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EXTRACTS FROM BACTERIOLOGY, VOLUME 3

[Following is the translation of pages 114-116 from Hallmann: Bakteriologie (Bacteriology), Vol 3, German. Additional bibliographic data not available in foreign language text.]

Procedure by Blaurock, modified by Paetzold, Archiv der klinischen und experimentellen Dermatologie (Archive of clinical and experimental dermatology), 1955, page 201:260 recommended: Fill a test tube up to one third with 33 percent blood serum agar see (2) and test for sterility. Then inoculate with a lancet the 48-hour gonococcus subculture and incubate at 36.5° C. As soon as growth of a colony appears, fill up another third of the test tube with sterile 1:5 blood serum mixture. Put a layer of liquid paraffin on top of this. The test tubes are kept in the incubator at 29-30° C. until taken for further use.

In typical cases of untreated gonorrhea the diagnosis of gonococci is easily accomplished and carried out with certainty in the original preparation (by dyeing with methylene blue and according to Gram). It should always be kept in mind that not all urethral suppurations are of a gonorrheal nature, but that infections of the urethra may also be caused by use of stronger mucous membrane stimulants, for instance, soap gonorrhea, beer gonorrhea through hop oil of new beer, and also so-called sterile suppurations may be caused by overly strong protective inoculations (prophylaxis). Also, there may be trichomonades (tr. vaginalis, not tr. hominis s. intestinals. Test in a fresh droplet of pus according to the procedure given on page 524) and other pus and infection stimulants, such as staphylococci, streptococci, pneumococci, influenza bacteria, as well as *Neisseria catarrhalis* (!) and certain mucous membrane spirochetes, for instance, sp. forans (as stimulant for Reiter's

disease, see page 399, which is related to urethra inflammation, conjunctive inflammation and inflammation of the joints). It follows from all of these facts that with the presence of numerous mixed bacteria, for instance in vaginal secretions, the diagnosis and with this also the clinical evaluation and the proof for complete recovery may be quite difficult. The growth of cultures, perhaps after provocation, must generally be asked for in all doubtful cases; this is particularly important in the case of female gonococous diseases of a chronic nature (best: taking of sample during menstruation); furthermore, it is important in the case of children's vulvovaginitis with gonococcus suspicion, infection of the rectum or wound infection. It should be noted that the culture methods are not to be preferred to the microscopic slide methods as far as positive yield is concerned (because with damaged gonococcus the culture might fail), but that a combined application of both methods promises optimum results. -- It is also impossible to distinguish with certainty gonococci from other Gram-negative diplococci (among others *N. catarrhalis*) on the basis of smears of the conjunctive secretion (page 36) or the prostate exprimate (Fig 50). A double check by means of a culture might become necessary here also.

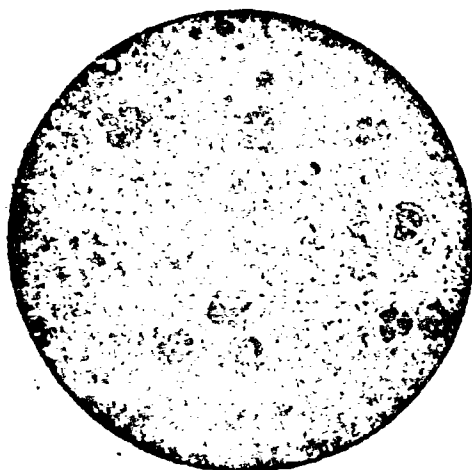


Fig. 50 Prostate exprimate in case of prostatitis gon.: Rare lipoid bodies, leucocytes, individual extracellular gonococci.

