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Rapid Method for Obtaining Anti-Rh Serum from Guinea Pigs.

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In order to prepare anti-Rh serum, Landsteiner and Wiener[1] recommended that guinea pigs be inoculated intraperitoneally with a suspension of washed *Rhesus* monkey erythrocytes equal to 2 cc of whole blood. The animals received 2 such injections 5 days apart, and were bled from the heart 7 days after the second inoculation. They stated that although most of the sera obtained in this manner could be used to show differences between Rh (+) and Rh (—) qualities of human blood, only "one or more" sera of a group of 10 animals thus immunized could be used diagnostically to determine the Rh characteristic. Recently these workers[2] suggested that the immunization of guinea pigs be accomplished by 5 successive inoculations of a suspension of *Rhesus* erythrocytes equal to 2 cc of whole blood given at 5-day intervals, and the sera collected by bleeding 7 days after the last inoculation.

In the present investigation anti-Rh sera were prepared according to 3 technics of immunization. The first anti-Rh serum was prepared according to the method suggested by Landsteiner and Wiener,[1] using adult guinea pigs weighing between 300 and 500 g. Twenty animals were given 2 intraperitoneal inoculations 5 days apart and were bled from the heart on the seventh day following the second inoculation. Two animals died during the immunization period from accidental causes, the remaining 18 yielded 3 diagnostically usable anti-Rh sera. Five of the 18 guinea pigs in this group were not exsanguinated during the bleedings, but were saved for further immunization. Six weeks after the first series of inoculations, these animals were given 2 doses of 2 cc of *Rhesus* blood at 5-day intervals and were bled 7 days following the last injection. Two sera of high titer and one serum of fair titer were obtained from these animals.

The second anti-Rh serum was prepared according to a modified method suggested by Landsteiner and Wiener.[2] A group of 16 adult guinea pigs weighing from 300 to 500 g were inoculated intraperitoneally with a suspension of erythrocytes equal to 2 cc of *Rhesus* blood. The animals were to be subjected to 5 separate inoculations 5 days apart. They were to be bled on the seventh day following the last inoculation. Nine animals failed to survive, 2 died within 4 hours, 4 died within 24 hours, and 3 died within 48 hours following the third inoculation. Post-mortem examinations revealed definite edema of the lung in all of these animals and it was concluded that death was due to hypersensitization. The administration of *Rhesus* monkey blood was discontinued for the 7 surviving animals. These were bled on the seventh day following the third inoculation. Three sera of high titer were obtained.

Although the modified technic of immunization (Landsteiner and Wiener) produced a high percentage of diagnostic sera in the animals surviving the third inoculation, the number of deaths from hypersensitization renders this method expensive.

The third anti-Rh serum was prepared by altering the above technics as follows: adult guinea pigs weighing from 300 to 500 g were given 5 intraperitoneal inoculations of *Rhesus* monkey erythrocytes in increasing doses at 48-hour intervals. The initial inoculation consisted of washed *Rhesus* monkey erythrocytes equal to 1 cc of whole blood. The inoculum was increased by 0.5 cc with each succeeding inoculation until the fifth or final inoculation, which consisted of 3.0 cc of washed erythro-

* Assisted by a grant from the Christine Breon Fund.

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2 Wiener, A. S., personal communication.

cytes. The animals were bled from the heart on the second day following the last inoculation. Five of 9 animals used in this group produced reliable diagnostic sera, and there were no deaths among them.

All of the anti-Rh guinea pig sera were prepared as suggested by Landsteiner and Wiener[1], and stored in the refrigerator. The serum from each immunized guinea pig was diluted 0.1 cc of serum in 0.9 cc of 0.85% buffered saline. Immediately before use each was absorbed for one hour at room temperature with 0.05 cc of washed human erythrocytes of group A and 0.05 cc of washed human erythrocytes of group B. After absorption and before using the serum as a test reagent, it was further diluted one to 3 with 0.85% buffered saline. Diagnostic tests were made using known Rh (+) and Rh (—) human erythrocytes as controls. In each test 2 drops of the diluted guinea pig serum were placed in a 7 mm test tube to which was added one drop of a 1.5% suspension of washed human erythrocytes. The mixture was allowed to remain at room temperature for one hour before the sediments were read. Sera giving clear-cut Rh negative and Rh positive sediments were considered reliable for diagnostic tests.

Summary. A modified rapid method for the preparation of diagnostically suitable anti-Rh serum obtained from guinea pigs is described.

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Measurement of Circulation Time (Photoelectric Method).

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Various methods have been proposed for the objective determination of the circulation time. This time is usually considered to be that which elapses between the injection of a particle of a foreign substance into the circulatory system and its arrival at the point of detection.

Many previous studies of the circulation time have been made with dyes. Meyer[1] first used blood containing methemoglobin as an indicator. Methylene blue was employed in animals by Stewart.[2] Koch,[3] Lian and Barras,[4] as well as Wollheim and Lange[5] employed fluorescein in humans. Thompson *et al.*[6] and Moore and Kinsman[7] used brilliant vital red for the study of the circulation time. Hamilton *et al.*[8] made use of phenol-tetra-iodophthalein sodium and Klein and Heinemann[9] employed congo red. Fishback[10] recently reported his experience with rabbits injected with fluorescein and other fluorescent substances.

Recently, we adapted a photoelectric method to the measurement of circulation time, the basic phenomena of which have been reported.[11] The principle involved is that a photoelectric cell is influenced by light trans-

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10 Fishback, D. B., *J. Lab. and Clin. Med.*, 1941, **26**, 12.

11 Jablons, B., *Science*, 1943, in press.