

## 13994 P

# Effect of Purified Protein Fractions on Sedimentation Rate of Erythrocytes.

SEYMOUR J. GRAY AND EARL B. MITCHELL. (Introduced by G. F. Dick.)

*From the Department of Medicine, University of Chicago.*

It has been established by Fahraeus<sup>1</sup> and others<sup>2</sup> that plasma fibrinogen and globulin increase the sedimentation rate of erythrocytes and that albumin retards the rate of sedimentation. Zarday and Farkas<sup>3</sup> modified normal whole blood by adding fibrinogen and globulin and found that the sedimentation rate was increased in proportion to the amount of protein added. The addition of albumin retarded the sedimentation rate. Coburn and Kapp<sup>4</sup> confirmed these results.

Studies of the relationship of the blood proteins to the sedimentation rate have been conducted heretofore with relatively impure protein fractions obtained by fractional precipitation with neutral salts.<sup>5</sup> Tiselius<sup>6</sup> has shown by electrophoretic analysis that these protein fractions are mixtures of albumin and alpha, beta or gamma globulins and observed that quantitative studies with proteins obtained by fractional precipitation were inaccurate. Thus the electrophoretically pure human protein fractions prepared by Dr. Edwin J. Cohn<sup>7</sup> present an opportunity to study the effect of the protein constituents of blood on the sedimentation rate of erythrocytes especially since no investigations have been made of the influence of the various fractions composing the serum globulin on the sedimentation rate of the erythrocytes.

**Method.** Four purified protein preparations were studied: electrophoretically pure human albumin, gamma globulin and fibrinogen, and a fraction containing alpha and beta globulins. The albumin and alpha and beta globulins

were isoelectric, while the gamma globulin and fibrinogen were in the salt form. Increasing amounts of these protein fractions, varying from 0.2 to 2.5% were added to heparinized whole blood, and the sedimentation rates were determined by the Wintrobe<sup>8</sup> method. The pH of the blood samples containing these proteins was unchanged.

**Results.** Fibrinogen was more effective than any of the protein fractions studied in increasing the sedimentation rate of erythrocytes. The addition of 0.2 g% of fibrinogen to whole blood increased the sedimentation rate from a normal value of 6 to 24 mm in one hour. The sedimentation rate was increased in proportion to the amount of fibrinogen added. The addition of 0.4 g% increased the sedimentation rate to 51 mm and 0.6 g% elevated the sedimentation rate to 54 mm in one hour. Thus the sedimentation rate of erythrocytes may be increased 300 to 800% by the addition of fibrinogen. (Table I.)

The fraction containing the alpha and beta globulins elevated the sedimentation rate less effectively than fibrinogen. The addition of 0.2 g% of this fraction to whole blood increased the sedimentation rate from a normal value of 7 mm to 14 mm in one hour, an increase of 100%. (Table I.) The sedimentation rate was elevated to 18 mm and 25 mm in one hour upon the addition of 0.4 and 0.8 g% respectively of alpha and beta globulins. (Table I.)

Gamma globulin proved least effective in increasing the sedimentation rate. The sedimentation rate in one hour was elevated from a normal value of 5 mm to 8, 11, and 27 mm upon the addition of 0.2 g%, 0.4 g%, and 0.8 g% respectively of pure gamma globulin. The addition of 0.4 g% of gamma globulin to normal blood was required to increase the sedimentation rate 100%. (Table I.)

<sup>1</sup> Fahraeus, R., *Acta med. Scandinav.*, 1921, **55**, 1.

<sup>2</sup> Kylin, E., *Acta med. Scandinav.*, 1935, **85**, 574.

<sup>3</sup> Zarday, I., and Farkas, G., *Z. f. d. ges. exp. Med.*, 1931, **78**, 367.

<sup>4</sup> Coburn, A. F., and Kapp, E. M., *J. Clin. Invest.*, 1936, **15**, 715.

<sup>5</sup> Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93, 109.

<sup>6</sup> Tiselius, A., *Biochem. J.*, 1937, **31**, 313, 1464.

