it is probable that even these diseases are toxæmias due to bacterial metabolic products.

It is to be regarded as an important finding that Petri and Maassen (A. G. A., VIII., 318) were able to demonstrate the sulphmethæmoglobin stripe in the fresh blood and œdema fluid of erysipelatous hogs —a sign that poisoning with sulphuretted hydrogen at least plays a part in the death of the animals. Similar evidence has also been obtained in malignant œdema.

Hoffa regards rabbit septicæmia as methylguanidin poisoning (Langenbeck's *Arch.*, 1889, p. 273). Emmerich and Tsuboi (*Münch. med. Wschr.*, 1893, No. 25) explain cholera as a nitrite poisoning, but this has been vigorously opposed.

These explanations are very interesting, but they do not seem to suffice, inasmuch as, apart from the toxic processes just mentioned, there are at least other specific processes in the blood and tissues of the animal. This is proven, among other things, by the development of specific protective substances ("anti-bodies").

In order that a pathogenic action may be observed the micro-organism must be in a condition of vigorous

virulence, the inoculation must be made upon a sensitive animal, and the proper channel of infection must be selected.

The virulence of bacteria varies like all their other functions (production of coloring matters, fermentation, etc.), and is best retained by constant inoculation from one sensitive animal to another. This is also done in many varieties by tolerably frequent transmission (about once a month) from one artificial nutrient medium to another, preferably with an occasional intermediate inoculation of an animal. On the other hand, the virulence usually suffers when, on account of rare inoculations, the cultures remain for a long time in contact with their accumulating metabolic products.

Attenuation of the virulence is easily effected:

(*a*) By making the cultures at somewhat too high a temperature. For example, at 42.5° anthrax is entirely deprived of virulence in three to four weeks, at 47° in a few hours, at 50°–53° in a few minutes. By the proper regulation of the action of heat the bacillus anthracis may be attenuated to such a degree that it will kill only mice, or mice and guinea-pigs, or these animals and rabbits.

Spores may also be "attenuated" by dry heat or brief, careful disinfection with steam.

(*b*) By cultures upon an unsuitable nutrient medium. The addition of phenol (1 : 600), potassium bichromate (0.04–0.02 per cent) was employed successfully to attenuate bacillus anthracis, iodine trichloride to attenuate the bacilli of diphtheria.

(*c*) By the action of sunlight, compressed oxygen, etc.

(*d*) By repeated inoculation of unsuitable animals. The bacilli of hog erysipelas become much less virulent from passing repeatedly through the rabbit; the organisms of variola (these are not bacteria) from passing through the body of the cow.

It is much more difficult to increase the virulence of those bacteria which have been attenuated. On the whole, it may be said that the virulence returns spontaneously so much more readily the more rapidly the attenuation has been effected.

Varieties which have slowly and spontaneously lost their virulence may often be restored to increased virulence in the following ways:

1. Culture in bouillon to which ascites fluid has been added (streptococci, diphtheria).

2. We first infect especially sensitive animals—particularly very young ones, such as young guinea-pigs—and, when these have succumbed, convey the germs (directly with the blood of the animals) to older and more resistant animals of the most sensitive species, later to more resistant species. Each passage through an animal increases the virulence until finally a certain maximum is reached.

3. Sensitive animals are infected with large amounts of the fresh bouillon culture of the bacteria. The metabolic products, which are introduced at the same time, then increase the predisposition for the injected organism.

4. Large amounts of the metabolic products of bacterium vulgare are injected with the bacteria (this has been especially useful in the case of staphylococci and streptococci). The explanation of the effect is the same as that of 3.

5. We inject—for example, with the attenuated bacillus œdematis maligni or anthracis—another variety which *per se* is almost entirely harmless—for example, bacterium prodigiosum.

6. We inject the culture, mixed with an injurious substance of non-bacterial origin—for example, lactic acid. In bacillus œdematis maligni this has produced increased pathogenic power, probably from local impairment of the anti-bacterial activity of the animal at the site of inoculation.

The susceptibility of different species of animals and of different individuals to different infectious diseases varies from birth in a striking and not easily explained manner.

Certain species are absolutely immune against special infection-producers.* For example, man against rinder pest, the cow against glanders, and all animals which have been tested against syphilis, malaria, and gonorrhœa.

A series of other diseases is conveyed very rarely and with difficulty to certain animals—for example, anthrax to certain varieties of pigeons, rats, and sheep. This constitutes relative immunity. The more vigorous and, as a general thing, the more mature an animal is, the more completely is its relative immunity developed. Noxious influences of all kinds (hunger, cold, excessive exertion, ingestion of

*It is especially remarkable that very closely allied varieties often exhibit astonishing differences. For example, the glanders bacillus can be conveyed very readily to the field mouse but not to the house mouse; the bacillus anthracis kills the house mouse with almost absolute certainty and is hardly pathogenic to the rat. Micrococcus tetragenus is pathogenic to the white variety of the house mouse, but is not virulent to the gray variety.

certain poisons) diminish the immunity to a considerable extent, so that a large number of organisms which are weakened in this manner succumb to a subsequent infection.

