point where the pancreatic duct had already been transplanted. This deprived the duodenum of both bile and pancreatic juice, leaving it only duodenal juices for protection against the acid gastric secretion. The dogs were now allowed to go on for an average of 78 days, during which period they were explored several times with no evidence of ulcer formation. At the end of this time when they were sacrificed their nutrition was good, there was no anemia, and no damage to the mucosa of the duodenum.

The number of these experiments is too small and the period of time too short to draw any definite conclusions, especially in view of the inconstant results, but they suggest that the bile is the most important of the 3 factors.

6103

A New Method for Determining the Fragility of Red Blood Cells.

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Prevailing methods for the determination of the fragility of erythrocytes are based upon the theory of their hemolytic stability when brought into contact with chemicals such as saponin and bile salts, with specific sera, or with hypotonic salt solutions. Theoretical as well as practical considerations have directed the development of these studies toward improving the technique for determining the resistance of the red blood cells to solutions containing different concentrations of various salts. Of the many variations in the original method of Ribierre,[1] that of Simmel,[2] or one of its modifications,[3] appears to be the best. However, the necessity of freshly prepared hypotonic salt solution of very exact concentrations, together with the labor in using the counting chamber and the necessity for setting up a control test with a known normal blood at each observation, are definite disadvantages.

We have devised a fragility test in which the difficulties mentioned are largely eliminated. The testing of the relative fragility of the

[1] Ribierre, P., *L'hemolyse et la mesure de la résistance globulaire; application à l'étude de la résistance globulaire dans l'ictère. Thèse de Paris,* 1903, No. 154.

[2] Simmel, H., *Arch. f. klin. Med.,* 1923, **142**, 252.

[3] Waugh, R. T., and Chase, W. J., *J. Lab. and Clin. Med.,* 1928, **13**, 873.

patient's cells in varying dilutions of his own plasma is the essential principle introduced in this new method.

Technique. Test tubes of 20-30 cc. capacity and capable of withstanding the high speed of the centrifuge are fitted with rubber stoppers. To each is added 2 mg. of powdered heparin, an amount sufficient to prevent the clotting of 10 cc. of blood for approximately 24 hours. After weighing out this quantity a few times to visualize the approximate volume involved, the quantity of heparin added to each test tube may be estimated without disturbing the accuracy of the test. Ten cc. of blood are drawn from one of the arm veins, using a dry (not rinsed in salt solution) 10 cc. syringe and needle. After discharging the blood into the heparin-containing tube, the latter is rocked back and forth for about one minute and put aside until the test is to be set up.

Thirteen agglutination tubes (11 mm. x 75 mm.) are placed in an appropriate rack. Tube No. 13 is half-filled with the whole blood. The remainder of the sample is centrifuged, and the clear plasma removed with a Wright's capillary pipette. Two-tenths cc. of the plasma is transferred into each of the tubes Nos. 1-12, inclusive, using a 1 cc. serum pipette graduated in 100 divisions and equipped with a rather sharp delivery end. Various quantities of fresh glass-distilled water are then added to each of these 12 tubes in amounts as shown in Table I, and mixed with the plasma previously added. Finally, 0.02 cc. of the whole blood from tube No. 13 is pipetted into each tube, and the tube immediately shaken. After standing 2 hours or more at room temperature the tubes are examined and the points at which hemolysis begins and is complete

TABLE I.
Plasma Dilutions with Calculated Equivalents in Salt Concentrations.

Tube No.	Dist. Water cc.	Salt Equivalents†	Tube No.	Dist. Water cc.	Salt Equivalents†
*	.06	.707	5	.28	.396
*	.08	.660	6	.30	.380
*	.10	.618	7	.32	.366
*	.12	.582	8	.36	.341
*	.14	.550	9	.40	.319
*	.16	.521	10	.44	.300
*	.18	.495	11	.48	.282
1	.20	.471	12	.52	.267
2	.22	.450	*	.56	.253
3	.24	.430	*	.64	.230
4	.26	.412	*	.72	.210

All tubes have 0.20 cc. plasma and 0.02 cc. whole blood added to the above amounts of distilled water to complete the test.

