A Biochemical Definition of Blood Viscosity:

Its Possible Significance in the Pathophysiology of Shock

FRANCIS E. ROSATO, M.D., LEONARD D. MILLER, M.D., MARK HEBEL, B.S.

From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

THE FACTORS influencing blood flow include the perfusion pressure, the cross sectional area of the vessels and the blood viscosity. The last mentioned factor has received minimal attention in studies elucidating the mechanisms in normal and abnormal blood flow. Recently, however, the factor of viscosity has increased significantly in investigations of the shock state,[10] hypothermic conditions,[9] the postoperative period,[3] and the physiology of flow in the micro-circulation.[8] For a review of some of the basic principles of viscosity and its measurement, beyond the scope of this report, the following excellent reviews are recommended.[1, 13, 14]

Although the blood hematocrit has been directly correlated with viscosity, changes in viscosity can be effected without altering the hematocrit.[7, 15] Particularly under conditions of low shear or "creeping flow," the plasma proteins have a profound effect on blood viscosity, probably through the mechanism of cell aggregation. In general, increasing the molecular weight and size or diminishing the electronegativity of the plasma proteins tends to promote red-cell aggregation and increase viscosity.[13]

An attempt is made here to reduce the protein-viscosity interaction to more specific terms, relating it to a particular carbohydrate moiety on the glycoprotein molecule. Since N-acetyl neuraminic acid (Table 1) has a terminal carboxyl group, this moiety carries a negative charge, found at the periphery of the glycoprotein molecule. The glycoproteins are found in all the serum electrophoretic fractions, with the exception of the albumin component.[11] With the use of the specific enzyme neuraminidase, a ready method is available for cleaving a negatively charged portion of the globulin proteins.

Methodology and Results. The experiment was divided into 3 parts; the methods and results of each are described.

I. Neuraminidase Action on Blood Serum in Vitro

Whole blood samples were collected from four anesthetized, healthy, adult mongrel

Submitted for publication November 20, 1967.

Supported by Public Health Service Research Grant No. CA-10287-01 from the National Cancer Institute.

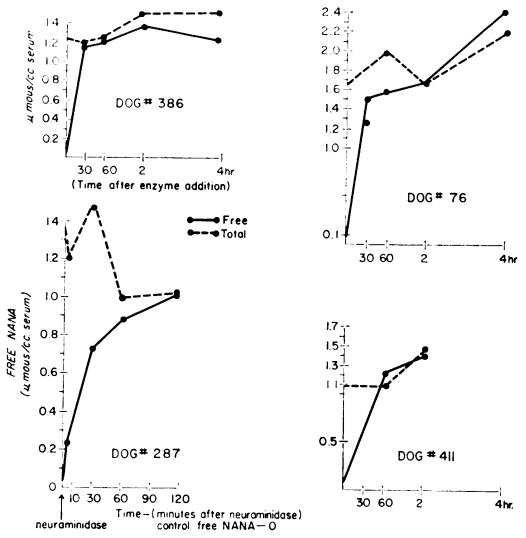


FIG. 1. *In vitro* action of neuraminidase on serum.

dogs by way of a femoral venous catheter. The samples were allowed to clot and the serum separated by centrifugation. Aliquots were then assayed for total (protein-bound) and free N-acetyl neuraminic acid according to the method of Warren.[12] Negligible amounts of free N-acetyl neuraminic acid were found. One cc. of Neuraminidase * from vibrio cholerae (500 units/cc.) was then added to 1 cc. of these serum samples and the serum-enzyme mixture incubated at 37° C. in a constant temperature water bath. Serial samplings at regular intervals were taken from the mixture and assayed for bound and free N-acetyl neuraminic acid. The results are shown in Figure 1. The enzyme is thus capable of liberating bound N-acetyl neuraminic acid from the serum proteins under the *in vitro* conditions described.