Hence in every newly isolated variety of bacteria whose pathogenic action we desire to prove it is necessary to experiment upon various animals if the experiments on those first selected proved negative. The principal animals for experimentation are: the white domestic mouse, white rat, guinea pig, rabbit, chicken, pigeon, and, for special purposes, the monkey. More rarely we employ the gray domestic mouse and rat, field mice, dogs, cats, cows, sheep, pigs, and horses. The most convenient animal, but one requiring good care, is the guinea-pig, characterized by suitable size, mildness, and modest consumption of food. Animal plagues are studied and explained much more readily than human diseases, because the animals are at our disposal for experimentation. In difficult cases various experiments in infection have also been made upon man.

The causes of congenital immunity (resistance) reside in protective arrangements of the organism which I cannot here consider in detail. It may be said, however, that the views formulated by Buchner as a compromise between the various opposing theories are in tolerable accord with all the facts. In an invasion of pathogenic germs into the resisting organism a part is destroyed by substances (alexins) already present in the serum (and derived from leucocytes); another part is destroyed by substances which are produced from leucocytes (or other tissues) under the influence of the bacteria. A part of

the germs which are destroyed by the leucocytes is absorbed by the latter secondarily, but some germs are undoubtedly ingested alive by the leucocytes. Metschnikoff — the most redoubtable antagonist of Buchner—insists upon the view that the latter process (phagocytosis), followed by subsequent death of the germs within the leucocytes, is the essential feature of natural immunity.

An increase of the congenital resistance to various infectious diseases has been effected in a number of ways. Thymus extract, spermin, abrin (toxic albuminoids from the paternoster pea), papayotin (albumin-dissolving ferment from the papaya), cinnamic acid, iodine trichloride, sodium carbonate, etc., when injected into animals have produced favorable effects, sometimes in one, sometimes in several infectious diseases. Indeed, an increased resistance has been observed from the injection under the skin, but especially into the peritoneal cavity, of an entire series of ordinary albuminous substances, such as blood serum and bouillon.

It is generally assumed that this effect depends upon increased stimulation of the leucocytes to the production of substances which are antagonistic to the bacteria.

According to the majority of writers there is a sharp contrast between this increased resistance and the specific immunity from a definite disease which develops when an individual has spontaneously acquired and passed through this infectious disease or when he has been purposely inoculated with:

(1) Naturally or artificially attenuated infection-producers of the same variety; or

(2) Extinct cultures of the micro-organism in question; or

(3) The blood serum or tissue juices of an animal immunized by the plans mentioned under (1) and (2). After (1) and (2) there develops an active immunity, after (3) a passive immunity.

According to the most widely entertained opinion specific immunity depends upon the presence of specific "antisubstances" (Behring) in the blood and tissues of the immunized animal. According to Buchner the "antisubstances" are derived from the injected bacteria cultures and are much more resistant than alexins to noxious influences. Thus tetanus antitoxin tolerates a temperature of 70°–80° and the action of sunlight and putrefaction without decomposing. Brieger and Ehrlich have extracted diphtheria antitoxin in a solid form from the milk of goats which were rendered immune against diphtheria. Whether it is an albuminoid or adheres to albuminoids, is not yet known. The antitoxins are best extracted (Brieger and Boer: Z. H., XXI., 266) by means of zinc chloride, but we have not yet succeeded in freeing them from the last traces of zinc. According to Emmerich the "antisubstances," which he calls "immune proteidins," are combinations of a substance furnished by the bacteria with body albumin from the immunized animal.

In some cases the character of immunity, the action of the "antisubstances," is purely antitoxic, a true antidote. The notion, first advanced by Behring and Kitasato, that toxin and antitoxin neutralize one another chemically (somewhat like an acid and its base) has not been corroborated. We have to deal rather

with an antagonistic action upon the cells of the body analogous to the action of atropine against morphine, except that the antisubstances possess a minimum toxicity or none at all. The proof that an ineffective mixture of toxin and antitoxin still contains a virus is furnished, for example, by the fact that guinea-pigs, upon whom antitoxin has less protective action than upon mice, can be poisoned with mixtures of toxin and antitoxin, which are entirely devoid of effect on mice (Buchner).

While the "antisubstances" of diphtheria protect very well against the diphtheria virus, they have no injurious effect on the diphtheria bacilli either *in vitro* or *in vivo*, *i.e.*, they are not bactericidal. The diphtheria bacilli may grow in the interior of an immunized organism but they are not harmful.

Entirely different in principle is the mode of action of the "antisubstances" in cholera. Here they are exquisitely bactericidal, but do not protect against large amounts of the cholera virus (R. Pfeiffer). According to Emmerich, this is also true of hog erysipelas and pneumonia.

Much attention has been devoted to the question of the specific action of the "antisubstances." Richard Pfeiffer, the strongest advocate of their absolutely specific action, has defended successfully the following standpoint in regard to the cholera vibrio and its allies: Every pathogenic organism furnishes, in the body of the actively immunized animal, "antisubstances" which exert a bactericidal action (often extremely pronounced) only against the organism in question but not against its closest allies. This specific action is so pronounced that Pfeiffer regards

it as the most valuable diagnostic measure, for example, in deciding the question whether an organism is to be regarded as a cholera vibrio or not. Pfeiffer made the same discovery in regard to bacterium typhi and its allies, and this is corroborated by Dunbar, Sobernheim, Löffler, and Abel.

It must not be forgotten, however, in opposition to these very interesting and surprising findings that a number of investigators (for example, Hüppe) do not recognize a sharp distinction between resistance and specific immunity, but acknowledge only quantitative, not qualitative, differences. At all events, we still have much to learn in this difficult field.

