printing one decimal point was misplaced. Both printings show 350,000A (350 μ), and $1cm^{-1} = 1/\lambda_{(cm)}$. Obviously the first is in error by a factor of 10, and the second is true only when $\lambda = 1cm$. These errors are much too trivial to damage the book; they are cited only as warnings to proofreaders.

The theory of infrared absorption and its relation to molecular structure are discussed to provide the background essential for detailed descriptions of techniques useful in analysis. A description of a spectrometer is followed by transmission curves for 363 organic compounds including hydrocarbons, alcohols, ethers, carbonyl compounds, nitrogen compounds, terpenes, organic chlorides and miscellaneous. These curves represent absorption spectra between 2,000 and 750 cm^{-1}; they may be used for studying correlations between molecular structure and spectral characteristics, for identification of unknown materials, and for determining in advance the possibilities of qualitative and quantitative infrared analysis of mixtures.

The bibliography, presumably contributed largely by the second-named author, probably represents the chief justification for the printing of this book. It contains 2,701 entries, and even though it is incomplete it is incomparable because it is the first large collection of titles associated with infrared, so far as this reviewer knows.

In the relatively new field of industrial applications of infrared spectroscopy this book will have a triple appeal, first as an outstanding example of success in a certain industry, second as a valuable catalogue of the absorption spectra of organic compounds, and third, as a source of published information of general and specific interest.

WILLIAM F. MEGGERS

SPECIAL ARTICLES

EXPERIMENTAL AND CLINICAL OBSERVATIONS ON INCREASED MECHANICAL FRAGILITY OF ERYTHROCYTES[1,2]

INTRODUCTION

SINCE the work of Meltzer and Welch[3] and of Rous and Turner[4] the liability of red blood cells to physical destruction by the motion of the circulation has received little attention. Recently, however, Dameshek and Miller,[5] Stats[6] and Tsai and associates[7,8] have observed increased mechanical fragility of agglutinated red blood cells. Shen, Ham and Fleming[9] noted the increased mechanical fragility of the red blood cells of previously heated blood. The present report is a preliminary account of a quantitative method for determining the mechanical fragility of erythrocytes, of some experimental factors affecting this property and of its relation to certain hemolytic anemias.

METHOD

The percentage volumes of red blood cells (hematocrits) of samples of defibrinated blood to be compared (including a normal control) were adjusted to approximately 40 per cent., if necessary, by removal or addition of serum. Seven cubic centimeters of each sample of blood to be tested were introduced into individual cylindrical 150 cc soft glass tonometers (permitting equilibration of the blood with gas mixtures), the length of the parallel portions of the sides of which was about 130 mm, the diameter about 28 mm. To each tonometer were added 50 glass beads uniformly 4 mm in diameter. The tonometers were then attached at each end to clips on the periphery of two wheels approximately 150 mm in diameter. These wheels were fixed at an appropriate distance apart on a horizontal axle which was rotated at 28 to 30 r. p. m., usually for 2 hours at room temperature.[10] Before rotation, an accurately measured 0.1 cc sample of blood (designated as sample *C*) was introduced into a test tube containing 1 cc of distilled water (complete osmotic lysis). At the start and at the termination of the period of rotation, additional 0.1 cc samples of the blood (samples *A* and *B*) were delivered into test tubes containing 1 cc of a 1.25 per cent. solution of

[1] From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.

[2] This investigation was aided by a grant from the John and Mary R. Markle Foundation.

[3] S. J. Meltzer and W. H. Welch, *Jour. Physiol.*, 5: 255–260, 1884.

[4] P. Rous and J. R. Turner, *Jour. Exp. Med.*, 23: 219–237, 1916.

[5] W. Dameshek and E. B. Miller, *Arch. Int. Med.*, 72: 1–17, 1943.

[6] D. Stats, *Proc. Soc. Exp. Biol. and Med.*, 54: 305–306, 1943.

[7] J. S. Lee, Y. C. Puh and C. Tsai, *Proc. Chinese Physiol. Soc., Chengtu Branch*, 2: 59–61, 1944.

[8] Y. C. Puh, J. S. Lee and C. Tsai, *Proc. Chinese Physiol. Soc., Chengtu Branch*, 2: 61–63, 1944.

[9] S. C. Shen, T. H. Ham and E. M. Fleming, *New Eng. Jour. Med.*, 229: 701–713, 1943.

[10] Recently, 50 cc rubber stoppered Erlenmeyer flasks, each containing 10 beads and 0.5 cc of oxalated blood, were found satisfactory when attached so that the beads rolled in the greatest internal circumference of the flasks.

sodium chloride (no osmotic lysis). After centrifugalization, the amounts of hemoglobin in the supernatants in these 3 test tubes were determined, as is usual, in the Evelyn colorimeter and were designated as *c*, *a* and *b*, respectively.

