Rœntgen rays has repeatedly been described as a diminution of antibody-production due to injury of the bone marrow, spleen or lymphatic tissues.[7-10]

Summary. The local formation of precipitins following the injection of purified egg-albumin into the rabbit's cornea was increased by exposure of the cornea to the dosages of Grenz ray used in these experiments. The effect was most marked when the exposures were made for several days prior to injection of antigen.

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Factors Influencing Sedimentation Rate of Erythrocytes.

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There is increasing use of the sedimentation rate of erythrocytes as a test in clinical medicine, but there is equal difficulty in correct interpretation of the results obtained due to the many factors that influence it. Much work has been done to elucidate the value of this test in different clinical conditions, especially in infections.

Fahareus[1] has reviewed the literature and studied many of the factors that influence the sedimentation velocity of erythrocytes, especially the amount of fibrinogen and globulin in the plasma. Many other publications add new evidence. Westergren[2, 3] states that a definite positive correlation exists between the plasma proteins and the sedimentation of erythrocytes. Zarday and Farkas[4] have modified blood by the addition of normal fibrinogen and globulin and found that the sedimentation rate was increased in proportion to the amounts added. When they added albumin the rate was decreased. Coburn and Kapp[5] arrived at similar conclusions and added that

[7] Benjamin, E., v. Sluka, E., *Wien. Klin. Wchnschrift*, 1908, **21**, 311.

[8] Läwen, 1909, quoted by Hektoen.[9]

[9] Hektoen, L., *J. Inf. Dis.*, 1915, **17**, 415.

[10] Murphy, J. B., and Sturm, E., *J. Exp. Med.*, 1925, **41**, 245.

[1] Fahareus, R., *Physiol. Rev.*, 1929, **9**, 255.

[2] Westergren, A., Theorell, H., and Widström, G., *Z. f. d. g. exp. Med.*, 1931, **75**, 668.

[3] Westergren, A., Juhlin-Dannfelt, C., and Schnell, R., *Acta Med. Scand.*, 1932, **77**, 469.

[4] Zarday, I., and Farkas, G., *Z. f. d. g. exp. Med.*, 1931, **78**, 367.

[5] Coburn, A. F., and Kapp, E. M., *J. Clin. Invest.*, 1936, **15**, 715.

albumin inhibited sedimentation almost completely. Lucia and coworkers[6-9] found that *in vitro* a consistent relationship cannot be established between the sedimentation rate and the globulin content of various dilutions of serum globulin. They also showed that *in vivo* the sedimentation rate is apparently accelerated as the globulin content of the serum increases, and that a cause and effect relationship between the quantity of the various serum proteins and the rate of sedimentation cannot be said to exist. Further, they have shown that beef serum albumin or human serum albumin prolonged the sedimentation time when dissolved in Locke's solution or added to plasma.

Such controversial statements are found in the literature concerning the effects of the different protein fractions on sedimentation rate.

We have briefly attempted to show some of the fundamental factors that influence sedimentation rate of erythrocytes. The method used in determining the sedimentation velocity is as follows: Whole oxalated blood was used. The Weintrobe hematocrit tube was filled to 1 cc. mark, the rate of sedimentation observed, the reading being recorded every 15 minutes up to 2 hours, when the final reading was taken. This last figure is here reported in millimeters.

Effect of Number of Erythrocytes. We made 2 sets of experiments, varying the number of erythrocytes, using the same plasma, with the following results:

A		B	
No. of cells	Sed. rate	No. of cells	Sed. rate
5,600,000	5.0	7,000,000	1.0
4,200,000	24.0	4,200,000	29.0
2,800,000	48.0	3,200,000	49.0
1,400,000	79.0	2,100,000	68.0
		1,050,000	85.0

These results show that there is a logarithmic relationship between the number of erythrocytes and the sedimentation rate, a result that might be expected from theory. It becomes of importance to report the blood count in showing the sedimentation rate, so that the results of one test can be compared with another.

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[6] Lucia, S. P., and Brown, J. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 189.

[7] Lucia, S. P., Gospe, S. M., and Brown, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 356.

[8] Lucia, S. P., Blumberg, T., Brown, J. W., and Gospe, S. M., *Am. J. Med. Sci.*, 1936, **192**, 179.

[9] Lucia, S. P., and Brown, J. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 598.

Effect of the Different Plasma Protein Fraction. Two sets of experiments were done, with the different protein fraction in similar concentrations, using approximately the same number of erythrocytes in each case, but from 2 different individuals. The results are as follows:

	A	B
Protein fraction	Sedimentation	rate
Fibrinogen	41.0	61.0
Euglobulin	42.0	64.0
Pseudoglobulin	5.0	12.0
Albumin	3.0	6.0

These results agree in general with those reported by different authors, except that in our case the euglobulin had a more accelerating effect than the fibrinogen, but for general consideration we can assume that their effect was similar. The graduation of the accelerating effect of the different protein fractions suggests strongly that there is a parallel effect to the electric charge of these proteins, it being that the less charge, the accelerating effect becomes more marked. The cells are more closely packed and the rouleaux are more tight, and often there is definite aggregation of the cells in strong globulin solutions. In the albumin solution, on the contrary, the cells are seen dispersed, the rouleaux are either very loose or broken, the shape of the cells often showing distortions when seen under the ultramicroscope.

