PUBLIC HEALTH LABORATORY NOTES

Abstracted by ARTHUR LEDERER, M. D.

New Culture Medium for the Isolation of the Glanders Bacillus.—To a glycerine (5 per cent) agar an aqueous solution of parafuchsin is added in the proportion of 1 to 10. Upon this medium B. mallei grows readily, decolorizing the medium. Many organisms are inhibited by the dye.—S. Kondo and H. Oguni, *Jour. Cent. Vet. Med. A.* (*Tokyo*), 30, No. 12 (1917); *Abst. Bact.*, 2, 204 (1918).

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New Specific Serum Reaction for the Diagnosis of Pregnancy.—The reaction gives 94 to 98 per cent correct results. One cc. of serum is added to 0.1 cc. of a combination of placenta and iron. Contact for three hours is permitted. The material is filtered and the filtrate is washed with water. Twenty drops of 18 per cent hydrochloric acid and an equal amount of 50 per cent potassium ferrocyanide are added to the filtrate and the mixture is shaken with 2 cc. of ether. A positive reaction is indicated by a reddish color.—F. Thoenen, *Muenchen. med. Wochenschr.*, No. 24 (1917); *Abst. Bact.*, 2, 254 (1918).

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Typhoid Vaccine as a Therapeutic Agent.—Commercial typhoid, or typhoid-para-typhoid vaccine, prepared for prophylactic inoculations, has unique and very marked therapeutic properties if injected intravenously. The conditions in which it has been applied were acute arthritis, chronic arthritis, gonorrheal arthritis, rheumatic fever, syphilitic affections, psoriasis, lichen planus and eczema.—W. C. Cadbury, *Med. Rec.*, 95, 144 (1919).

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Simple Method of Carrying Meningococcus Cultures.—The composition of a liver-agar favoring the growth of meningococcus is given. The culture is planted into the water of condensation taking care not to tip it over the surface of the slant. After 24 hours at 37° C., carefully tilt the tube so that the water of condensation wets the slope for the distance of about 5 mm. or less and incubate again. The organism will not grow above the old growth as far as the liquid has wetted the slant. This can be repeated until the entire slant is covered. Thus a culture may be kept alive approximately three to four weeks.—J. Bronfenbrenner and M. J. Schlesinger, *Jour. Med. Res.*, 39, 217 (1918).

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The Micro Plate.—Description of method of observing bacterial reactions in very small quantities of medium. The method is serviceable only with solid or semi-solid media. The reactions which may be observed are gelatin liquefaction, amylolytic action, hydrogen sulfide formation, reduction of nitrates, and sugar fermentations.—J. Bronfenbrenner and M. J. Schlesinger, *Jour. Med. Res.*, 39, 267 (1918).

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Some Simple Culture Media for B. Influenzæ.—Very profuse growth of *B. influenzæ* can be obtained: 1, from blood boiled in agar; 2, from agar to which has been added some of the clear colorless fluid resulting from boiling blood in water; 3, from agar to which has been added blood which has been broken down by a strong mineral acid. If a strong mineral acid is used to break down the blood, sterile blood is not required. The substance which stimulates the growth of *B. influenzæ* is not the colored fraction of hemoglobin. By the addition of brilliant green to the medium pneumococci, streptococci, and staphylococci are inhibited to a much greater extent than *B. influenzæ*. Cultures of *B. influenzæ* can be kept alive for a considerable period on a blood meat medium.—A. Fleming, *Lancet* (London), No. 4978 (1919).

On the Vitamines Available for the Cultivation of Bacteria.—A solution practically free of the albumins and coloring matter of the blood, which contains the substance or group of substances (vitamines or hormones of growth) of indeterminate nature necessary for the cultivation of Pfeiffer's bacillus, can be prepared from washed blood corpuscles or from the whole blood. This solution loses its properties if heated above 100° C. It can be filtered through filter paper or through a filter candle.