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For the detection of "culture gonococci", the proof of typical gonococcus colonies is, in general, satisfactory. These colonies (exhibit the oxidase reaction and) consist of Gram-negative diplococci with the characteristics given on page 112, if meningococci of the type of the test material can be excluded. To complete the diagnosis, it is possible to make a subculture from the suspected colonies (in the case of oxidase-positive colonies prior to the black coloration so that the germs will not be damaged too badly) and if one deals with a pure culture (Gram preparation), fermentation behavior according to the table on page 106 may be tested. To be sure, the sugar series alone will not yield complete certainty because other diplococci (those of the oral cavity) exhibit an almost identical fermentation behavior. Subsequently, the growth on agar nutrient should be checked; gonococci do not grow on an agar plate.

Neisseria meningitidis, diplococcus (*Neisseria micrococcus* or *streptococcus*, respectively) intracellularis (meningitidis), triv. meningococcus (Mg.). The bacterium was first (1887) discovered by Weichselbaum in the spinal fluid and recognized as the stimulant of epidemic meningitis; this disease occurs today in Germany only sporadically. Sometimes other manifestations of the disease, for instance in form of epididymitis and otitis are observed besides the bacteriemic states (meningococcemia). - The epidemic paralysis of the neck, being a "contagious paralysis of the neck", has to be reported to the authorities according to the law covering epidemics and must therefore be distinguished from other meningitis diseases (pneum meningitis, streptococcus, tuberculosis, influenza, coli bacteria, viruses, etc.). However, clinical and bacteriological differentiation might be difficult because, at times, a secondary immigration of other pus stimulants might underlie the actual meningococcus infection; this occurs sometimes to such an extent that these germs dominate the overall picture of the disease and indicate a different type of meningitis.

The pharyngeal membrane, especially the tonsil, is to be regarded as the entrance for contagious paralysis of the neck. The transmittal occurs presumably by healthy meningococcus carriers who have meningococci on their pharyngeal membrane just as a person suffering from paralysis of the neck carries them. The meningococci are spread by droplet infection (coughing, sneezing, speaking, snoring) and perhaps also by dissipation (handkerchiefs, etc.). Attempts to prevent occurrence or spreading of the disease by detection and isolation of meningococcus carriers are

futile in view of the wide distribution of such carriers. Examinations of the surroundings (pharyngeal smears) should therefore be limited to the closest neighbors (members of the family, school classes, roommates) and should not be carried beyond this point.

Several types may be distinguished serologically; these types are distributed, approximately, as follows: type I 25 percent, type II 63 percent, type III 16.6 percent, type IV 11.6 percent. Type II is therefore the most frequently detected type. Type I and the remaining types are much rarer by far. But it is noteworthy that type I exhibits the greatest pathogenesis (Wüstenberg).

[Following is the translation of page 369 from
Hallmann: Bakteriologie (Bacteriology), Vol 3.]

Morphology: In fresh cultures Gram-positive, movable rods, thicker and bigger than tetanus bacillus ($3-10\mu$ in length; the bacilli are smaller in the case of type E and exhibit vacuols). Otherwise the form of the rods might vary. Chainlike formations are frequently formed in liver broth cultures. Decomposition phenomena are often observed in nutrient agar, at times to such an extent that rods formed in the smear can hardly be detected any longer. The spores which form best in sugar-free culture media (liver broth, ordinary blood plate) are most frequently located at the end of the rod (but not always as in the case of tetanus) and enlarge here the body of the bacillus. It is characteristic that the spores are not round (as tetanus), but longitudinal and egg-shaped so that spore-containing rods have the form of a tennis racket or a spoon (Fig 167). As a rule, the spores are very resistant and can often undergo heating to 100°C . for five hours (at pH 7.0; there is lower resistance in an acidic reaction).

Culture (pages 347-353). Strongly anaerobic (highly oxygen-sensitive!). The optimum temperature varies for the different species (35°C . or 20°C . but even at 50°C . growth is observed in a liquid culture medium; optimum toxin formation for type A at approximately $20-25^{\circ}\text{C}$. and for the remaining types in liquid media at $28-35^{\circ}\text{C}$). - Types A and B do not exhibit growth on liver-agar; type E grows on human blood agar or sheep blood agar in form of small, flat, transparent, pale grey colonies with smooth or irregular edge and sometimes slightly elevated center. There is a tendency towards film formation on moist nutrient agar. Hemolysis formation is variable. Similarly, on a Fortner plate, the appearance of the colonies is rather variable with the individual types and also in the course of recultivations (Fig. on page 371): partly slightly arched, smooth

colonies with fibrous edge, partly level asbestos fiber-like,
rough colonies, partly hazy growths, especially on moist
media.