II. Effect of Neuraminidase on Whole Blood Viscosity

Ten cc. samples of whole blood were collected from healthy, adult, male patients about to undergo relatively routine operations (herniorrhaphy, hemorrhoidectomy, etc.). To each sample was added 0.15 cc. of heparin sodium (100 mg./cc.). The diluent used to suspend the neuraminidase (0.05M sodium acetate acetic acid buffer; pH 5.5; 0.9 Gm./deciliter sodium chloride; 0.1 Gm./deciliter calcium chloride) was prepared. To a 1.5 cc. aliquot of the patient's heparinized whole blood was added 0.5 cc. of the enzyme neuraminidase. To another 1.5 cc. blood aliquot from the same patient 0.5 cc. of the enzyme diluent was added. The samples were each incubated for one half hour at 37° C., frequently agitated, and viscosity readings then taken, utilizing a Brookfield micro (cone-plate) viscometer.[14] Viscosity readings were taken at different shear rates after initial shearing at 230 inverse seconds for 4 minutes. Samples were allowed to equilibrate at 37° C., using a constant temperature water bath, connected to the cone-plate apparatus by in-flow and out-flow portals. Hematocrits were determined in duplicate on the blood-enzyme-diluent mixtures after each test using the Wintrobe microhematocrit. One cc. samples were used and results expressed in centipoise units.

Six samples were tested in this fashion and the results summarized in Table 2. Listed is the change in hematocrit and in blood viscosity when the enzyme-treated sample is compared to the non-enzyme (diluent) treated sample. Results are also shown graphically in four representative assays in Figure 2. As can be seen, the enzyme has a consistent effect in increasing the whole blood viscosity, particularly at the lower shear rates.

III. Neuraminic Acid in Shock

In view of the demonstrated *in vitro* effect of neuraminidase on whole blood viscosity, it seemed logical to investigate any possible changes in N-acetyl neuraminic acid in the shock state, since this is charac-

* Hoescht Chemical Company, Cincinnati, Ohio.

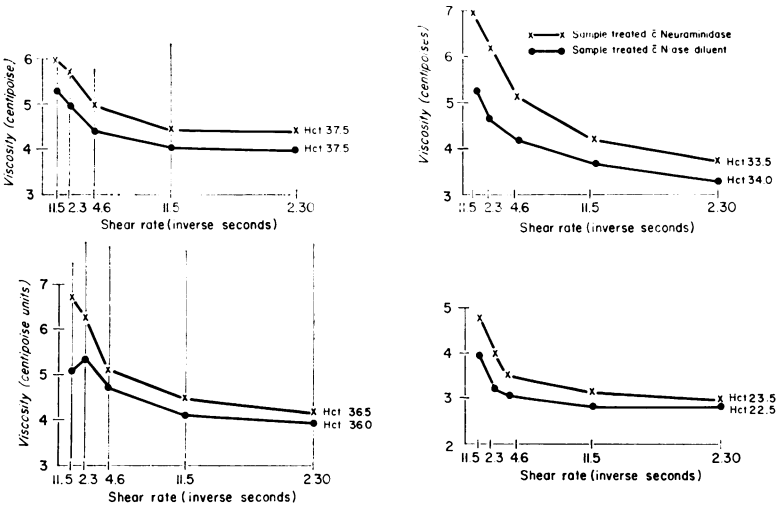


FIG. 2. Chart showing effect of neuraminidase on blood viscosity.
x ——— x Sample treated with neuraminidase.
• ——— • Sample treated with neuraminidase diluent.