The authors recommend the following procedure for its preparation: Dilute the defibrinated blood with four volumes of physiological salt solution; heat this dilution for 15 minutes in a water bath at 80° C., with frequent shaking; filter through filter paper or centrifugalize. If the work thus far has not been carried out aseptically, filter through a Chamberland F. The aqueous solution obtained, added to ordinary culture media in the proportion of 5 to 10 per cent, promotes the abundant cultures of Pfeiffer's bacillus. Even with the weak proportion of 1 per cent, growth is obtained, but the cultures develop slowly and the colonies remain small. The blood of birds, the eel or the horse yields the same results. It is the cellular element, not the serum, that contains the vitamine principle.—Agulhon and Legroux, *Compt. Rend. Acad. des Sciences*, Paris, 147, 597 (1918).

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Hemoculture of Typhoid Bacilli in Peptone Glucose Bile.—Bile, to which has been added 1 per cent of peptone and 1 per cent of glucose, is a better medium for the enrichment of typhoid bacilli from the blood than is bile alone. The authors advise the introduction into a certain number of the tubes of a sterile piece of broken glass rod, which with shaking promotes the mixture of the fresh blood and the pepton-glucose bile. If the blood has already coagulated, then only the coagulum is planted in the bile tube and there broken up. (See *Bull. Inst. Pasteur*, 15, 608 (1917).)

If bacterial growth develops, the medium assumes a sanious aspect, becomes clouded and assumes gradually the tint of old chocolate (15 to 24 hours). The paratyphoid bacilli, in contradistinction to the typhoid, gives rise to formation of small bubbles of gas.—Tribondeau and Dubreuil, *Compt. Rend. Soc. de Biol.*, Paris, 81, 130 (1918).

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Method of Staining Spirochætes with Formolated Gentian Violet.—

1. Make a thin smear on a slide; dry.
2. Ruge's acetic formol (formol, 2 parts; acetic acid, 1 part; distilled water, q. s. for 100 parts), allow to act for 5 minutes cold; renew this mordant two times.
3. Replace the acetic formol with an aqueous solution of chromic acid (10 per cent) and allow to act for 10 minutes.
4. Wash in absolute alcohol for 2 minutes and flame.
5. Two minutes exposure to formolated gentian violet (hot) (gentian violet, 1 part; formol, 4 parts; alcohol, 10 parts; distilled water, q. s. for 100 parts).
6. Wash rapidly in water.
7. Blacken the violet with Lugol for 5 minutes.
8. Wash, dry in the cold and mount.

The cellular elements have their protoplasm stained violet and the nucleus black. The bacteria are black, the spirochætes, violet, but more often black, even those which, like the spirochæte of hæmorrhagic icterus, take stains difficultly.—P. Spehl, *Compt. Rend. Soc. de Biol.*, Paris, 81, 305 (1918).

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Quantitative Wassermann.—The degree of lysis secured in the reaction is compared with known concentrations of hemolyzed blood.—K. Taege, *Muenchen. Med. Wochenschr.*, No. 47 (1917).

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Complement Fixation in Tuberculosis.—A series of tests was carried out on 570 soldiers, using a suspension of living bacilli as the antigen, prepared somewhat after the method of Miller. With two slightly different methods the author obtained 74 and 77 per cent of positive reactions in cases with active pulmonary tuberculosis. It was also noted that the test was positive in 21.8 per cent of clinically non-tuberculous cases. The latter figure, which is rather high, may be due to the lighting up of dormant lesions sufficiently to produce antibodies without signs or symptoms.—Fidlar, E., *Lancet* (London), Dec. 21, 1917, 844. (*D. G.*)

Selection Hypothesis (An attempt to furnish a uniform explanation for Immunity, Tissue Immunity and the Manifestations of Immunity).—This article is in the nature of a preliminary communication, edited under the form of propositions, concerning a work which the author promises will appear shortly *in extenso*.

