blood flow (Fig. 2 B). During the following phase peripheral vasoconstriction apparently results in a marked elastic recoil of the blood, causing a diastolic regurgitation. The resultant average peripheral flow is thus reduced to 10% of the normal (Fig. 2 C). The contour of the velocity curve is obtained by joining the *upper edges* of the individual dots. The area corresponding to negative flow is, therefore, not as great as might appear at the first glance.

The intravenous injection of 25 mg of Papaverin (Fig. 3) is followed by an almost immediate rise in peripheral blood flow reaching a maximum of 230% of the normal (Fig. 3 B, Sec. 44) and persisting at an elevated level for an appreciable period of time (Fig. 3 C). Fig. 3 B shows an interesting gradual change of the diastolic contour of the flow tracing which indicates a transient diastolic regurgitation of short duration. But, as Table I shows, the average flow remains above normal even in those sections where the regurgitation is greatest.

In the beginning of record 3 B there is a noteworthy prolongation of the diastolic interval which is followed by a vigorous systolic thrust.

Summary. A modified electro-magnetic flow meter has been described which may be applied to investigation of a wide variety of cardiovascular problems involving flow and pressure measurements in intact animals.

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New Media for the Growth of *Bartonella bacilliformis.*

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Liquid, semi-solid and solid media, tissue cultures, and developing chick embryos have been utilized for the growth of *Bartonella bacilliformis.*[1-5] Of these media, the semi-solid leptospira medium of

1 Noguchi, Hideyo, and Battistini, Telémaco S., *J. Exp. Med.*, 1926, **43**, 851.

2 Samper, Bernardo, and Montoya, Juan Antonio, *Rev. Fac. Medicina de Bogotá*, 1940, **9**, 197.

3 Jiménez, J. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 402.

4 Pinkerton, Henry, and Weinman, David, PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 587.

5 Jiménez, J. F., and Buddingh, G. John, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 546.

Noguchi and nutrient blood agar have aided (1) in proving *B. bacilliformis* as the etiological agent of Oroya fever and *verruga peruana,* the two entities of Carrion's disease in Peru, (2) animal experimentation, (3) studies on transmission, and (4) limited immunological studies with the organism. Further progress, particularly with the biochemical characteristics of the organism and the immunology of the disease, has been handicapped by the slowness and relatively sparse growth of bartonella in leptospira medium, and the difficulty of making suspensions with organisms when grown on blood agar. Thus, it was believed that the development of methods for cultivating greater quantities of the organisms would stimulate attempts to solve some of the current problems of bartonellosis.

A fruitful approach to the problem of improving known media and devising new ones, appeared to be linked with the need in leptospira medium for serum and particularly a solution of hemoglobin from laked red blood corpuscles. The necessity for these ingredients indicated that certain vitamins and growth-promoting factors found in the cellular elements of the blood might be the key substances needed for growth.* Furthermore, *B. bacilliformis* in Oroya fever invades and multiplies within cells of the reticulo-endothelial system,[4] a fact which suggested the possible advantageous use of ingredients in monocyte media[6, 7] for enhancing the growth of bartonella.

Extensive experiments with protein digests; blood, yeast, and liver extracts; peptones; vitamins and developing chick embryos have yielded numerous new media that surpass the results obtainable with leptospira medium and blood agar. The preferred media described below are considered practical, giving growth of *B. bacilliformis* in measurable quantities.

Five strains of *B. bacilliformis* from Peru were used for the experiments and were isolated as follows: Strain 1, March 10, 1939, from a child with acute Oroya fever; Strain 2, August 3, 1939, from the proboscis of a wild fed sandfly, *Phlebotomus verrucarum,* taken at Huinco in the Santa Eulalia valley; Strain 3, August 24, 1940, from an adult during a third recurring attack of *verruga peruana;* Strain 4, August 7, 1940, from an adult in the Dos de Mayo Hospital, Lima, who had contracted Oroya fever in the Department of Ancash; Strain 5, August 7, 1940, from an adult in the Dos de Mayo Hospital, Lima, who had contracted acute Oroya fever in

* Jiménez (1940) has recently claimed that *B. bacilliformis* requires the x factor from blood but not the v factor from yeast for growth.