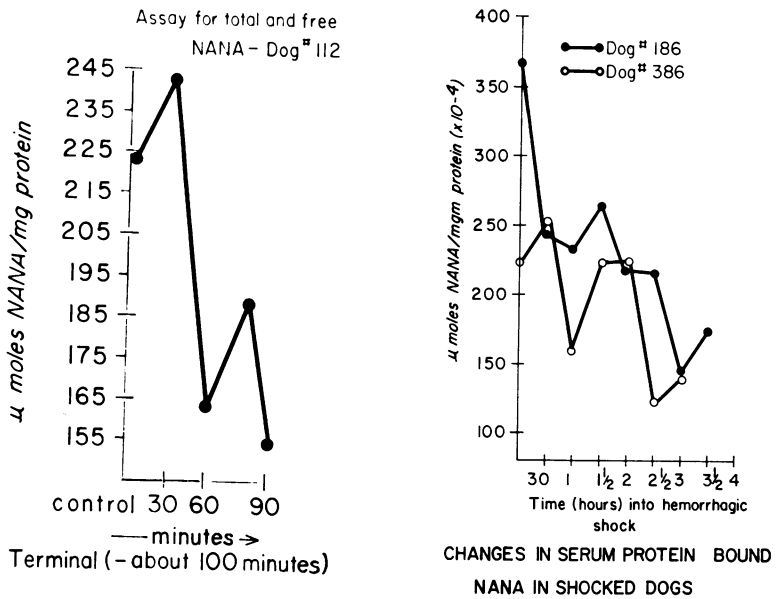
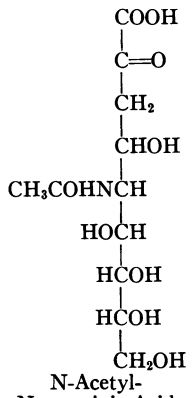


FIG. 3. Chart showing levels of total N-acetyl neuraminic acid in the serum of dogs subjected to hemorrhagic shock.

terized by an eventual increase in blood viscosity. Accordingly, three adult, healthy mongrel dogs were anesthetized with Nembutal and bled into a reservoir, until a mean pressure of 30 mm. Hg was reached. This level of mean pressure was maintained throughout the experiment by alterations in the height of the reservoir. Samples were collected from an indwelling femoral vein catheter at half hour intervals, after the level of 30 mm. Hg had been achieved. The samples were immediately centrifuged and the serum frozen. Assays were carried out within 48 hours of collection in all experiments. N-acetyl neuraminic acid determinations were carried out by the Warren technic[12] and the serum protein by the Biuret method.[4]

The results are seen in Figure 3. In each instance there was a progressive fall in the

TABLE 1.



N-Acetyl-
Neuraminic Acid

TABLE 2. *Effects of Neuraminidase on Blood Viscosity*

Patient	Δ Hct	Δ Visc. (cp) with Shear Rate (sec^{-1}) 230	115	46	23	11.5
#1	+0.50	+0.50	+0.57	+0.98	+1.35	+1.7
#2	+0.50	0	+0.18	+0.40	+0.75	+0.8
#3	+0.50	+0.35	+0.40	+0.60	+0.85	+0.80
#4	−1.0	+0.12	+0.25	+0.33	+0.95	+1.6
#5	+ .20	0	+0.07	+0.12	+0.70	+0.30
#6	+0.50	0	0	0	+0.40	+0.60

serum levels of N-acetyl neuraminic acid, as expressed in terms of micromoles N-acetyl neuraminic acid/mg. protein.

Discussion

A definite increase in whole blood viscosity, especially at low shear rates, occurs after addition of neuraminidase. This would seem to be due to the specific action of the enzyme in liberating the negatively charged N-acetyl neuraminic acid portion of the protein molecule. These effects are more pronounced at lower shear rates, consonant with the non-Newtonian character of blood. One might expect even more pronounced effect in the *in vivo* situation, since shear rates of arterioles of 100 microns diameter approximate 10 inverse seconds and would certainly be much lower in the capillary circulation.[1]

Neuraminidase, produced by a variety of bacteria and viruses, specifically cleaves the ketosidic bond between N-acetyl neuraminic acid and the next internally located sugar[5] (Fig. 4). As expected, the enzyme action, in releasing the negatively charged residue, results in an elevation of the isoelectric point.