The mechanical fragility (M. F.) of a sample was determined by means of the formula, M. F. $= \frac{b-a}{c-a}$, and expressed as a percentage value. In most instances no significant amount of cell fragmentation without release of hemoglobin resulted from the trauma, as was indicated by the absence of hemoglobin-containing cell fragments in the sediments of the traumatized samples, as well as by the unaltered osmotic resistance of the cells remaining intact. Accordingly, the percentage of hemoglobin liberated was considered to be equivalent to the percentage of cells destroyed by the trauma.

RESULTS

Technical Factors. When a sample of normal human blood was divided into 6 parts, their respective M. F. values were 2.8, 3.0, 2.9, 3.2, 2.9 and 2.8 per cent. Simultaneous determination of the M. F. of samples of defibrinated blood from each of 6 normal subjects gave the following values: 3.0, 3.4, 3.2, 3.4, 3.2 and 2.6 per cent.

The percentage volume of red blood cells had a decided effect on the proportion of hemoglobin liberated by trauma. Thus, for portions of a sample of normal human defibrinated blood with hematocrits adjusted to 9.6, 28.6, 50.0, 68.8 and 90.4 per cent., the M. F. values were, respectively, 1.6, 2.1, 3.8, 6.8 and 14.3 per cent. It was recognized that even with identical hematocrits, differences in the mean corpuscular volume of erythrocytes might affect slightly the comparative M. F. values. However, because the M. F. was expressed in terms of percentage of hemoglobin liberated, rather than in absolute values, differences in the mean corpuscular hemoglobin concentration of two samples could not distort the evidence as to the percentages of cells destroyed.

Spheroidicity. From the fact that the spherical form of the erythrocyte is "critical" for rupture of the cell membrane by osmotic forces it was anticipated that if normal erythrocytes were rendered relatively spheroidal by immersion in hypotonic solutions, their mechanical fragility would increase sharply as the final spherical form was approached. This was found to be the case for washed cells suspended either in progressively hypotonic solutions of sodium chloride or in serum progressively diluted with water. At tonicities equivalent to 0.85, 0.62 and 0.44 per cent. sodium chloride solution, the M. F. values of erythrocytes were as follows: in sodium chloride solutions, 6.5, 10.1 and 23.9 per cent.; in serum, 2.3, 6.4 and 15.0 per cent., respectively. In order to compensate for the differences in the amount of swelling of the red blood cells caused by the various hypotonic media, the proportion of cells in each sample was finally adjusted to approximately 40 per cent.

Cohesion. In theory, increased cohesion between red blood cells should increase their liability to rupture by mechanical trauma. When 43 per cent. by volume of washed human erythrocytes were suspended in complement-inactivated serums with various titers of iso-agglutinins, the M. F. of the samples remained between 3.1 and 3.7 per cent. for titers from 0 to 1:250. However, at titers of 1:512 and 1:1024, the M. F. values were 5.4 per cent. and 13.2 per cent., respectively. The M. F. values of a blood sample with a hematocrit of 42 per cent. and a serum titer of cold agglutinins of 1:640 were found at temperatures of 37°, 24° and 15°–20° C. to be 0.8, 3.6 and 23.6 per cent., respectively. Control M. F. values for a normal blood were 1.8, 1.8 and 4.0 per cent., respectively. When one portion of a sample of the blood of a patient with sickle cell disease and a hematocrit of 44 per cent. was fully oxygenated, the M. F. was 4.6 per cent. The other portion of the sample was kept in a nitrogen atmosphere (erythrocytes sickled) during rotation in the tonometer, and showed an M. F. of 18 per cent.

The increased cohesion of the red blood cells was reflected by the increased "viscosity" of the blood sample as measured in the Ostwald instrument. By contrast, in experiments with bloods in which the viscosity of the serum was greatly increased by the addition of gelatin, the mechanical fragility of the erythrocytes was not increased; nor, despite rouleaux formation, was the mechanical fragility detectably augmented by the increased serum viscosity in a case of multiple myeloma.

Membrane. Finally, changes in the strength of the membrane of the red blood cell were found to affect the mechanical fragility. Thus, as already observed by Rous and Turner,[4] when 45 per cent. by volume of erythrocytes from defibrinated human blood were suspended in isotonic salt solution their M. F. increased to 5 per cent., but when the cells were resuspended in serum, it returned at once to its initial value of 2.1 per cent. Again, human erythrocytes, 36.2 per cent. by volume, were incubated under sterile conditions in a medium (isotonic) containing 5.4 grams of sucrose and 0.425 grams of sodium chloride per 100 cc. After 42 hours, although the osmotic fragility of all the erythrocytes in the sample had *decreased* to below normal, their M. F. had *increased* from 4.8 to 31 per cent.

