lymph nodes have been reported elsewhere[15] and will not be repeated here. On examination of the sections of normal nodes by methyl-green and pyronine, almost no pyronine stained material could be found.

The histologic differences among the stimulated nodes were primarily those of degree. In these nodes, one day after the injection of antigen, a few young lymphocytes were seen to contain red granules in the cytoplasm, and in many of these cases, clearly defined red nucleoli. Such cells were progressively more numerous on the second, third and fourth days. On these latter days there was an occasional crescentic accumulation of pyronine-staining material at the very edge of the nucleus of cells which showed this material, and occasionally cells were seen which showed no cytoplasm except for a sector of material solidly stained red, contiguous to about one-quarter of the circumference of an otherwise denuded nucleus.

No plasma cells were seen in the greatly enlarged cortex of the stimulated lymph nodes by either method of staining. The few plasma cells noted were in the medullas of lymph nodes, which were fairly similar in area to those of control nodes, and the number of these cells did not differ remarkably, in these preparations, between control and stimulated nodes.

The great majority of cells with pyronine-staining granules were young lymphocytes. A few more mature, smaller, lymphocytes showed these granules, on the third and fourth days, and a number of the cells with these granules had nuclei so vesicular as to suggest that they were transitional forms between the reticulum cells on one hand and clearly recognizable young lymphocytes on the other. Within this range the distribution was continuous: A complete series of cells with pyronine-staining cytoplasmic granules could be picked out of a given section, from transitional forms to mature lymphocytes. Large lymphocytes of various stages were certainly much more in evidence than the mature cells among those with ribonucleic acid granules.

It is well to recall that the pyronine-staining granules are not the new protein, or antibody, itself, but a part of the mechanism which produces this protein. Accordingly it is quite consistent with our interpretation that the ribonucleic acid granules are found primarily in young rather than old lymphocytes.

Summary. The identification of the cell within lymphatic tissue which produces antibody has been approached by the application of a new histochemical technic for localization of cytoplasmic ribonucleic acid. The latter has been found to be an invariable concomitant to the formation of new protein in tissue. In lymph nodes actively engaged in the production of antibodies a wide range of lymphocytes, largely younger forms, was found to have cytoplasmic granules and nucleoli stained with pyronine, which is used to identify ribonucleic acid. Such granules and nucleoli were found also in transitional forms between reticulum cells and lymphocytes, but in no other cell types.

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Viscosity Studies of Erythrocytes from Persons with Sickle Cell Disease.

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The viscosity of the erythrocytes of sickle cell disease varies with changes in oxygen tension as Ham and Castle[1,2] have shown. The variation in viscosity is due to changes in

1 Ham, T. H., and Castle, W., *Trans. Assn. A. Phys.*, 1940, **55**, 127.

2 Ham, R. H., and Castle, W., *J. Clin. Invest.*, 1940, **19**, 788.

the red cell itself and not to influences of the suspending medium.[3]

It was the purpose of this study to investi-

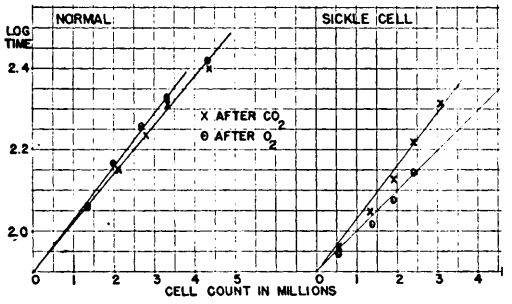

FIG. 1.

Log. of time of flow in seconds plotted against red cell count in millions, after exposure to O_2 and CO_2.

gate the possibilities of the viscosimetric method as as objective means of measuring the sickling tendency of the red cells of individuals or groups of individuals under varying conditions.

Methods. An Ostwald viscosimeter with a capillary bore of 0.48 mm in diameter and a length of 7.18 cm was used. The upper reservoir of the viscosimeter was calibrated at a volume of 2 ml. The viscosimeter was suspended in a water bath at 37° C.