<sup>7</sup> Cohn, E. J., *Chem. Rev.*, 1941, **28**, 395.

<sup>8</sup> Wintrobe, M. M., *Internat. Clin.*, 1936, **2**, 34.

TABLE I.  
Effect of Pure Protein Fractions on Sedimentation Rate of Blood.

| Amt of protein<br>added<br>(g %) | Sedimentation rate<br>(mm in 1 hr) |    |                                |    |                   |    |         |    |
|----------------------------------|------------------------------------|----|--------------------------------|----|-------------------|----|---------|----|
|                                  | Fibrinogen                         |    | $\alpha$ and $\beta$ Globulins |    | $\gamma$ Globulin |    | Albumin |    |
| 0                                | 6                                  | 15 | 7                              | 2  | 5                 | 10 | 51      | 52 |
| .2                               | 24                                 | 45 | 14                             | 3  | 8                 | 16 |         |    |
| .4                               | 51                                 | 50 | 18                             | 4  | 11                | 18 | 46      |    |
| .6                               | 54                                 | 56 | 24                             | 8  | 17                | 20 | 40      | 50 |
| .8                               |                                    |    | 25                             | 11 | 27                | 27 | 36      |    |
| 1.0                              |                                    |    |                                |    |                   |    | 32      | 49 |
| 2.0                              |                                    |    |                                |    |                   |    | 23      | 40 |

Albumin inhibited the sedimentation rate of erythrocytes. The addition of 2.0 g% of pure human albumin to normal blood retarded the sedimentation from the original rate of 18 mm to 11 mm in one hour. The inhibition of sedimentation was more pronounced when 2.0 g% of albumin was added to blood with a markedly elevated sedimentation rate; the sedimentation rate was retarded from the original value of 51 mm to 23 mm in one hour.

**Summary.** Purified fibrinogen increased the erythrocyte sedimentation rate more effectively than the other protein fractions studied; 0.2 g% increased the sedimentation rate 300%. The fraction containing alpha and

beta globulins was next most effective; 0.2 g% increased the sedimentation rate 100%. Gamma globulin proved least effective; 0.4 g% was required to increase the sedimentation rate 100%. Pure human albumin inhibited the sedimentation rate of erythrocytes, the inhibition being most pronounced in the presence of a markedly elevated sedimentation rate.

The authors wish to express their gratitude to Dr. Edwin J. Cohn for supplying the purified protein fractions used in this study, which were prepared under contract with the Office of Scientific Research and Development upon recommendation by the Committee on Medical Research.

### 13995 P

#### Milk Influence and Leukemia in Mice.\*

ARTHUR KIRSCHBAUM AND LEONELL C. STRONG.

*From the Departments of Anatomy, University of Minnesota Medical School, Minneapolis, and Yale University School of Medicine, New Haven.*

It has been established that an "influence" contained in the breast milk of mice of high mammary cancer strains is important in determining the development of mammary cancer in these stocks.<sup>1,2</sup> This "maternal influence"

was originally demonstrated in F<sub>1</sub> hybrid mice representing reciprocal crosses between animals of high and low mammary cancer strains.<sup>3</sup> (F<sub>1</sub> hybrids with mothers of the high mammary cancer strain developed more breast cancer than F<sub>1</sub> hybrids whose mothers were of the low mammary cancer strain.) MacDowell and Richter<sup>4</sup> reported a similar

\* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, the Fluid Research Fund of Yale University School of Medicine, The Anna Fuller Fund and a grant from the Research Funds of the Graduate School of the University of Minnesota (grant administered by Professor Hal Downey). Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 165-1-71-440.

<sup>1</sup> Bittner, J. J., *Science*, 1936, **84**, 162.

<sup>2</sup> Bittner, J. J., *Science*, 1942, **95**, 462.

<sup>3</sup> Staff, Jackson Memorial Laboratory, *Science*, 1933, **78**, 465.

<sup>4</sup> MacDowell, E. C., and Richter, M. N., *Arch. Path.*, 1935, **20**, 709.