The high viscosity of ovine submaxillary mucin was believed to be due to its high N-acetyl neuraminic acid content. The electrostatic repulsion caused by these charged groups along the molecule could impart to it an extended and more rigid shape. In treating this mucin with neuraminidase the protein molecule, now cleared of its N-acetyl neuraminic acid components, would be free to assume a more compact configuration with resultant decrease in viscosity. Theoretically, the same thing could be accomplished by lowering the pH and thereby suppressing ionization of the carboxyl group, and this was shown to be the case by Gottschalk.[6]

In serum or plasma the same effects of N-acetyl neuraminic acid might be expected and in samples so tested by us a definite, although small, decrease in viscosity was seen after addition of the enzyme to dog serum (Fig. 5). However, when evaluating the effects of the enzyme on whole blood, as seen before, the results are consistently to increase viscosity, probably by the mechanism of increased red cell aggregation. With the loss of the electro-negative protein charge, and possibly the loss of the surface negatively from the red cell as well,[2] cell dispersion is lessened and aggregation results. The viscosity of erythrocytes in plasma, particularly at low shear, has been shown to be greater than the addition of the viscosity of the same cells in saline to the plasma viscosity—it is more than an additive effect. In this model it seems that a summation of the slight decrease in serum viscosity brought about by neuraminidase is more than outweighed by

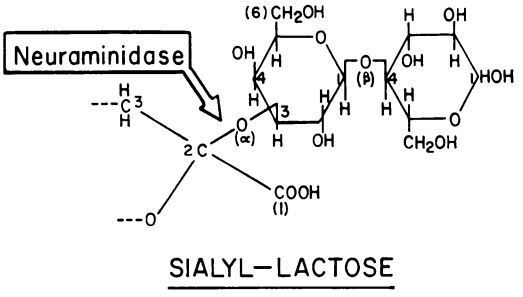


FIG. 4. Site of enzyme action.

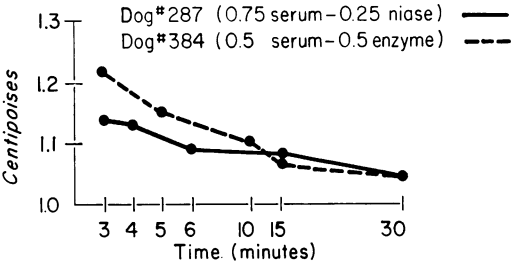


FIG. 5. Effect of addition of enzyme to serum viscosity *in vitro*.

the effect of cell aggregation produced by the same enzyme. Further, the increased viscosity appears attributable to loss of the electro-negativity contained in the N-acetyl neuraminic acid group. The value of this study resides in its demonstration of this chemo-electrical determinant of viscosity.

A number of intriguing speculations immediately present themselves in assessing the biologic role of N-acetyl neuraminic acid in the shock state. As was shown, there is a persistent and progressive diminution in the concentration of total N-acetyl neuraminic acid in dogs subjected to hemorrhagic shock. In these same experiments the concentration of free N-acetyl neuraminic acid remained negligible and unchanged. This may be due to decreased hepatic synthesis although the proof of such a hypothesis remains to be elucidated. Certainly, such a decrease in total N-acetyl neuraminic acid is a maladaptive phenomenon in hemorrhagic shock and contributes to the increased viscosity.

The enzyme, neuraminidase, is widely distributed in both viruses and bacteria, especially in those normal inhabitants of the respiratory and gastrointestinal tracts. Its present role is thought to be an aid in accommodating a parasite to its host, specifically by preventing confinement of the parasite in a coating of host mucin. However, if this enzyme is elaborated by intestinal bacteria and if, through breakdown of the intestinal barrier in shock, it gains access to the general circulation then a possible mechanism is operative to cleave N-acetyl neuraminic acid and further increase blood viscosity.