Freshly drawn oxalated blood was centrifuged and the erythrocytes washed 3 times in Tyrode's solution. Three ml of cell suspension in Tyrode's solution was placed in the lower reservoir. The appropriate gas was passed through the suspension to effect cell saturation. The time of flow through the capillary was measured with a stop watch.

Results. In Fig. 1 the viscosities of various cell suspensions from a normal subject (No. 1) and from a subject with the sickle cell trait (No. 7) are shown. Viscosity, expressed as the logarithm of the time in seconds, is plotted against the cell count in millions.

Tables I-VI show the results of various studies of blood subjects with sickle cell disease.

TABLE I.
Viscosimeter Time Intervals and Cell Counts for Red Cell Suspensions.

Subject	After O_2	After CO_2	Time difference in seconds	Cell count in millions
1 (Normal)	217	207	— 10	3.9
2 "	233	229	— 4	3.5
3 "	152	151	— 1	2.3
4 "	262	253	— 9	4.5
5 "	271	259	— 12	4.3
6 "	244	228	— 16	4.6
7 (S. C. T.)	192	272	+ 80	4.4
8 (S. C. T.)	236	498	+262	3.8
9 (S. C. T.)	197	321	+124	3.1
10 (S. C. T.)	132	151	+ 19	2.0
11 (S. C. T.)	150	185	+ 35	2.4
12 (S. C. A.)	182	267	+ 85	2.2
(A) (Year later)	139	200	+ 61	2.0
13 (S. C. A.)	141	157	+ 16	2.1
(A) (Year later)	139	200	+ 61	2.0
14 (S. C. A.)	189	281	+ 92	4.2
15 (S. C. A.)	152	175	+ 23	2.1
16 (S. C. A.)	202	320	+116	2.5

TABLE II.
Effect of Nitrogen

Subject	Time in seconds		
	After O_2	After N_2	After CO_2
17 (S. C. A.)	152	182	186

Discussion. Several methods for the determination of the viscosity of blood have been used.[4,5] Both the capillary method and the concentric ring method indicate that the

[3] Huck, J. G., *Johns Hopkins Hosp. Bull.*, 1923, **34**, 335.

[4] Fahreus, R., and Lindquist, T., *Am. J. Physiol.*, 1931, **96**, 562.

[5] Brundage, J. T., *Am. J. Physiol.*, 1934-1935, **110**, 659.

TABLE III.
Effect of Change of pH. Phosphoric Acid Added.

Subject	After O_2		After CO_2		Cell count in millions
	pH	Time in secs.	pH	Time in secs.	
18 (S. C.A.)	7.8	160	6.35	221	2.5
Acid added	6.25	138	6.10	192	2.0

TABLE IV.
Effect of Carbon Monoxide.

Subject	Time in secs.			Cell count in millions
	After O_2	After CO_2	After CO	
10 (a)	132	147	133	2.0
(b)	106	112	107	1.4

TABLE V.
Effect of Repeated Washing of Cells with Tyrode's Solution and Normal Saline.

Subject	Solution	Time in secs.		Cell count in millions
		After O_2	After CO_2	
19 (S. C. A.)	Tyrode's, 3 ×	160	195	1.9
	Tyrode's, 20 ×	112	130	1.7
	Normal saline, 20 ×	119	134	2.4
	On ice—24 hr			
	Normal saline, 20 ×	111	120	1.2

TABLE VI.

Subject	Time in secs.		Cell count in millions
	After O_2	After CO_2	
20 (a)			
S. C. A. (Heparin)	128	145	2.3
(b) (Oxalate)	123	140	2.0
(c) (NaCN added to *b*)	126	140	2.0

apparent viscosity of blood will vary with the procedure used for its determination. The size of the capillary and variations in pressure are important in the capillary tube method. The speed of rotation is an important variable in the concentric ring method. In this particular investigation an Ostwald viscosimeter with a relatively wide bore was used so that time intervals would be short and there would be a minimum of sedimentation of red cells. Actual viscosities were not calculated and differences are indicated by varying time intervals.

