

river water have likewise been examined. In all cases, the value of the typing method of Craigie and Yen has been evident. Epidemiological studies have repeatedly verified the original investigations of Craigie and Yen regarding the stability of types and the separation of epidemic and endemic cases. Application of the typing results to epidemiological studies is being made as often as is possible and the results show that the typhoid typing method is a valuable and sensitive tool in the control of typhoid fever. Properly applied, the procedure is a useful adjunct to epidemiological studies. Future possibilities in applying the method are dependent on the aid and co-operation of all concerned.

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Carrion's Disease. I. Some Growth Factors Necessary for Cultivation of *Bartonella bacilliformis*.

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The medium of preference for the isolation of *Bartonella bacilliformis* from the blood of Oroya fever cases and for the routine of stock cultivation is still the one originally used for these purposes by Noguchi: namely, his semi-solid serum agar for leptospira.^{1, 2} This medium however, is not suitable for the production of vaccines. In attempts to grow the organism on solid media it soon became apparent that the addition of blood was necessary.^{3, 4} It seemed of

¹ Noguchi, H., and Battistini, T., *J. Exp. Med.*, 1926, **43**, 851.

² Noguchi, H., *J. Exp. Med.*, 1926, **44**, 553.

³ Aldana, L., *Bacteriología de la Enfermedad de Carrion Tesis*, Lima, Peru, 1930.

⁴ Lawkowic, W., *Trans. Roy. Soc. Med. Hyg.*, 1939, **32**, 601.

interest then, to determine whether one or both the x and v growth factors contained in the blood were necessary for its successful cultivation.

Four strains of *Bartonella bacilliformis* were studied. Two were obtained from Dr. M. Hertig in Peru. They were originally isolated from the blood of 2 fatal cases of Carrion's disease. These strains were pathogenic for monkeys one year after isolation. A third strain was supplied by Dr. D. Weinman of the Harvard Medical School. It had been shown to be pathogenic for the monkey for a period of several years. These 3 cultures were received in Noguchi's medium. A fourth culture was isolated by us from a blood specimen sent by Dr. H. Colichon in Peru, taken from a recovered case of Carrion's disease which no longer showed the organisms in the blood cells. Smears of this blood made in this laboratory were negative. The morphologic, staining and cultural characters of all these strains were similar. None of the strains grew in infusion broth or on agar slants even when heavily inoculated.

As a source of the v factor an aqueous extract of yeast prepared according to Lwoff⁵ was used in a concentration of 0.1% in 1% glycerine infusion agar. The x factor was furnished by the addition of 5% defibrinated rabbit's blood which was then autoclaved at 120° C for 20 minutes.⁶ Agar plates containing x, v, and xv were inoculated. Cultures of *H. influenzae* and parainfluenza were used as controls on the media. The cultures have been followed for 5 transfers. At each transfer plain infusion broth and agar slants were also inoculated with the bartonella strains and the influenza controls and in each instance no growth occurred. The incubation temperature varied between 25° and 28° C; the pH of the medium between 7.2 and 7.6. Desiccation of the medium was always fatal to growth of this organism. The plates were sealed with rubber.⁷ On this medium numerous small translucent colonies of *B. bacilliformis* could be easily seen with the naked eye by the 3rd or 4th day.

The results may be briefly stated as follows: The presence of both factors x and v in the glycerine agar insure an excellent growth for *B. bacilliformis* which becomes apparent on the third day. The medium containing the x factor alone is sufficient to support a good growth in all the transfers but it appeared on or sometime after the fourth day. The basic medium containing the v factor alone is in-

⁵ Lwoff, A., and Lwoff, M., *Proc. Roy. Soc. London*, 1937, **122**, 352.

⁶ Thjotta, T., and Avery, O. T., *J. Exp. Med.*, 1921, **34**, 97, 455.

