Demonstration that Red Blood Cell Slowing Factor Found in Cancer Serum by Microelectrophoresis is an a_1 Component. (27895)

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We have reported(1) that carcinoma, some forms of inflammatory disease and pregnancy caused retardation of the microelectrophoretic mobility of the red blood cell. It was shown that this slowing was caused by factors in the serum that fell into 2 categories, distinguishable from each other by the maximum slowing effect exercised upon red cells with normal electrophoretic mobility, difference in stability at various temperatures and loss of activity with dilution(2). The factor present in the serum of pregnant women and patients with chronic inflammatory disease, characterized by a maximum slowing of normal cells from 1.33 μ/sec/volt/cm to 1.14 μ fell into one category, that in serum of persons with cancer, infectious mononucleosis and infectious hepatitis, characterized by a maximum slowing effect of 0.89 μ that could not be further reduced(3) fell into the second category. For convenience, the factors are referred to as SF-114 and SF-89 respectively.

We report here the finding, demonstrated by paper electrophoresis technic, that SF-89 may be a component of the a_1 or a_2 protein, depending upon the disease that has produced it. Thanks to this finding it is now possible to differentiate the SF-89 factor of cancer (a_1) from the SF-89 factor of infectious mononucleosis (a_2), constituting yet another demonstrable difference between the slowing factors of malignant neoplasm and those of other diseases. It also paves the way toward isolation of the cancer slowing factor, since ample quantities of the a_1 fraction can now be collected.

Materials and methods. Group O blood of persons in health was collected into tubes containing ethylenediaminetetraacetic acid to prevent clotting. The red cells were separated by centrifugation, washed in 1% saline 3 times, tested to see that mobility was normal, and then used to detect the presence or absence of slowing factors in the unknown serum.

Blood to be analyzed for slowing factor was collected into a plain tube, allowed to clot and the serum separated. Thirty lambda of serum was placed on each of 6 strips of Whatman chromatography paper #3 mm. Current and voltage used for separation was 10 milliamperes, 150 volts for 6 hours, and the buffer was barbital buffer 0.05 M, pH 8.6. One of the 6 strips was stained with bromphenol blue to identify the areas occupied by albumin, a_1, a_2, β and gamma.

This strip was aligned with each of the 5 unstained strips, the corresponding areas marked off and cut apart. Strips of each fraction were pooled and soaked in 10 ml of 1% saline, giving an eluate of each fraction that derived from 150 lambda of serum.

One-tenth milliliter of normal red cells prepared as above was added to 4 ml of each eluate and incubated at 37°C for one hour. The cells were then separated by centrifuging at low speed for 5 minutes, added to veronal buffer pH 9, ionic strength 0.172, and then tested by microelectrophoresis for degree of altered mobility(4).

Results. Table I shows the normal mobility of the red blood cells of persons in health, the retardation characteristic of the red cells of pregnant women and a number of diseases, the effect exercised by the whole serum of each upon the normal red cell, and the protein fraction that contains the slowing factor.

Table I also shows that with some exceptions 2 slowing factors are present in the serum of various diseases, including cancer. Whereas in cancer the slowing effect of the

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TABLE I. Occurrence of Slowing Factors in Serum of Pregnant Women, of Persons in Health and with Various Diseases.

	Mobility of RBC, μ	Effect of serum on normal RBC	Distribution of slowing factor				
			Alb.	α_1	α_2	β	γ
Normal (10)	1.33	0	0	0	0	0	0
Pregnancy (5)	1.21 $\pm$.02	1.14	0	0	1.14	1.21–1.25	0
Lobar pneu. (1)	1.21	1.14	0	1.14	1.23	1.28	0
Pulmonary T. B.	1.17	1.14	0	0	1.14	1.25	0
Myocard. inf. (1)	1.19	1.14	0	1.21	1.14	0	0
Scleroderma (1)	—	1.14	0	0	1.14	0	0
Syphilis (1)	—	1.14	0	1.14	0	0	0
Portal cirr. (1)	1.19	1.14	0	0	1.14	0	0
Inf. hepatitis (10)	1.19	.89	0	.89	1.00–1.12	0	0
Inf. mono. (5)	1.17	.89	0	1.12–1.25	.89– .94	0	0
Malignant neoplasm (10)	1.16	.89	0	.89	1.10–1.25	0	0

factor in α_1 is uniformly the same, 0.89 μ, the factor in α_2 produces slowing that varies from 1.05 μ to 1.25 μ. It occurred to us that an explanation for the difference between the α_1 and α_2 factors, as well as the variability in α_2, might be that the SF-89 was spread diffusely over both the α_1 and α_2 zones but was more concentrated in the former, and that it diminished in gradient fashion from the head of α_1 backward through α_2. To test this, 2 experiments were done. Table II

TABLE II. Distribution of SF-89 Factor Throughout the α_1 Area.