Technical Appendix.

The following recommendations and brief descriptions furnish all the technical directions which are given in a thorough course of bacteriology. We have given only the most necessary data and those which in our experience have proved most practical.

I. MICROSCOPICAL EXAMINATION OF BACTERIA.

1. Hints on Microscopical Technique.

For bacteriological examinations we use almost exclusively the modern microscope with Abbé's illuminating apparatus, iris diaphragm, a low-power lens, and an oil immersion lens.

A. Low magnifying power (sixty to one hundred times) and narrow diaphragm are used for careful examination of plate cultures. For this purpose we either raise the cover* and examine the colony from

* Our plate cultures are always poured into cups.

above, or, if we do not wish to soil the plate by opening it, place it upon the cover and examine the colony from below. This does not give such characteristic appearances in all cases.

B. High magnifying power. Oil immersion (seven hundred to twelve hundred times) is used in the observation of individual bacteria. Upon the preparation is placed a drop of oil of cedar, the tube of the microscope pushed down by means of the coarse adjustment until the lens just touches the surface of the oil, and then adjust it accurately on the preparation with the micrometer screw.

(*a*) Unstained Preparations. Narrow diaphragm. They are examined in two ways:

1. A drop of a fluid pure culture or a drop of water mixed with a trace of pure culture is placed between the slide and cover-glass; or

2. In the hanging drop. A platinum loopful of fluid pure culture, or a loopful of bouillon mixed with a trace of pure culture, is placed on a cover-glass, and this laid (reversed) upon a slide which has been hollowed out so that the drop lies in the cavity. The cover-glass is then fixed to the slide by applying a trace of water to the four corners of the cover-glass or by applying vaseline, if prolonged observation is required.

FIG. 13.

(*b*) Stained Preparations. Open diaphragm. Abbé's illuminating apparatus. To observe double-stained section preparations we require wide diaphragm for the bacteria and narrow diaphragm for the tissues.

C. Cleansing of the preparations and the micro-

scope. The immersion oil is always brushed off gently, and now and then the lens is rapidly cleansed with xylol and chamois skin; prolonged action of xylol loosens the setting of the lens. Xylol also removes dried particles of oil from the cover-glasses of old preparations.

2. The Most Important Solutions for Making Preparations.

A. Staining Solutions.

1. Watery alcoholic solution of fuchsin and methyl blue. A concentrated "stock solution" is made by pouring absolute alcohol over the powdered coloring matters (fuchsin, methyl blue) in bottles, shaking, letting them stand for a few hours, and then filtering. Of this saturated solution one part is mixed with four parts distilled water and filtered before using. In order to obtain good preparations it is better to stain for a longer time with weak solutions than for a shorter time with strong solutions.

2. Carbolized fuchsin (Ziehl's solution):

Fuchsin	1.0 gm.
Acid. carbolic. liq.	5.0 "
Alcohol	10.0 "
Aq. dest.	90.0 "

3. Aniline fuchsin: 4.0 aniline oil (anilin. pur.) are well shaken for several minutes with 100 aq. dest., then filtered until all the water runs off clear (then the funnel is removed because otherwise the oil will pass through). In this aniline water are dissolved 4.0 gm. fuchsin and it is then again filtered.

4. Aniline gentian (Ehrlich's solution): To 100 c.c. aniline water add 11 c.c. of an alcoholic concentrated gentian violet solution (stock solution). This solution does not keep long.

5. Löffler's methyl blue: To 100 c.c. water, which contains 1 c.c. of a one-per-cent potash lye, add 30 c.c. of a concentrated alcoholic solution of methyl blue. The staining power is increased by the addition of the alkali.

6. Bismarck brown: Prepare like No. 1. (Stains tissues, but bacteria poorly).

7. Alum carmine: To 100 c.c. of a five-per-cent alum solution add 2 gm. carmine, boil for an hour, and filter.

B. Differentiation Measures.

1. Distilled water.
2. Absolute alcohol.
3. Iodine-potassium iodide solution (Gram).

Iodin. pur	1.0
Potassii iodidi	2.0
Aq. destil	300.0

4. Sulphuric acid (twenty-five per cent).
5. Acetic acid (three per cent).
6. Acid alcohol.

Alcohol (ninety per cent)	100 c.c.
Distilled water	200 "
Pure hydrochloric acid	20 gtt

C. Mordants for the Flagella.

Löffler's mordant:

10 c.c. alcoholic solution of fuchsin.
50 c.c. cold saturated ferrosulphate solution.
100 c.c. twenty-per-cent tannin solution.

2. Bunge's mordant:

25 c.c. of a twentyfold diluted officinal ferric chloride solution.
75 c.c. saturated watery solution of tannin.

To this solution is added, immediately before using, enough of a three-per-cent solution of hydrogen peroxide to produce a reddish-brown color, and it is then filtered (we have always dispensed with the peroxide).

D. Substances Used for Clearing Up and Mounting.

1. Xylol.
2. Canada balsam.
3. Dammar varnish.

3. Preparation of Stained Specimens of Bacteria.

A. Smear Preparations.

1. Ordinary Stain with Fuchsin or Methyl Blue. This may be used for all bacteria with the exception of the tubercle bacillus.