The viscosity of blood depends upon a number of factors related to the composition of blood itself, most important of which are the protein content of the plasma and the number and character of the erythrocytes. Red cells separated from plasma and resuspended in Tyrode's solution display viscosity changes due only to changes in the characteristics of the red cells.

Changes in viscosity of suspensions of red cells from persons with sickle cell disease are easily demonstrable and are compared in Table I with normal cell suspensions. It will be seen that such viscosity changes occur in cell suspensions from subjects with sicklemia (S.C.T.) as well as from subjects with sickle cell anemia (S.C.A.)

There are compared, in Fig. 1, the viscosity measurements of cell suspensions from normal individuals and subjects with sickle cell disease, after exposure to oxygen and to carbon dioxide. It is shown that the viscosity of sickle cells differs from that of normal cells

after treatment with carbon dioxide. Increase in viscosity with nitrogen is shown in Table II.

Variations of the hydrogen ion concentration do not seem to affect the viscosity of the abnormal cells (Table III).

Carbon monoxide reacts with hemoglobin in much the same way as oxygen although a far more stable compound, carbon monoxide hemoglobin, is formed. Murphy and Shapiro[6] found that exposure of cells from a subject with sickle cell anemia to carbon monoxide apparently prevented their sickling. In order to confirm this, cells which had the sickling tendency were treated with carbon monoxide and studied in the viscosimeter. The sickling process was inhibited by this treatment as it was no longer possible to produce viscosity changes with carbon dioxide or nitrogen (Table IV).

Tomlinson and Jacob[7] report an inhibition of sickling by repeated washings and by the addition of cyanides to sickle cell blood. It could not be demonstrated by the viscosimetric method that repeated washings inhibited the sickling tendency (Table V). The addition of cyanide or the use of various anticoagulants showed no influence on the sickling tendency as viscosity changes could still be demonstrated after their use (Table VI).

Summary. A viscosimetric method is described for the study of the sickling tendency of red cells from subjects with sickle cell disease. This method does not differentiate between sickle cell anemia and sickle cell trait. Data are compared with observations of other investigators.

6 Murphy, R. C., and Shapiro, S., *Ann. Int. Med.*, 1945, **23**, 376.

7 Tomlinson, W. J., and Jacob, J. E., *J. Lab. and Clin. Med.*, 1945, **30**, 107.

16604 P

Studies of Typhus Fever in Peiping by the Complement Fixation Test.

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Recently, the complement fixation reaction in typhus fever, first studied by Castaneda,[1] has been refined so that not only typhus may be differentiated from other rickettsial diseases such as Rocky Mountain Spotted Fever,[2] but may itself be classified according to epidemic or murine nature.[3] Inasmuch as we have been interested in the etiology of typhus fever in this area, and have reached certain tentative conclusions based on the studies of the biology of the rickettsia strains locally isolated,[4] we wish now to use the complement fixation test to complete our studies. Unfortunately so far, owing to the scarcity of clinical cases seen in the past year, the number of sera examined has been small. But the results seem to be sufficiently consistent to warrant a preliminary report at this time.

Method and results. The antigens used in the present study, kindly supplied by the Lederle Laboratory Division of the American Cyanamid Company,[5] consist of washed rickettsial bodies. The method of complement fixation test is that of Kolmer's modified by reducing all ingredients to one-half the volume of the regular method. Sera were

1 Castaneda, M. R., *J. Immunol.*, 1936, **31**, 285.

2 Bengtson, I. A., *Am. J. Pub. Health*, 1945, **35**, 701.

3 Wishart, F. O., and Malcomson, M. E., *Canad. Pub. Health J.*, 1946, **37**, 369.

4 Liu, W. T. *et al.*, *Am. J. Hyg.*, 1942, **35**, 231.

5 We are indebted to Dr. R. B. Watson, of the International Health Division of the Rockefeller Foundation for securing these antigens.