Acquired immunity is the result of a conflict between the cells of the body and the virus. Among the cells, there are strong and weak cells; the weak are the ones first attacked by the virus, then destroyed. This operates, then, to the advantage of the more resistant cells and of those cells which have come to replace the disappeared cells from which they inherit an increased resistance, thus establishing an immunity.

After passages of the microörganisms through the animal, their virulence is increased. The weaker microörganisms succumb in the conflict with the cells; the stronger survive, as well as those derived from them.

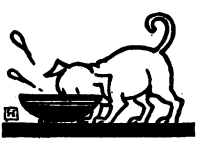
According to this hypothesis of the survival of the fittest, the degree of the immunity is in proportion to the number of cells destroyed and to the number of cells replacing those destroyed. If, then, as a result of a natural infection with typhoid, cholera or smallpox, the immunity acquired is more substantial than that conferred by artificial vaccination, it is because in the course of the naturally contracted infection there are many more cells destroyed and subsequently replaced than is the case with vaccination.

The author attempts to explain also upon his hypothesis of selection of the origin of antibodies, their specificity, the reaction of fixation of complement, normal antibodies, and anaphylaxis.—L. v. Liebermann, *Deutsch. Med. Wahnschr.*, Leipz. u. Berl., 44, 313 (1918).

Experimental Production of Amœbic Dysentery in the Guinea-pig.—Reference is made first to the work of Lynch on the amœbiasis of the Norway rat. Chatton fed three guinea-pigs material containing the cysts of *dysenteriæ*. One animal died at the end of 20 days from cæcal amœbiasis. With a scraping from a portion of this cæcum, he inoculated three other guinea-pigs through the rectum; one of these died in 9 days from an amœbiasis of the same type as that of the guinea-pig of the first lot. There were no clinical symptoms of dysentery and no lesions in the rectum; only the cæcum was involved.

The amœbæ recovered from the lesions had the characteristics, nuclear among others, of *dysenteriæ* and differed as much from the amœba normally present in the guinea-pig as from the *Amœba coli*. Chatton presents figures of these non-pathogenic amœbæ side by side with the amœbæ recovered from the experimental lesions.

In a later work published the next year in the same journal, Chatton describes the lesions obtained and states that they have never been observed before in the guinea-pig. They consist of hyperplastic reaction of both epithelial and connective-tissue cells. The epithelial reaction is a Lieberkühnal hyperplasia of the cysto-adenomatous type; the hyperplasia of the glands caused the rupture of the muscular mucosa; the sub-mucosa was infiltrated, greatly thickened and ultimately became hyperplastic itself. This connective-tissue reaction is a sarcomatous neoplasm of the fusocellular type with new formation of vessels by the neo-plasmatic cells themselves; by reason of the rôle of the lymphoid cells in its genesis and from the absence of metastases, Chatton calls it lympho-sarcomatoid hyperplasia. These hyperplasias were associated with necrotic lesions and inflammatory reactions, such as are observed in man and the cat. The author thinks that the amœbæ, attracted by the mucus, penetrate the mucosa by the glandular ducts and attack the culs-de-sac where the muciparous cells predominate.—Ed. Chatton, *Bull. Soc. Path. Exot.*, 10, 794 (1917); 11, 28 (1918).



Bacteriology and Pathology of Influenza.—Because of the variety of clinical types of cases observed the authors are of the opinion that the activating agent is not a single one, but that several organisms are implicated, and that the malady is not a simple but a compound disease.—Whittingham, H. E., and Sims, C., *Lancet* (London), Dec. 28, 1918, 866. (*D. G.*)

Typhoid Fever Occurring After Prophylactic Inoculation.—Occasional cases occur in which the usual preventive inoculations against typhoid fever fail to protect against the disease, most probably on account of the ingestion of virulent organisms in massive doses. To eliminate such occurrences, sanitary precautions should prevail; but they can succeed only by constant attention to the guarding of food and drink against contamination. No false sense of security from typhoid vaccination should be permitted to relax vigilance in this direction.—C. P. Brown, F. W. Palfrey and L. Hart, *Jour. A. M. A.*, 72, 463 (1919).