In either event, that is, decreased hepatic synthesis or increased entry of enteric neuraminidase into the circulation, the viscosity changes are likely to be a secondary rather than a primary pathophysiologic event in the progression of the shock state.

Summary

With an *in vitro* system the enzyme neuraminidase was capable of liberating approximately 90% of the neuraminic acid moiety from serum protein. Under identical circumstances the enzyme neuraminidase was also found to elevate whole blood viscosity, measurements being made on a cone-plate microviscometer. From these data it would seem that whole blood viscosity is, at least in part, related to protein binding of N-acetyl neuraminic acid and that liberation of N-acetyl neuraminic acid into the free state parallels increases in blood viscosity. In dogs subjected to hemorrhagic shock brought about by bleeding into a venous reservoir there was found to be a progressive decrease in the serum levels of N-acetyl neuraminic acid. Since the enzyme neuraminidase is elaborated by most intestinal bacteria it is possible that breakdown of the intestinal barrier in shock allows entrance of the enzyme into the general circulation with resultant cleavage of protein bound N-acetyl neuraminic acid and subsequent increase in blood viscosity.

References

1. Behar, M. J. and Alexander, S. C.: In Vitro Effects of Inhalational Anesthetics on Viscosity of Human Blood. Anesthesiology, **27**: 567, 1966.
2. Eylar, E. H., Madoff, M. A., Brody, O. V. and Oncley, J. L.: Contribution of Sialic Acid to Surface Charge of Erythrocyte. J. Biol. Chem., **237**:1992, 1962.
3. Gelin, L. E.: Disturbance of the Flow Properties of Blood and Its Counteraction in Surgery. Acta Chirurgica Scandinavica, **122**:287, 1961.
4. Gornall, A. G., Bardawill, C. J. and David, M. M.: Determination of Serum Proteins by Means of the Biuret Reaction. J. Biol. Chem., **177**:751, 1949.
5. Gottschalk, A.: Neuraminidase in Enzymes. Edited by P. D. Boyer, H. Lardy and M. Myrback. 7 Vol., Vol. 4, Second Edition. New York, Academic Press, 1960, p. 461.
6. Gottschalk, A. and Thomas, M. A. W.: The Significance of N-Acetyl Neuramic Acid for the Viscosity of Ovine Submaxillary Gland Mucoprotein. Biochem. Biophys. Acta., **46**: 91, 1961.
7. Litwin, M. S.: Blood Viscosity in Shock. Amer. J. Surg., **110**:313, 1965.
8. Merrill, E. W., Gilliland, E. R., Cokelet, G., Shin, H., Britten, A. and Wells, R. E., Jr.: Rheology of Blood and Flow in the Microcirculation. J. Applied Physiology, **18**:255, 1963.
9. Rand, P. W., Lacombe, E., Hurt, H. E. and Austin, W. H.: Viscosity of Normal Human Blood Under Normothermic and Hypothermic Conditions. J. of Applied Physiology, **19**:117, 1964.
10. Shoemaker, W. C.: Studies on Viscosity Alterations in Shock. Arch. Surg., **87**:355, 1963.
11. Spiro, R. G.: Glycoproteins: Structure, Metabolism and Biology. New Eng. J. Med., **269**:556 and 616, 1963.
12. Warren, L.: The Thiobarbituric Acid of Sialic Acids. J. Biol. Chem., **234**:1971, 1959.
13. Wells, R. E., Jr.: Rheology of Blood in the Microvasculature. New Eng. J. Med., **270**: 832, 1964.
14. Wells, R. E., Jr., Denton, R. and Merrill, E. W.: Measurement of Viscosity of Biologic Fluids by Cone Plate Viscometer. J. Lab. Clin. Med., **57**:646, 1961.
15. Yao, S. T. and Shoemaker, W. C.: Plasma and Whole Blood Viscosity Changes in Shock and After Dextran Infusion. Ann. Surg., **163**:973, 1966.