We place upon the cover-glass or slide a loopful of distilled water, mix with it a trace of pure culture (best from a solid nutrient medium) and then spread the drop in a very thin layer. After the fluid has evaporated the preparation, with the layer turned up, is rapidly drawn three times through the flame in order to fix the bacteria on the glass (not to burn them) and the layer of bacteria is covered with the staining solution. After a brief interval (one minute), perhaps after feebly warming the glass, the preparation is washed with water and allowed to dry (some-

times after cautious warming). By means of a drop of Canada balsam the dry cover-glass is finally fixed to the slide with the bacterial layer downward.

2. Gram's Stain.

(1) Making the smear preparation as above.

(2) Staining with Ehrlich's solution three minutes.

(3) Washing off with water.

(4) Differentiation with iodine-potassium iodide solution one minute.

(5) Decolorizing with absolute alcohol up to colorlessness (usually one to two minutes).

(6) Drying and mounting.

For the species which are adapted to Gram's stain, *vide* the table. In our experience the common opinion that every variety of bacteria may be prepared invariably either well or not at all according to this method is erroneous. For example, we observed among the fluorescents, which are usually described in literature as unstainable, that three varieties out of twelve stained very beautifully after twenty-four hours' culture. Indeed, according to Zimmermann, all fluorescents may be stained in young cultures.

In like manner we were able to stain the bacillus of symptomatic anthrax which has often been regarded as incapable of staining. The contradictory statements may be explained in part by the fact that the material employed has varied greatly in age, and also that the differentiation with alcohol was effected in different ways. But tyrothrix tenuis, which has been regarded as unstainable by Gram's method, was found to stain very well on a subsequent test of the same culture with the same technique. At all events

at each staining a fresh preparation of anthrax bacillus should be stained at the same time and all preparations differentiated with alcohol for an equally long time (one or two minutes). We can then judge very well whether one variety of bacteria retains or gives off the coloring matter.

3. Capsule Preparation. According to Johne we proceed in the following manner:

(1) Heating the preparation with two-per-cent solution of gentian violet until steam is given off.

(2) Washing with water.

(3) Moistening with two-per-cent acetic acid for six to ten seconds.

(4) Washing with water.

By this method a very distinct membrane around the intensely colored bacterium cell can often be demonstrated in varieties which are not regarded as "capsular bacteria." The capsules are seen best on examination in water.

4. Staining of Flagella. The flagella, which are almost always invisible when unstained, are generally prepared according to Löffler's method:

(1) Rubbing up a trace of young agar streak culture (not bouillon) in a very small drop of water; spread out well, dry rapidly.

(2) Heating of the preparation with mordant until steam is produced (do not boil) for one-half to one minute.

(3) Washing off in a vigorous stream of water.

(4) Washing off in alcohol in order to remove the remains of the mordant adherent at the edges.

(5) Dropping of the staining fluid (a few crystals are dissolved in 10 c.c. aniline water, and then one

per cent soda lye is added drop by drop until the clear fluid just begins to grow opaque) and heating for one minute until steam is evolved.

(6) Washing off in water, drying, mounting in Canada balsam.

The manipulations must be carried out with the most scrupulous cleanliness, and the cover-glasses must be especially well cleaned with acids and alcohol. The cultures must be young, although it is not necessary, as some authors maintain, to make the staining only in cultures that are twenty-four hours old. We have often obtained very good preparations even at the end of twelve days. The mordants are usually prepared fresh.

According to Löffler, it is necessary, in the case of the majority of bacteria, to add a definite amount of acid or alkali to the mordant in order to obtain well-stained flagella. Löffler advises that to 16 c.c. of the mordant there be added for:

	Drops.	Soda lye.
Cholera vibrios	½ to 1	1 per cent
Spirillum rubrum	9	1 "
Bacterium typhi	20 to 22	1 "
Bacillus subtilis	28 to 38	1 "
Bacillus œdematis maligni	36 to 37	1 "
Bacterium pyocyaneum	5 to 6	Equivalent sulphuric acid.

Our results show that in the majority of cases we obtain very useful pictures with the unchanged mordant and that the addition of alkali or acid is by no means material. Similar experiences have been had by other writers, for example, Lucksch, Günther, A. Fischer, Nicolle and Morax, but our investigations have not been concluded.

Bunge has recently employed a somewhat different method which also gave us good results, but, like Löffler's method, occasionally left us capriciously in the lurch.

(1) Preparation of the specimen, according to Löffler.

(2) Heating with Bunge's mordant for one minute until steam is produced.

(3) Careful cleaning with water and drying.

(4) Warming slightly with carbolized gentian violet or carbolized fuchsin.

(5) Washing in water, drying, and mounting in Canada balsam.

Most of our specimens are prepared with Bunge's mordant which is several months old.

5. Staining of Endospores.*

According to Hauser:

(1) Preparation of the specimen. (It should be drawn ten times, instead of three times, rapidly through the flame.)

(2) Staining with watery fuchsin or carbolized fuchsin (Ziehl's solution); the preparation, over the flame, is covered freely with the staining fluid, and heated (not boiled) one to two minutes until there is an indication of simmering. The evaporating staining fluid is replaced constantly by fresh fluid.

(3) Washing with acid alcohol,† until the red color of the preparation is almost gone.

*Arthrospores possess no undisputed color reactions. For metachromatic corpuscles, Ernst's and Bunge's granules, preliminary stages of spores, *vide* page 71.