* Tubes not numbered are set up only when needed for exceptional cases.

† See footnote p. 837.

are recorded. At this reading it is also noted whether the plasma in tube No. 13 is free from spontaneous or traumatic autohemolysis —if not, the results must be rejected, new blood obtained, and the test repeated. It is best to use the same pipette for measuring the plasma, distilled water and blood.

Application of the Test. Blood samples have been secured from 50 healthy individuals varying from 10 to 60 years. In no case has hemolysis been noted in tubes 1-3 inclusive, nor was a higher dilution required for complete hemolysis than that represented by tube No. 10. It is, therefore, believed that when hemolysis occurs in more concentrated plasma than that represented by tube No. 4, pathological fragility is indicated, and when hemolysis has not started in the dilution represented by tube No. 5, or is not complete in that of tube No. 10 an abnormal increase in erythrocyte resistance is present. The salt equivalents* for the limits of normal as established by this test are 0.300-0.412.

Inter-plasma controls have been made on normal and pathologic bloods to determine definitely whether the increased ease of hemolysis in certain individuals is a property of cells or plasma. While the important determination is of course the effectiveness with which the homologous red blood cells resist the hemolyzing environment of their own plasma, which this test assures quite irrespective of the salt-equivalent-resistance-value, it has been of interest to analyze the constancy of the isotonicity of the blood plasma from individual to individual. In no single instance in the 100 bloods studied to date, including both normal and pathologic fragility ranges, has the blood plasma influenced the index of hemolysis for homologous or isologous erythrocytes.

Among the 50 individuals, representing a variety of diseases, from whom blood samples have been tested, there were 3 cases of congenital hemolytic icterus. As originally tested in dilutions of their own plasma these erythrocytes all showed a marked increase in fragility above the established normal values. The cells from the cases of congenital hemolytic icterus, when tested against plasma from normal individuals, showed hemolysis within exact limits established by tests with their own plasma. Conversely, the cells from the normal individuals were tested in the plasma from the cases with hemolytic jaundice, but no increase of fragility was demonstrated.

* These values are calculated as percentage of sodium chloride, assuming that 0.9 gm. sodium chloride per 100 cc. distilled water yields a solution isotonic with mammalian blood.[4]

[4] Starling, E. H., Principles of Human Physiology, Philadelphia, Lea and Febiger, 5th Edition, 1930.

These results showed that the cells both from the normal and jaundiced patients retained their respective characteristic fragilities without reference to the source of the plasma.

Using the technique described in this paper, a detailed study of the index of hemolysis in both normal and diseased individuals will be presented elsewhere.

6104

An Intravascular Lesion in Poliomyelitis Induced by Feeding in Macacus Cynomolgus.

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Wickman[1, 2] dwelt at some length upon the lesions of the central nervous system associated with acute poliomyelitis, and as a result of the accuracy of his original observations his descriptions of the changes encountered have been little altered in subsequent years. Wickman noted that the vascular lesions of acute poliomyelitis were more marked in the veins than in the arteries, and that the frequently encountered round cell infiltration was situated in the lymph channels of the vessel wall. It was also recognized, however, that the lymphocytic infiltration was often of sufficient intensity to extend beyond the adventitial limits of the vessels into the surrounding tissues. Flexner and Amoss[3] pointed out that in cases of acute poliomyelitis induced by intravenous administration of virus the vascular lesions were more extensive than in instances of infection by other routes.

The lesion described below was encountered in the meningeal vessels of a *Macacus cynomolgus* which developed acute poliomyelitis after having been fed with virus-infected milk. The material used was a 10% milk suspension of recently glycerinated brain and spinal cord from monkeys which had succumbed to typical poliomyelitis. Six daily feedings of 30 cc. were administered by mouth with the aid of a medicine dropper, the milk being fed slowly so that the monkey could swallow it easily.

[1] Wickman, I., *Studien über Poliomyelitis acuta.* Karger, Berlin, 1905.

[2] Wickman, I., *Die akute Poliomyelitis bzw. Heine-Medinsche Krankheit.* Springer, Berlin, 1911.

[3] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, **20**, 249.