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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2 Stewart, G. N., *Am. J. Physiol.*, 1921-22, **58**, 27.

3 Koch, E., *Deut. Arch. klin. Med.*, 1922, **140**, 39.

4 Lian, M. C., and Barras, E., *Bull. Soc. Med. Hop. Paris*, 1939, **54**, 157.

5 Wollheim, E., and Lange, K., *Verhandl. deut. Ges. inn. Med.*, 1931, **43**, 133.

6 Thompson, W. O., Alper, J. M., and Thompson, P. K., *J. Clin. Invest.*, 1928, **5**, 605.

7 Moore, J. W., and Kinsman, J. M., *J. Lab. and Clin. Med.*, 1939, **22**, 165.

8 Hamilton, W. V., Moore, J. W., Kinsman, J. M., and Sperling, R. G., *Am. J. Physiol.*, 1928, **84**, 338.

9 Klein, O., and Heinemann, J., *Zentrbl. inn. Med.*, 1929, **50**, 490.

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

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

mitted through animal tissues, the intensity of which may be diminished by intravenously injected dyes.

Certain non-toxic dyes, such as 2 to 5 ml of a 1% solution of methylene blue or 5 ml of 0.5% Evans' blue, when injected intravenously, act as screens impeding the transmission of light, as observed by the deflection of the indicator of a sensitive galvanometer connected with a photoelectric cell. The time elapsing between the injection of the dye into the vein of the arm or leg, and its arrival at the point where the light and photoelectric cell have been placed, is determined by a stop watch, or can be recorded by connecting the leads from the photoelectric cell to a recording galvanometer.

In our studies, the circulation time was measured in humans from arm vein to ear lobe, and in rabbits from the ear vein of one ear to the opposite ear, using methylene blue almost exclusively. In humans, all determinations were made in the supine position. The ear unit was placed on either ear with the light source behind the ear. As the light was turned on, the needle of the galvanometer indicated the amount of current generated by the light transmitted through the tissues. Methylene blue was then injected rapidly into the arm vein, which was about at the level of the heart. The arrival of the dyed blood at the point of detection was thus indicated by a definite deflection of the needle of the galvanometer.

Objective determinations of the circulation time are made possible by this procedure, without the disadvantages inherent in other methods. Moreover, we have observed no venous thrombosis, as has been reported in other procedures. A few precautions, however, need emphasis. The needle employed should be of a sufficiently large bore (No. 18-20 gauge) to permit rapid injection of the dye. Methylene blue (1% solution) rarely produces a reaction even when injected in less than 1 to 2 seconds. In some individuals, 2 ml is sufficient to produce a deflection of the needle; however, in large subjects and patients with congestive heart failure, it has been found advisable to use as much as 5 ml. After the needle is in the vein, it is necessary to wait a few seconds before injecting the dye to stabilize the indicator on the galvanometer.

This method was found effective in animals and in humans. In normal rabbits the circulation time was approximately 6 seconds. In a series of 50 humans we obtained the following results:

Group A. In 14 patients without evidence of cardiac involvement the time varied from 9 to 16 seconds.

Group B. In 18 patients suffering from cardio-vascular disease (rheumatic heart disease, hypertensive cardio-vascular disease and coronary heart disease) who showed no subjective or objective evidence of decompensation, the time was similar to that of group A, namely, 9 to 15 seconds.

Group C. In 15 patients with decompensated cardio-vascular disease the time ranged from 20 to 45 seconds.

Group D. In 3 patients with hyperthyroidism the time ranged from 8 to 9 seconds.

All patients were in the 2nd to 8th decades of life, the majority in the 4th, 5th and 6th.

Summary. An objective method for the determination of circulation time is presented, which appears to have many advantages over previous methods with respect to accuracy, safety and facility to obtain recorded data. The use of an intravenously injected dye and a light source transmitted through animal tissue to a photoelectric cell, makes determinations possible, by noting the deflection of the needle in a sensitive galvanometer or other sensitive measuring device. The time elapsing between the injection of the dye and the deflection of the needle is the circulation time. Under experimental conditions, the normal circulation time ranged from 9 to 16 seconds, and in decompensated cardio-vascular disease as high as 45 seconds was recorded.