†Instead of acid alcohol we may also use thirty per cent nitric acid, five or twenty-five per cent sulphuric acid, but these must be allowed to act for a shorter period.

(4) After-staining with methyl blue (a few seconds). The spores remain red, the bacilli blue.

6. Staining of Tubercle Bacilli. This is done according to the same principles as the staining of spores. The preparation is treated in the flame with a deeply staining solution and then everything with the exception of the tubercle bacilli is decolorized with some acid solution.

(*a*) We may manipulate exactly as in spore staining (according to Ziehl-Neelsen), except that the preparation is drawn only three times through the flame. This method is the only one employed by us. Another favorite method is the one recommended by A. Fraenkel and Gabbet, in which decolorization and after-staining are effected at the same time. Then the preparation which has been stained with hot carbolized fuchsin, and washed in water, is placed in the following solution:

Sulphuric acid	1
Distilled water	3
Methyl blue, q.s. until the most intense blue color is produced.	

We then wash carefully in water, dry, and mount in Canada balsam.

However convenient this method may be, it is better, for those who are not very experienced, to stain, differentiate with acids, and after-stain separately, because in this way success is more assured.

(*b*) Ehrlich-Koch's method is also often employed. The dry preparation is drawn through the flame, treated with aniline gentian solution for one to two minutes over the flame and heated with acid (usually thirty per cent nitric acid) for one to four seconds,

and with sixty per cent alcohol for a few moments. It is then dipped for several minutes in a watery solution of Bismarck brown and washed off in water. The tubercle bacilli then appear violet on a brown background.

In this form the method is suitable for cover-glass preparations from pure cultures and tuberculous sputum with many tubercle bacilli. If very few or no bacilli are found in the first preparations, we must adopt some method for increasing their numbers. We mention two of the innumerable recommendations:

(*a*) According to Strohschein:

Five to ten cubic centimetres of the sputum are mixed with a threefold amount of Wendriner's borax-boracic acid solution,* and after vigorous shaking allowed to settle for four to five days. The mixture becomes fluid and the bacilli settle at the bottom. Such sputum may be used for examination even after the lapse of years.

(*b*) According to Dahmen, modified by Heim:

The entire sputum is cooked from fifteen to twenty minutes in a beaker glass in the steam chamber, then allowed to cool, the opalescent fluid is poured off, and the crumbly sediment is used for smear preparations.

B. Section Preparations.

1. Universal method, according to Löffler, adapted to the large majority of bacteria.

The section, which lies in alcohol, is conveyed

*Eight grams borax dissolved in hot water, 12 gm. boracic acid added, and then 4 gr. borax; after crystallization the solution is filtered.

(spread upon a spatula of German silver or glass) to Löffler's alkaline methyl blue solution for from five to thirty minutes, and is then placed for a few seconds in one-per-cent acetic acid. After the differentiation the section is placed in absolute alcohol, xylol, and Canada balsam. We must try how long the acetic acid may be allowed to act, and must accelerate the dehydration in alcohol as much as possible; the bacilli should be blackish-blue, the nuclei blue, the protoplasm bluish.

2. Nicolle states that by the following method he has obtained very good section staining of objects which are stained with difficulty—for example, in glanders, typhoid fever, etc.:

Löffler's blue, one to three minutes.

Washing in water.

Treatment with ten-per-cent solution of tannin for a few seconds.

Washing in water.

Absolute alcohol, oil of cloves, xylol, Canada balsam.

3. According to Gram:

(1) Ehrlich's solution, three minutes.

(2) Iodine-potassium iodide solution, two minutes.

(3) Alcohol, one-half minute.

(4) Alcohol containing three per cent hydrochloric acid, ten seconds.

(5) Alcohol, several minutes until maximum decolorization.

(6) Xylol; finally mounting in Canada balsam.

If the tissues are to be stained in a contrasting color, the section is placed, after the maximum decolorization with alcohol, in a watery solution

(10 : 100) of Bismarck brown for a few minutes, then in absolute alcohol for fifteen to twenty seconds, then in xylol, and finally in Canada balsam.

4. Botkin maintains that Gram's stain is facilitated by washing in aniline water preparations which have been stained with aniline gentian. The preparations, when taken from the iodine solution, subsequently stand the action of the alcohol very much better. Bacillus œdematis maligni and bacterium pneumoniæ Friedländer can be stained in this way.

5. Kutscher's modification of Gram's method:

A concentrated solution of gentian violet is made in a mixture of:

Aniline water	1 part.
Alcohol	1 "
Five-per-cent carbolized water	1 "

This concentrated solution is poured drop by drop into a watch-glassful of water until a shimmering layer forms on the surface. The sections are placed in this for ten to fifteen minutes, are then washed off in distilled water, placed one minute in iodine-potassium iodide, then in alcohol, xylol, and balsam. Malignant œdema and symptomatic anthrax can also be stained by this method.

6. If tubercle bacilli are to be stained in sections we use carbolized fuchsin or aniline gentian solution as in cover-glass staining, but we dispense with the heating and instead allow the staining fluid to act for fifteen to thirty minutes.

4. Production of Section Preparations.

At the autopsy small pieces of the organs are thrown at once into an abundance of absolute alcohol and kept there two to three days, the alcohol being renewed two to three times. In most cases the organs are then ready for cutting. For this purpose the firmer part of the kidneys, liver, and muscles are placed on a piece of cork with liquefied commercial gelatin * and then again placed, with the cork, in absolute alcohol. At the end of twenty-four hours the organ may be cut with the microtome. More delicate organs must be embedded in celloidin or paraffin; before staining, the paraffin is removed completely by washing repeatedly in turpentine or xylol and the preparation is placed in absolute alcohol after removal from the xylol.