[Following is the translation of pages 376-377
from Hallmann: Bakteriologie (Bacteriology),
Vol 3.]

Course of the Examination (See also page 346):

(1) Morphological characteristics (Fig 168/169):
large, thick, straight, immovable (without flagellum!)
rod with rounded off edges. In fresh cultures Gram-positive,
in aged cultures more or less Gram-negative (especially in
carbohydrate cultures). Characteristic arrangement of the
rods in cover glass replica preparations: they lie close
together at the edge of the colony and are groupwise inter-
locked. - Capsule formation is observed in the tissue (ani-
mal body) and in albuminous media (serum addition). - Spore
formation only sluggish and only in non-acidic (sugar-free)
medium, for instance in liver broth cultures of a few days
by addition of chalk or, better still, in alkaline cerebral
pulp or on agar with 2-3 percent soda addition. The spores
which are located in terminal positions rather than in
central positions do not bring about a swelling of the rods.

(2) Culture characteristics (Table on pages 358/359).
Anaerobic growth, however absence of oxygen is not quite as
essential as in the case of tetanus bacillus or botulin bacil-
lus (growth already at 40 mm air pressure). - In liver broth
rapid and abundant growth with strong turbidity and gas
formation (no proteolysis). - Livermilk curdles into a solid
lump which appears highly shrunken and perforated by gas
bubbles and torn; but this lump is not digested. As in case
of the living organism, a typical butyric acid odor arises.
On glucose agar occurs characteristic growth (growth form I,
Figs 154/155 on page 356); the colonies with complete anae-
robiosis are slightly strawberry-colored and, after 20-24
hours incubation, up to 2 mm long, round, arched, shining
with moisture; as a rule, their color changes when oxygen

is admitted from grey and clay-brown to olive green or reseda green; the center is usually lighter. The colonies are uniformly surrounded by a practically opaque corona (hemotoxin). This corona is approximately 1 mm wide and of dirty-brown color. On coagulated albumin: round to irregular colonies, smooth (occasionally rough varieties), surrounded by a broad, opaque precipitation zone without brightness effect above the colony or the precipitation zone. There occurs gas and acid formation in the usual sugar media, but sugar alcohols or glycosides are not attacked or only irregularly. After precipitation of the glycerine or inulin fermentation, several types (insignificant for human pathology) may be distinguished. In iron-chloride-sulfite-gelatine (orientating fast culture, see Fig. 170) there is increasing black coloration. - Best media to stimulate spore formation: alkaline dextrose-ascite-liver broth according to Bethge.

(3) Animal experiment. The virulence of individual strains for the guinea pig is variable. Ordinarily there is shortly after subcutaneous injection of 1-2 cubic centimeter liver broth culture into the abdomen a painful infiltration and progressive degradation of the muscles occur. A large undulating pouch which lifts up the fur and which lies between the outer skin and the abdominal or pectoral cavity is formed; this pouch contains gas and a small amount of a meat broth-like, odorless liquid. The muscles below are dry and scaly or decompose like pulp; they are red-brown. When the blister bursts prematurely towards the outside, the animals stay alive, usually, but they appear to be severely undernourished. However, in most cases they perish from infection within 1-3 days. With intravenous injection of 0.5 cubic centimeter of a 24-hour liver broth culture into the femoral vein of a guinea pig and immediate killing and subsequent storage for 20 hours at room temperature, the animal is observed to be tremendously swollen and stretched to bursting. The inner organs, especially the liver, contain gas ("foam organs") or they are torn from gas and the entire organism is permeated with bacilli.

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