1st ¼ α_1	2nd ¼ α_1	3rd ¼ α_1	4th ¼ α_1
.89		.94	
.94	.89	.89	.94
.96 .92	.89 .89	.89 .89	.89 .98

shows distribution of SF-89 when a series of α_1 areas prepared from the same serum were divided into halves, quarters and eighths and the eluate from each tested for factor. It can be seen that the SF-89 factor is uniformly distributed throughout α_1.

Next, increasing amounts of α_2 fraction were added to equal aliquots of test cells and the slowing effect compared. As shown in Table III, the α_2 from 300 lambda of serum

TABLE III. Slowing Effect upon Normal Red Cells of Increasing Amounts of α_2 from Serum of a Patient with Carcinoma of Stomach.

Concentration	Degree of slowing
α_2 from 300 lambda of serum	1.33 μ ⟶ 1.17 μ
" 900 " "	1.33 μ ⟶ 1.03 μ
" 1500 " "	1.33 μ ⟶ 1.03 μ

retarded the cells from 1.33 μ to 1.17 μ; from 900 lambda to 1.03 μ; and from 1500 lambda to 1.03 μ. An almost 2-fold increase of α_2 having failed to retard the mobility below the retardation caused by 900 lambda indicates that the maximum slowing ability of the α_2 is 1.03 μ. Thus the slowing factors carried by α_1 and α_2 differ not only with respect to mobility but also with respect to maximal slowing effect they are able to exercise upon normal red blood cells.

Discussion. By a large series of microelectrophoretic determinations it has been shown that infectious mononucleosis, cancer and infectious hepatitis produce a factor in the blood that retards mobility of normal red cells used as test cells from 1.33 μ/sec/volt/cm to 0.89 μ. The factor in serum from cancer and infectious hepatitis migrates with the α_1 serum protein, that of infectious mononucleosis with the α_2 protein. The findings are so consistent as to make the technic of value in differential diagnosis of infectious mononucleosis, infectious hepatitis and cancer, but to date we have not been able to differentiate the SF-89 of cancer from that of infectious hepatitis. Judiciously correlated with other criteria, however, the presence of SF-89 should cause no confusion in distinguishing between infectious hepatitis and cancer.

It is interesting that the SF-114 of cervicitis and syphilis is found in the α_1 component, while that of tuberculosis, portal cirrhosis and scleroderma migrates as an α_2 component. Whether or not the factors in

α_1 and α_2 that produce the same retardation (Table II) are identical is not clear, since there is no other clarifying physical or chemical evidence.

We do not know what portion or constituent of the red cell membrane or possible extraneous coats is acted upon or modified by the slowing factors. Eylar *et al.*(5) report that modification of the sialic acid component of the cell membrane by neuramidase may result in as much as a 94% retardation of the red cells of humans. Our experiments furnish no evidence either for or against operation of a sialic acid mechanism. Also the physiological role played by the SF-89 factor is obscure, but it is invariably present. The slowing factor in cancer that travels with the α_2 protein fraction, differs in activity as well as in mobility from the factor that travels with the α_1 fraction.

Conclusion. The red blood cells of persons with malignant neoplasm move more slowly in an electric field than do cells of persons in health. They do so because their surface electrical charge has been altered by a factor present in the blood serum, called for convenience the SF-89 factor. By paper electrophoresis it has been demonstrated that this factor is located in the α_1 protein fraction of the serum.

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Studies on Fibrinolysis: I. A Circulating Substance Capable of Converting Plasmin-Sensitive to Plasmin-Resistant Clots.* (27896)

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It has been observed that clots produced *in vivo* can develop resistance to lysis by fibrinolytic agents(1). This phenomenon has been attributed to clot organization. The object of this study is to investigate the mechanism of acquired clot resistance to fibrinolysis.

Materials and methods. Fibrinogen. Crude human fibrinogen, Fraction I (Cohn), was obtained from American Red Cross. Purified "Profibrinolysin-Free" human fibrinogen was obtained from Cutter Laboratories, Berkeley, Calif. 0.6% Solutions of fibrinogen were prepared in either 0.05 M imidazole buffer, pH 7.35(2), or a 1:25 dilution (v/v) of this buffer in normal saline. The method of Back *et al.*(3) was utilized for iodination of fibrinogen I^{131}.

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Bovine topical thrombin (Parke Davis & Co.) was rendered plasminogen-free by the following procedure: 2.0 g crude thrombin was suspended in 200 ml cold 0.15 M phosphate buffer pH 7.3. The solution was stirred slowly and 4.0 g calcium phosphate (anhydrous) was added slowly at 4°C. The suspension was then brought to 20% (w/v) with ammonium sulfate and allowed to stand for 30 minutes. Precipitate was separated by centrifugation and supernatant dialyzed against 16 liters of water. Siliconized or plastic equipment was employed for preparation and storage of thrombin. The final product was diluted to give a 15-second clotting time when 0.1 ml was added to 0.5 ml of 0.6% fibrinogen. The solution was stored at −20°C. *Streptokinase* was obtained from Lederle Laboratories *(Varidase). Urokinase* was obtained from Leo Pharmaceutical Prod-