II. CULTURE OF BACTERIA.

1. Nutrient Media.

A. Non-albuminous (according to C. Fraenkel and Voges).

Common salt	5 gm.
Neutral commercial sodium phosphate	2 "
Ammonium lactate	6 "
Asparagin	4 "

are dissolved in 1,000 gm. of distilled water. We may add ten per cent gelatin or one per cent agar, and thus obtain a non-saccharine nutrient medium which

* One part of gelatin is dissolved in two parts of water.

is suitable to the majority of bacteria. The addition of milk sugar gives a milk-sugar nutrient medium which is free from dextrose (Lehmann and Neumann).

B. Albuminous.

1. Peptone water. In 1 litre of water are dissolved 10 gm. dried peptone, and 5 gm. sodium chloride, and sterilized together.

2. Milk. Fresh milk (best, fresh centrifugal milk) is placed in test tubes and sterilized in the steam chamber for one-half hour on two successive days. Milk which contains the spores of the subtilis group is often incapable of sterilization.

3. Litmus whey (Petruschky). Casein is cautiously precipitated from milk by giving it a very feeble acid reaction with diluted hydrochloric acid, the filtrate is boiled and filtered, and the neutralized fluid mixed with some litmus. This whey is not easily prepared (*vide* Heim: "Lehrbuch," p. 210).

4. Hay decoction. About 10 gm. dry hay are boiled in a litre of water. The filtered solution is placed in test tubes, and sterilized for two hours on three successive days (kept over night in the incubating chamber) in order to destroy the very resisting spores.

5. Beer wort (not neutralized) is allowed, after sterilization, to settle for a few weeks, then poured off clear into test tubes, and again sterilized.

6. Nutrient bouillon.

(*a*) From meat: 500 gm. lean beef are boiled upon the flame for one-half hour with 1,000 gm. of water in an enamelled pot, filtered, the filtrate reduced to 1,000 gm. and 10 gm. peptone with 5 gm. sodium chloride added; this is placed in the steam chamber until dissolved, and the whole is then neutralized with

normal soda lye (indicator, phenolphthalein).* We then filter, pour into test tubes, and sterilize.

(*b*) From meat extract: 10 gm. meat extract are dissolved in 1,000 gm. water, 5 gm. sodium chloride and 10 gm. peptone are added, the solution is neutralized and well sterilized several times.

7. Potato water for tubercle bacilli: 500 gm. peeled potatoes are rubbed upon a grater, allowed to remain over night in 500 gm. water in the refrigerator, decanted, filled up to 1000 gm., cooked for an hour in the water-bath, filtered, four per cent glycerin is added, and the mixture sterilized.

8. Gelatin nutrient media.

(*a*) Meat water-peptone gelatin (ordinary "gelatin" or "nutrient gelatin" of the laboratories).

To 1,000 gm. bouillon (*vide* nutrient bouillon) are added 100 gm. gelatin, 10 gm. peptone, 5 gm. sodium chloride, the mixture is heated in the steam chamber until all the ingredients are liquefied, neutralized with normal soda lye, sterilized, and filtered. After the melted gelatin is placed in test tubes it is again sterilized.

(*b*) Meat-water gelatin: the same as under (*a*), but without peptone and sodium chloride.

(*c*) Beer-wort gelatin is made by adding ten per cent gelatin to the wort; it should not be neutralized.

(*d*) Plum-decoction gelatin: 500 gm. dried plums are cooked in 500 gm. water, the fluid is poured off, and the plums are again cooked with 500 gm. water.

* Illustration: Ten cubic centimetres bouillon require for saturation 2.2 c.c. one-tenth normal soda lye; 1,000 c.c. bouillon require for saturation 220 c.c. one-tenth normal soda lye, or 22 c.c. normal soda lye.

Both fluids are then mixed, filtered, and ten per cent gelatin is added. Not to be neutralized.

(*e*) Herring gelatin. Two salt herring, unwashed, are boiled in 1,000 gm. water and ten per cent gelatin is added to the filtrate; not to be neutralized.

(*f*) Potato-water gelatin, according to Holz, for bacterium typhi: 500 gm. potatoes are thoroughly washed, peeled, finely grated, and squeezed through a linen cloth. The opaque juice may be allowed to settle for twenty-four hours and then filtered, or, as we always prefer, filtered at once through pure animal charcoal. After heating one hour in the steam chamber ten per cent gelatin is added to the clear fluid, this is again heated in the steam chamber, filtered, poured into test tubes and sterilized on three successive days.

(*g*) Potassium iodide potato-water gelatin (Elsner): One per cent iodide is added to the gelatin. The best way is to add a well-sterilized solution in the requisite amounts to gelatin which has just been made ready for use.

9. Nutrient agar. To 1,000 gm. bouillon add 10 gm. very finely divided agar, boil for one hour on the fire in a glass retort until completely dissolved; the water which has evaporated is replaced and then 10 gm. peptone and 5 gm. sodium chloride are added. After heating again in the steam chamber the fluid is neutralized, filtered by means of the hot-water funnel, placed in test tubes, and again sterilized.