Effect of Albumin-Globulin Ratio and Total Protein. Wishing to test the statement of several authors as to the positive correlation between the plasma proteins and sedimentation velocity, we determined the index of correlation with 100 cases between the albumin-globulin ratio and the sedimentation rate. We also correlated the total protein percent with the sedimentation rate. Our results are shown in Table I. There is a negative correlation without significance between the albumin-globulin ratio and the sedimentation rate, the same being true with the total protein percentage. The large value for sigma (standard deviation) in the sedimentation rate and the albumin-globulin ratio in proportion to the mean average further demonstrate the broad distribution of the observed data.

TABLE I.
Index of correlation between sedimentation rate and A/G ratio.

	Mean Aver.	σ	Index of Corr.
Sed. Rate (x)	14.3	13.60	$0.04(x-y)$
A/G (y)	2.0	1.28	$\overline{0.08(x-z)}$
T.P. (z)	7.20	0.67	

A/G Albumin-globulin ratio.
T.P. Total protein percentage.

Effect of Different Metal Ions. To avoid the Ca effect on coagulation we used heparin as an anticoagulant in these experiments. To 1 cc. of the blood we added 0.1 cc. of a 0.1 M solution of the chloride salt of Na, K, Ca, and Mg. The results here given are the average of many experiments:

Na—7.0; K—5.8; Ca—5.4; Mg—3.3

The inhibiting effect of the different ions follows the Hofmeister series, increasing with the series. The effect of valency seems to explain only partly the results observed, for it seems that there is a specific effect by the metal ion itself.

Effect of Lecithin and Cholesterol. Suspensions of purified lecithin and cholesterol were made, and equal amounts (0.8 mg.) were added to the various tubes, keeping a control to which the same amount of Locke's solution was added. Table II shows the results of these experiments.

TABLE II.
Effect of lecithin and cholesterol on sedimentation rate.

Control	22.5	21.5	21.0	25.0	10.0	22.4	17.0	31.0	16.0	4.0
Lecithin	14.0	1.5	2.0	1.2	1.5	8.9	14.8	18.6	8.0	2.1
Cholesterol	24.8	29.0	21.5	21.8	11.0	23.5	18.5	32.2	17.0	4.4

These results show the marked retardation produced by lecithin and to a less extent the accelerating effect of cholesterol. Under the ultramicroscope we observe the dispersion of the red cells and the modification of their shape (spherical) produced by the lecithin, while the cholesterol increased the closeness of the rouleaux. This difference in effect is probably due to the change in electrical charge and hydration of the red cells by these substances. The intensity of effect on the different bloods produced by the same amount of the lecithin and cholesterol is difficult to explain.

Effect of Erythrocytes from Different Individuals in the Same Plasma. The blood was centrifuged and the corpuscles washed with Locke's solution, then equal amounts of erythrocytes were measured in each one of the different tubes that contained an equal amount of plasma. The results of the sedimentation rate are shown in Table III.

The results show that there is a notable difference in the different erythrocytes. Whether this difference is due to chemical nature

TABLE III.
Effect of different erythrocytes on the same plasma.

Case	1	2	3	4	5	6	7	8	9	10
Sed.	8.6	24.6	7.2	15.0	32.0	26.6	19.0	1.8	8.0	6.5

or not is difficult to say, but it is more probable that there are differences in the size, charge and hydration of the corpuscles. In order to study this problem further, we studied the effect on sedimentation rate of the same erythrocytes in different plasmas. We give the refractive index and the viscosity of each one of the plasmas to give a general idea as to their protein composition. Table IV gives the result of this experiment.

TABLE IV.
Effect of the same erythrocytes on different plasmas.

Case	1	2	3	4	5	6	7	8	9	10
R. I.	1.347	1.350	1.350	1.347	1.348	1.348	1.346	1.348	1.346	1.350
Vis.	1.73	2.02	2.02	1.72	1.77	1.66	1.77	1.77	1.72	2.08
Sed.	8.6	43.8	29.6	13.0	21.0	12.0	33.0	25.4	49.6	26.8

Here we observe a similar difference from the one observed above. There is a marked difference between the effect of the various plasmas on the same erythrocytes, suggesting that there are other factors that influence the sedimentation rate than the ones already considered.

Effect on Sedimentation from Erythrocytes of Different Species on its Homologous and Different Plasmas. The plasma was separated from the blood of a horse, an ox, and a human case, and the cells washed with Locke's fluid several times. Equal amounts of plasma from the different specimens were placed in tubes to which equal amounts of cells suspensions from each individual were added. A count was made to determine the number of erythrocytes in the final dilution and the mixtures were placed in the hematocrit tubes. The result of this experiment is shown in Table V.

TABLE V.
Effects of different erythrocytes on different plasmas.

Erythrocytes Number	Human 3,100,000	Horse 4,530,000	Ox 3,200,000
Human Plasma	40.0	41.2	2.0
Horse "	56.5	52.5	3.0
Ox "	60.2	36.5	1.4

This experiment clearly demonstrates that ox corpuscles are much slower in sedimenting than horse or human cells in the various plasmas. Human erythrocytes sediment faster in the different plasmas than either horse or ox, but more marked in ox plasma. The reason for the horse cells sedimenting slower in ox plasma is not clear. It is clearly demonstrated that there are intrinsic factors in the plasma and on the erythrocyte that affect sedimentation velocity which can not be predicted, making the sedimentation test theoretically difficult to evaluate.