⁷ Petroff, S. A., and Steenken, W., Jr., *J. Exp. Med.*, 1930, **51**, 834.

sufficient for successful cultivation. However it is possible to show some restricted growth on the first or second transfers due probably to the x factor carried over with the inoculum. Further transfers on the medium were unsuccessful.

The small number of experiments with only 4 strains imposes caution in the matter of making definite statements regarding the x and v factors, but it may be inferred from these results that *B. bacilliformis* is incapable of synthesizing the x factor but can produce its own cozymase. This is indicated by the results obtained with the x factor alone. It is interesting in this connection to note that one member of the Hemophilic group, *H. canis*,^{8, 9} is similar in this respect to these strains of *Bartonella bacilliformis*.

The macroscopic appearance of *B. bacilliformis* colonies can be of two kinds: 1. Minute, round, mucoid clear colonies which are sometimes confluent; 2. An opaque finely granular yet mucoid film which has a tendency to outgrow the original boundaries of the inoculum. In both types the growth adheres so tenaciously to the medium and to the loop that it is difficult to make a suspension. The suspensions are homogeneous.

On microscopic examination this organism presents great pleomorphism, a character mentioned by many investigators.¹⁰ Mixtures of small bacillary and finely granular shapes sometimes occurring in masses, can be seen. It is not possible at this time to identify the micro- and macro-scopic characters in their relation to the phenomenon of dissociation.

During the experiments on the growth factors v and x, other solid media were tried. Several are worthy of mention since to our knowledge their use for isolation has not been reported in the literature. A blood-glucose-cystine agar gave excellent results. This medium was made by adding 0.1% cystine and 1% glucose to autoclaved beef infusion agar. The medium was then sterilized in flowing steam one hour for 2 days. Defibrinated fresh rabbit's blood was added up to 5% concentration before pouring the plates. One of the strains studied was isolated on this medium directly from the patient's blood specimen. The colonies were numerous, discrete and easily discernible on the third day of incubation. The addition of 0.1% tryptophane did not enhance the growth promoting properties of this medium. Blood glucose agar gave much slower and scantier growth.

⁸ Rivers, M. T., *Johns Hopkins Hosp. Bull.*, 1922, **33**, 140.

⁹ Fildes, P., *Brit. J. Exp. Path.*, 1923, **4**, 265.

¹⁰ Pinkerton, H., and Weinman, D., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 591.

Another medium which gave excellent results was a 5% defibrinated rabbit's blood infusion agar to which 1% glycerine had been added. Growth of all the strains on this medium was abundant on the third day. Microscopic examination showed compact masses larger than those seen in the smears from the cystine medium. No changes in the blood pigment were observed in any of the media employed in this investigation.

For comparison it may be stated that a 4 or 5 day growth on either the cystine or glycerine blood agar medium can be favorably contrasted with that obtained in a 15- to 20-day-old culture grown in Noguchi's medium.

Summary. Solid media for the rapid isolation of *Bartonella bacilliformis* are described. It is shown that this organism requires the x growth factor of blood. No growth was obtained with the v factor alone.

11698 P

A New Intravenous Galactose Clearance Test for Differentiation of Obstructive from Parenchymatous Jaundice.*

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Galactose is an ideal substance for testing hepatic function because it is utilized exclusively by the liver and because its utilization is independent of insulin. Nevertheless the usual method of performing the galactose test by giving this sugar orally and determining its excretion in the urine gives unreliable results, due in part to wide variations in the rate of intestinal absorption^{1, 2} which are especially significant because of the absence of a renal threshold for galactose, and in part to difficulties in measuring the galactose in the urine of patients with marked bilirubinuria. Therefore we have modified the test by administering galactose intravenously and determining its clearance from the blood.

Technique: One cubic centimeter of a 50% solution of galactose per kilogram of weight is injected intravenously. The injection

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¹ Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1938, **123**, 577.

² Althausen, T. L., *Am. J. Digest. Dis.*, 1939, **6**, 544.