10. In order to make grape-sugar or milk-sugar agar, two per cent of the corresponding substance is added with the peptone and sodium chloride. As

bouillon agar generally contains traces of grape sugar, we have for some time made a milk-sugar agar which is free from grape sugar, according to the plan described under *A*.

11. Glycerin agar. To the nutrient agar is added five per cent glycerin, the mixture poured into test tubes and sterilized.

12. Sugar-chalk agar. Mix melted sugar agar with finely powdered, dry, sterilized carbonate of lime until the mixture becomes cloudy and opaque, inoculate the bacteria into it, and pour out in plates.

13. Potatoes. After careful washing the potatoes are peeled, cut into discs 1 cm. thick, and sterilized several times in high Petri's dishes. We may also perforate the peeled potato with a large cork borer and divide the cylinder by an oblique cut into two wedges. The pieces are then placed in a test tube at the bottom of which is a little dry cotton (to absorb the water of condensation) and sterilized several times in the steam chamber.

14. Blood serum. The blood, taken from the slaughtered animal under proper precautions, is allowed to stand for twenty-four hours in well cleaned glass cylinders in the refrigerator; on the following day the serum is removed by means of large sterile pipettes. It is placed in bottles, one per cent chloroform is added, and is then allowed to stand for a few weeks, being shaken occasionally. For use, we place the serum, which has been poured into tubes, in the incubating chamber for a few days in order that the chloroform may escape completely. It is employed either in the fluid state or after it has been made rigid at a temperature of 65°.

15. Löffler's serum mixture for diphtheria bacilli. Three parts of beef or sheep serum are mixed with one part calf's bouillon, which contains one per cent grape sugar, one per cent peptone, and one-half per cent sodium chloride.

16. Entirely different from the other media is that first devised by Kühne, modified by various writers, and finally made somewhat more practicable by Stutzer and Burri. We refer to the silicic acid nutrient medium. Gelatinous silicic acid, which is merely mixed with a few salts, is an important nutrient medium for certain organisms (for example, the nitrate-producers) on account of the lack of organic nutrient substances. For the somewhat complicated manipulation, *vide* Stutzer and Burri (C. B., Vol. I., Part V., 722).

2. The Employment of the Different Nutrient Media Depends upon the Following View-Points :

I. Fluids (bouillon, sugar bouillon, milk, non-albuminous nutrient solution).

1. To produce cultures *en masse*.

2. To obtain bacterial solutions containing an accurately determinable number of bacteria (counting by means of plates).

3. To observe the development of membrane and sediment.

4. To study the metabolic products.

II. Solid Nutrient Media.

1. Gelatinous nutrient media. The most extensive use is made of gelatinous, transparent nutrient

media (agar and gelatin) and for the following reasons:

(*a*) They may be used as fluids and as solid media: as fluids they permit the separation, as solid substances the fixation, of the isolated germs and their separate growth into colonies.

(*b*) On account of their transparency they permit a macroscopic as well as a microscopic observation of the cultures; they permit a differential diagnosis of the varieties and an early recognition of any impurities.

They are used particularly:

(*a*) For plate cultures, *i.e.*, as a proof of positive separation and for the enumeration of individuals and varieties.

(*b*) To secure characteristic macroscopic cultures, which will serve for differential diagnosis.

(*c*) For permanent cultures or collections of living bacteria.

The special advantages of agar and gelatin are:

(*a*) Gelatin. Advantages: Easily produced, easily formed into plates (at 25°); its property of liquefaction by certain bacteria possesses great diagnostic importance. Disadvantages: As it melts at 25°, it cannot be used in hot weather and at incubating temperature.

(*b*) Agar. Advantages: Practicable at incubating temperature (*i.e.*, for the rapid culture of bacteria [spores] and particularly of thermophile bacteria). Disadvantages: Difficulty of preparation; not so easily formed into plates. The cultures are often not very characteristic.

2. Blood serum and glycerin agar. Used for the

culture of pathogenic varieties, which thrive with difficulty or not at all upon other nutrient media. Plate cultures are only possible with glycerin agar and mixtures of agar and serum.

3. Potatoes.

(1) To obtain macroscopically characteristic cultures of great durability and for differential diagnosis.

(2) Occasionally for the development of spores.

3. A Few Words on the Manipulation of Ordinary Cultures.

The platinum needle must be brought to a glow throughout its entire length each time before using and before putting it away.

(*a*) Fluid cultures are inoculated with a loopful of pure culture.

(*b*) Gelatin and agar stick cultures are made with a straight needle without a loop, only one puncture to each tube but extending nearly to the bottom.

(*c*) Agar and gelatin streak cultures and potato cultures are made by a gentle superficial stroke upon the surface with the platinum loop. In the case of the potato it is sometimes necessary to rub the culture in.

(*d*) Gelatin plate cultures.

1. To isolate definite germs in the pure culture. We melt three gelatin tubes; put into the first, after it has been cooled to 30°, a loopful of a fluid culture or a trace of a solid culture; shake the tube while turning it upside down, and then convey from this one or two loopfuls of liquefied gelatin into a second tube. After shaking this, two to three loopfuls are

placed in a third tube, and the contents are then poured into three dry sterilized plates, lifting the cover briefly and gently inclining the plate to and fro, in order that the gelatin may be distributed as uniformly as possible. In making inoculations from one tube to another it is advisable to hold them in an inclined position in order to guard against the entrance of foreign germs. The plates are then placed in the culture chamber at a constant temperature of 22° (or they may be kept at the temperature of the room) and at the end of two to three days the individual colonies which have developed are observed macroscopically and also microscopically with low (50) magnifying powers. As a general thing only two of the three plates are serviceable for observation, one at least is sown too thick or too thin.