6 Baker, Lillian E., *Science,* 1936, **83**, 605.

7 Baker, Lillian E., and Ebeling, Albert H., *J. Exp. Med.,* 1939, **69**, 365.

the Santa Eulalia valley. Strains 1, 2, and 3 were isolated by Dr. Marshall Hertig during studies on the transmission of Carrion's disease, and Strains 4 and 5 were isolated by Messrs. Eiseman, Howe, and Plimpton during preliminary immunological studies with Carrion's disease. Sincere acknowledgments are expressed for the cultures and also for the arrangements, cordial coöperation, and the provision of generous facilities for investigation by Director Telémaco Battistini, Instituto Nacional de Higiene y Salud Publica, Lima.

The liquid tryptone-serum medium is mixed as follows: 75 cc of a sterile 1% solution of tryptone† in distilled water adjusted to pH 7.6-7.8, 25 cc of fresh rabbit serum, and 0.2 cc of a sterile (Berkefeld N) mixture of ascorbic acid‡-glutathione solution (10 mg ascorbic acid and 40 mg glutathione in 20 cc Ringer's solution[7]). Place 8 or 10 cc of this mixture in small sterile Erlenmeyer flasks (50 cc), incubate for sterility and then inoculate with bartonella. Growth (maximum at 28°C) takes place on the bottom of the flask, becoming visible as a finely divided sediment within 24 to 48 hours and reaching a peak on about the tenth day. Depending upon the size of the original inoculum, an amount up to 0.1 cc of packed organisms can be harvested from each flask. These organisms can be resuspended in 3-5 cc of buffered saline solution pH 7.8-8.0 to make a homogeneous suspension for use in testing immune sera for agglutinins.

The semi-solid medium is made as follows: Base agar—2 g Bacto-agar, 8.5 g sodium chloride, 1000 cc distilled water. Dissolve ingredients and adjust to pH 7.6-7.8. To 100 cc of this base medium, dissolved and cooled to 45°C, add 10 cc of sterile 5% aqueous tryptone adjusted to pH 7.6-7.8, 10 cc of fresh sterile rabbit or sheep serum, and 0.2 cc ascorbic acid-glutathione solution. Dispense in sterile test tubes, incubate, insert rubber stoppers to prevent evaporation if desired, and inoculate. At 28°C, growth becomes grossly visible at 36 to 48 hours and very heavy at about the fifth day. Minute inocula that fail to grow out on leptospira medium develop very readily on this medium. The medium is colorless and transparent, characteristics which facilitate early detection and description of growth. If tubes are kept at room temperature, the period of growth is lengthened and subcultures can be made with safety at intervals of 6 weeks to 2 months.

The solid medium is made as follows: Base agar—20 g Bacto-

† Courtesy Difco Laboratories, Inc.

‡ Courtesy Merck & Co.

agar, 20 g tryptone or proteose peptone No. 3,§ 5 g sodium chloride dissolved in 1000 cc distilled water and adjusted to pH 7.6-7.8. To 75 cc of sterile and dissolved base agar cooled to 45°C, add 25 cc of fresh (rabbit or sheep) serum or, for economical reasons, 25 cc of whole defibrinated blood from rabbits or sheep, and 0.2 cc ascorbic acid-glutathione solution. Place sufficient quantities in test tubes to make a short butt and a long slant. When slants are set, place sterile rubber stoppers in the tubes and incubate for sterility. Inoculate the slant with *B. bacilliformis* by the loop method and incubate at 28°C. Growth follows the streak of the loop, becoming grossly visible within 24 to 48 hours and reaching a maximum in 10 to 14 days. Heavy growth takes place also in the water of condensation. The transparency of the medium containing serum facilitates observation of growth and general morphology of colonies. Single colonies of the 5 strains that have been tested are consistent in their morphology, being smooth, convex, mucoid, and opaque, and reaching a maximum diameter of 1-1.5 mm. However, growth is usually in a sheet, having a characteristic mucoid and opaque appearance. Since the surface of the slant must be moist to obtain maximum growth, the organisms can be harvested from the slants very easily by washing and careful scraping. Organisms grown on this medium can be used for making suspensions, although they are not as easily dispersed as those grown on the liquid medium. If defibrinated blood instead of serum is used in the medium, the quantity of growth is about the same, but the inclusion of blood cells or laked solutions of blood is not necessary for growth or maintenance of bartonella by subculture.

The media described above can be made easily and they give relatively rapid and luxuriant growth of *B. bacilliformis* when compared with results on older types of media. Thus, measurable quantities of the human bartonella can now be grown to permit bacteriological and immunological studies, the results of which hold promise of leading to methods for the control of Carrion's disease. This possibility assumes added significance when it is remembered that new foci of Carrion's disease have been reported in Colombia and Ecuador in recent years, and that no satisfactory treatment for the disease is known.

§ Courtesy Difco Laboratories, Inc.