Clinical Applications. So long as the blood moves,

there is kinetic energy potentially available for mechanically destroying red blood cells. The increased mechanical fragility of red blood cells which have been rendered nearly spherical suggests a teleological reason for the biconcavity of mammalian erythrocytes; namely, the inevitability of rupture, were a nearly spherical cell to be deformed in traversing a narrow capillary. The increase in mechanical fragility with increase in hematocrit may be a factor in limiting the concentration of red blood cells normally in circulation.

The correlation between increased osmotic fragility (spheroidicity) and increased mechanical fragility, already observed under experimental conditions, was found to occur in congenital hemolytic jaundice. In one case in which the osmotic fragility of the erythrocytes was characteristically increased, their mechanical fragility was also augmented, so that with a hematocrit of only 30.2 per cent. the M. F. was 12.8 per cent. At splenectomy, the osmotic fragility of the blood in the spleen was found to exceed that of the peripheral blood. After splenectomy, as the evidence of increased blood destruction diminished, the osmotic and mechanical fragilities of the red blood cells declined progressively, until 39 days after the operation both were approximately normal.

In patients with thermal burns and hemoglobinuria, the erythrocytes have been found to be relatively spheroidal and to be increased in osmotic fragility.[9] In addition, such erythrocytes exhibit increased mechanical fragility, as do those of samples of human blood momentarily heated *in vitro* to from 52° to 58° C. Similarly, the erythrocytes of heated dog's blood are osmotically and mechanically fragile and are rapidly destroyed on re-injection into the animal.[9]

In the absence of increased osmotic fragility and cohesion between erythrocytes, increases in mechanical fragility are presumably on the basis of diminished strength of the cell membrane. This was found to be the situation with respect to patients with pernicious anemia tested prior to treatment with liver extract. Thus, in 3 such cases, osmotic fragility values were normal, but the M. F. values were 3.7, 3.9 and 4.3 per cent. The M.F. of the erythrocytes of a patient in advanced remission induced by liver extract was 2.1 per cent.; that of a normal control was 2.0 per cent. In these experiments the hematocrits were all adjusted to approximately 25 per cent.

In agreement with others,[5,6,7,8] it is suggested that the cohesion of erythrocytes may lead to their prompt mechanical destruction while in motion in the circulation. *In vitro,* increased mechanical destruction was shown to occur in the presence of iso-agglutinins and cold agglutinins, and in experiments with sickled erythrocytes. It has already been suggested[11] that such types of erythrocyte cohesion may cause sequestration of erythrocytes in the spleen and other tissues, with consequent progressive increase in their spheroidicity and osmotic fragility. Incubation of erythrocytes, at least *in vitro,* increases both their osmotic and mechanical fragilities. Consequently, if certain red blood cells temporarily sequestered (incubated) in the spleen, escape before their osmotic destruction occurs, they may still be readily destroyed when re-subjected to the traumatic motion of the circulation, because of their increased mechanical fragility.

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PROGRESSIVE ASCENDING PARALYSIS IN DOGS DUE TO DEFICIENCY OF A VITAMIN B COMPLEX FACTOR FOUND IN YEAST[1]

THE thirty-eight dogs used in this study received a synthetic B complex free diet composed of casein (water and alcohol extracted) 40 per cent., sucrose 36, cotton seed oil 18, cod liver oil 2, mineral salts 4 per cent. This was altered in the case of the positive control animals to contain dried brewers' yeast at a level of 10 per cent. as a source of the B complex. The others had their B complex requirement met by seven or eight of the following synthetic vitamins: (1) thiamine hydrochloride; (2) riboflavin; (3) pyridoxine; (4) nicotinic acid; (5) pantothenic acid; (6) para-aminobenzoic acid; (7) inositol, and (8) choline.[2]

The incidence of paralysis varied considerably on the different deficiencies, but it was greatest in the animals receiving all the synthetic B complex factors listd above where eleven out of twelve animals became paralyzed.

The paralysis comes on gradually, the early signs being a peculiar gait and an arching of the neck; then the hind legs show marked spasticity. There are often several bouts of transient paralysis with spontaneous recovery before the final progressive stage is reached. It is then rapidly progressive and ascending, the hind legs becoming involved first, then the fore legs, then the neck and, finally, the respiratory center. The paralysis is at first spastic and later becomes almost completely flaccid. It is rapidly fatal if untreated.

[11] T. H. Ham and W. B. Castle, *Tr. Asn. Am. Phys.,* 55: 127–132, 1940.

[1] Reported at the meeting of the American Chemical Society, September 12, 1944, New York City.

[2] SCIENCE, 98: 520, 1943. The amounts of inositol and choline were subsequently increased to 300 mg per dog per day.