2. If we wish to ascertain the number of colonies, for example, in a specimen of water, we place in three test tubes of melted gelatin, 1 c.c., 0.5 c.c., and 0.1 c.c. of the water, shake and pour into three dishes. To ascertain the number of germs, we use Wolffhügel's counting apparatus if very many germs have developed. If the germs are few the following plan is simpler: The plate is laid upside down (upon the cover), the bottom is divided with ink into sextants, and each visible colony is marked with a dot. Plates upon which the number of germs in drinking-water are to be ascertained must be counted several times (on the second, third, and fifth days). When the fluid is very rich in germs (for example, sour milk, ditch water, etc.), 1 c.c. is first placed in 100 c.c. of sterilized water and the mixture then manipulated as described above. Solid bodies are first rubbed up in

water. When air is to be examined a definite volume is sucked through a tube of sterilized sand, the latter carried into sterilized water, and plates are then formed.

(*e*) Agar plate cultures are made in the same way. The agar should not be poured into the dishes when too cool, because otherwise it coagulates at once into an irregular surface; if used when too warm, the inoculated bacteria will die. In recent times it has been recommended that in making agar plates the nutrient medium should first be allowed to become rigid in the dish, and then the mass to be examined is smeared superficially upon it with a sterilized platinum loop, a strip of filtering paper or a platinum brush. In this way we obtain only characteristic superficial colonies.

(*f*) Sugar-agar-agitation cultures: The contents of the tube are melted in the water-bath, then cooled to about 40°; a loopful of pure culture is then introduced, the tube well shaken, and when it becomes rigid the culture is placed in the incubating chamber.

4. Anaërobic Cultures.

We have employed almost exclusively Buchner's method, *i.e.*, the absorption of oxygen by pyrogallic acid and potash lye.*

(*a*) For stick cultures: Upon the bottom of a glass cylinder, which must be somewhat longer and wider than a test tube, is placed a heaping teaspoonful of pyrogallic acid and 20 c.c. of a three-per-cent potash

* Sensitive varieties are said to thrive still better in a hydrogen atmosphere.

lye; place in it the infected stick culture and close the cylinder at once with a soft-rubber stopper or a ground-glass stopper which is sealed with paraffin. According to Kitasato the anaërobics which are less sensitive to oxygen may be cultivated in saccharine agar in a high stick culture, even without pyrogallic acid. A wire with a small loop is pushed into the layer of sugar agar (8 to 10 c.c. high), and the wire turned on its long axis before withdrawal.

(*b*) For plate cultures we use, instead of the glass cylinder, a wide exsiccator with a ground cover; fill the lower part with sand and the pyrogallic-acid mixture, and then manipulate as before.

III. EXPERIMENTS ON ANIMALS.

A. Infection.

1. Subcutaneous inoculation. A shallow incision is made with a pair of scissors on some part of the skin, after it has been washed with a 0.1-per-cent solution of corrosive sublimate; the inoculating matter is carried beneath the skin by means of a stout platinum wire with a loop. Mice are generally inoculated above the root of the tail; they are simply held by the tip of the tail, and allowed to hang into a glass which is covered up in great part by a piece of wood. Guinea-pigs and rabbits are inoculated on the side of the thorax.

2. Subcutaneous injection is generally effected by means of Koch's rubber ball injection syringe or Strohschein's syringe. A fold of skin is picked up at some part of the body, and the needle inserted in the longitudinal direction. If several cubic centi-

metres are to be injected, the following simple method may be adopted: A short piece of rubber tube provided with an injection needle is fastened to a graduated pipette, the entire apparatus sterilized, the pipette filled, and the fluid blown in by the aid of the mouth or a rubber bulb.

3. Peritoneal injection is made by perforating with a sterilized hollow canula, at one puncture, the abdominal wall, then cautiously advancing the needle and injecting the fluid.

B. Observation.

Mice may be kept in sterilized glass vessels closed with cotton and wire netting; larger animals must be kept in sterilized cages or stalls.

C. Autopsy and Disposal of the Cadaver.

Autopsies must be made immediately after death, or, at least, the animal placed on ice. The animal, lying on the back, is tied or nailed through the legs to a board, the abdomen and chest are throughly moistened with corrosive sublimate, and then the abdominal cavity is opened with a previously sterilized knife. The abdominal walls are separated and from the spleen, liver, and kidneys some blood (or tissue juice) is removed with a sterilized platinum loop. This is smeared at once upon prepared agar plates. The organs are carefully cut out, avoiding contact with the intestines, and are placed in absolute alcohol for further examination. Then the thorax is opened with a pair of scissors, blood taken from the heart and lungs, and these organs are placed in alcohol. Before each operation the instruments must be carefully

brought to a glow. It is better to have on hand numerous instruments which have been sterilized at 130°. The hands must be kept perfectly clean.

After the autopsy it is best to cremate the cadaver. If this is not feasible, the body is wrapped in cloths dipped in a solution of corrosive sublimate and buried in a hole in the ground at least one-half metre deep, which is filled in with quicklime.

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