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Filtrable Poison Produced by B. Influenzæ.—It has been found that the influenza bacillus produces a filtrable poison which is lethal to rabbits. It also deteriorates very quickly. An immunity can be produced against it. The serum of immune animals appears to be antitoxic both *in vitro* and *in vivo*. J. T. Parker, *Jour. A. M. A.*, 72, 476 (1919).

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Differentiation of Streptococcus Hemolyticus.—The hydrogen ion concentration at which the human strains of *S. hemolyticus* cease to grow is different from that which limits growth of hemolytic cultures from bovine sources; 95 per cent of all the strains examined were accurately and rapidly classified by the determination of their final hydrogen ion concentration in 1 per cent glucose broth. The human type of *S. hemolyticus* reaches a final hydrogen ion concentration of P_H 5.2 to 5.0, and the bovine type of P_H 4.5 to 4.3.—O. Avery and G. E. Cullen, *Jour. Exp. Med.*, 29, 215 (1919).

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Staphylococcus Aureus Pneumonia.—Pneumonia associated with *S. aureus* occurred in almost every organization at Camp Jackson. In general, where the total incidence of pneumonia was high there was a correspondingly larger number of *S. aureus* infections. The treatment of this infection of the lung is extremely ineffectual. The prognosis is always grave, though occasionally patients survive. —H. T. Chickering and J. H. Park, *Jour. A. M. A.*, 72, 617 (1919).

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Complement Fixation in Influenzal Pneumonia.—The complement binding factor of serum from influenzal pneumonia patients is probably a very weak one. The results suggest strongly that the influenza bacillus forms specific antibodies that will fix complement when this organism is used as an antigen. Polyvalent antigens did not give more positive results than a monovalent one. Serums that gave positive tests were also found more suitable for the serotherapy of influenzal pneumonia patients. Since some of the reactions were inconstant, it must be concluded that the complement fixation test in influenzal pneumonia cannot yet be accepted as a diagnostic test. The influenza bacillus found in more than 80 per cent of the necropsies was used as an antigen.—F. H. Rapoport, *Jour. A. M. A.*, 72, 633 (1919).

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Transmission of Infection Through the Eye.—The eyes offer a relatively large surface area for the reception of droplets sprayed from the mouth of other persons. An organism introduced into the conjunctival sac may be recovered from the nose in five minutes, and from the stool in 24 hours. The upper respiratory tract of a person wearing a properly constructed mask may be infected by exposing the eye briefly to direct droplet spray. This portal of entry is of importance in the transmission of acute respiratory infections.—K. F. Maxcy, *Jour. A. M. A.*, 72, 636 (1919).

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Complements in Wassermann Reaction.—The complements of fresh guinea-pig serum varied greatly in fixability. Human complement gave much weaker positive results than did guinea-pig complement. Guinea-pig complement left on the clot and kept in the refrigerator remained fairly constant for three days, while complements which had been removed from the clots deteriorated more rapidly. Ramy's method of preserving complement by sodium acetate was a total failure in the author's hands. Glycerol seemed to prevent deterioration of complement for a few days.—E. H. Ruediger, *Jour. Inf. Dis.*, 24, 121 (1919).

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New Method for the Identification of Tubercle Bacilli in Sputum.—The method consists in diluting the sputum with an equal quantity of water and adding a drop of ammonia, then allowing the mixture to stand in a water bath at 50° C. for between 15 to 20 minutes, afterwards adding an aluminum sulfate solution in the proportion of 0.5 cc. to 10 cc. of the sputum mixture, and finally centrifugalizing the deposit and examining it by staining in the ordinary way. The experiments showed this method to be superior to all others tried.—K. E. F. Schmitz and K. Brauer, *Centralbl. Bakt. I. Orig.*, 81, 359 (1918); *Rev. Bact.*, 8, 107 (1918).

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Simplified Confirmatory Test for B. Coli.—It is suggested that in the confirmatory test for B. coli, Endo slants be employed instead of Endo plates. The inoculations from the lactose fermentation tubes are made in the butt as well as on the slant. It is claimed that the colonies on the slant appear more typical. Ærobic and anærobic growth may be detected at the same time.—*R. L. Kahn, Jour. Bact.*, 3, 555 (1918).

Study of the Pfeiffer Bacillus.—A serum with pronounced specific properties was obtained from animals immunized with the Pfeiffer bacillus. The serum had a distinct curative action and prevented the death of guinea-pigs after inoculation with lethal amounts. The serum of the immunized animals (goats) was toxic in the case of blood withdrawn too soon after the last injection of bacteria. Intraperitoneal injection of the serum killed guinea-pigs and subcutaneous injection caused edema. Serum from blood withdrawn 20 days after the last injection did not show any toxicity.—A. Latapie, *Compt. Rend. Soc. Biol.*, 81, 833 (1918); *Chem. Abst.*, 13, 233 (1919).

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Variation in Diphtheria Group.—Twenty-five cultures of members of the diphtheria group isolated from various sources were studied. Variations were noted in morphology, in fermentative reactions and in virulence in subcultures derived from one parent strain. The virulence of a strain is not closely correlated with its morphology, nor is it correlated with its fermentative reactions.—F. D. Meader, *Jour. Inf. Dis.*, 24, 145 (1919).

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New Method of Diagnosis of Malaria.—The method depends upon complement deviation in cases of malaria. After a large number of experiments a specific antigen for malaria has been prepared which acted well, diluted 1 to 30 in normal saline. Recently the author used an antigen composed of ten different strains of benign tertian malarial parasites with satisfactory results, and this gives a positive reaction with known cases of benign or malignant tertian malaria. The antigen must be titrated before use to estimate the minimum dose of complement required. The patient's serum is diluted 1 to 10 with normal saline. The test is carried out in the usual way using positive and negative controls. The method used is long fixation in the cold, the tubes being kept overnight in the ice chest. In the morning the sensitized red cells are introduced and the results read after fifteen minutes in the water bath at 37° C. After very prolonged quinine treatment and in cases where there has been no attack of malaria for many months, the blood serum usually gives a negative result. The serums of syphilitic patients give a positive result with this antigen, and it is necessary to exclude this disease.—J. Gordon Thomson, *Brit. Med. Jour.*, No. 3023, p. 628 (1918).

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Agglutination in the Diagnosis of Glanders.—Fifteen strains of B. mallei were tested and one obtained which is constant in its agglutinability with positive sera. The best culture medium is glycerin-potato-agar. No phenol is added to the stock nor to the dilution. A negative reaction by a single agglutination test if not confirmed by the ophthalmic and the complement fixation tests does not prove the case negative. All three tests must be performed in order to pronounce the horse negative. A reaction of less than 1 to 1000 by agglutination is not indicative of glanders in the horse.—Olga R. Povitzky, *Jour. Immunol.*, 3, 463 (1918).

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Reaction of B. Coli on Endo Medium.—During the growth of B. coli on Endo medium adsorbed lactose is broken down first. After 10 to 15 hours incubation, the trace of adsorbed lactose is probably transformed into lactic and other organic acids, and the colony is colored red. On further incubation, the organic acids are probably reduced by the bacteria to aldehyd and alcohol, which volatilize from the surface, leaving the nonvolatile fuchsin behind, thus producing a metallic sheen. Following this, the organisms attack the nitrogenous material. Ammonia and other substances are produced in which the fuchsin goes back into solution, and ultimate decolorization takes place.—R. L. Kahn, *Jour. Bact.*, 3, 547 (1918).

You'll feel mighty lonely when the membership campaign is over, if your name isn't on the roll of honor.