VISCOSITY CHANGES IN SYSTEMS CONTAINING CHICKEN RED CELL NUCLEI AND BILE SALTS

by

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In the course of an investigation of the hemolysis of chicken red cells, SHATTUCK[1] described an increase in viscosity which occurs shortly after the liberation of Hb from the cells by sodium taurocholate, sodium glycocholate, or sodium oleate (*i.e.*, shortly after hemolysis, or liberation of Hb from the cytoplasm, an event which may be followed, in the case of nucleated red cells, by karyolysis or destruction of the nucleus). Further investigation has shown that this viscosity increase is worth describing in detail, because it presents such remarkable quantitative relations.

METHODS

A suspension in 1% NaCl of washed red cells from heparinized chicken blood is made, and its volume concentration is adjusted to 0.08. One volume is added to 7 volumes of water. The mixture is allowed to stand for a few hours at 25° C, and is then centrifuged at about 4000 r.p.m. for 30 minutes. The Hb-containing supernatant fluid is discarded, leaving hemolysed ghosts. These are suspended in 2 volumes of NaCl-buffer (phosphate, pH 5 to 8) bringing the final concentration to $\varphi = 0.04$. With respect to nuclear material, the volume concentration of this suspension is about $4 \cdot 10^{-3}$, the volume of a nucleus being about 0.1 the volume of a cell. More dilute suspensions are prepared by diluting with NaCl-buffer. Microscopically, the ghost consists of a more or less intact nucleus surrounded by the outlines of the cytoplasm.

A series of solutions of sodium taurocholate* of concentration 40 g/l, 20 g/l, ... 1.25 g/l are prepared in NaCl-buffer at pH 7.0. A volume of one of these solutions, *e.g.*, that containing 5 g/l, is mixed with a volume of the suspension of $\varphi = 0.04$, and the viscosity of the mixture is measured 5, 10, 15, 20, and 30 minutes after the mixing. The viscosity increases with time, reaching a maximum in about 5 minutes; after about 15 minutes, it begins to decrease (see Fig. 1). Similar viscosity measurements are made for mixtures of equal volumes of other concentrations of the bile salt with the suspension of $\varphi = 0.04$; other series of viscosity measurements are then made for mixtures of equal volumes of the bile salt solutions and the suspension diluted so that φ is 0.02, 0.01, 0.005, 0.0025, and 0.00125. Finally, the way in which the viscosity depends on pH is found by repeating the measurements, or as many of them as may be necessary to give an outline of the pH dependence, with solutions of the bile salt in NaCl-buffers of different pH. Sodium glycocholate or sodium oleate may then be substituted for sodium taurocholate, and new series of viscosity measurements obtained.

The viscosity measurements are made at 25° C in Ostwald viscosimeters with an outflow time for water of about 1.5 minutes. The outflow time for the more viscous taurocholate-suspension mixtures is from 3 to 5 minutes; the flow is non-Newtonian, but this aspect of the apparent viscosity relations has not been investigated. The apparent specific viscosity, η_{sp}, is found from the viscosity η of the system and the viscosity η_0 of water ($\eta_{sp} = \eta/\eta_0 - 1$).

* The sodium taurocholate and the sodium glycocholate used were obtained from two sources, British Drug Houses and Bios Laboratories, New York City. The latter specimens were purified to the extent that neither bile salt contained more than 0.1% of the other or of cholesterol. This does not mean that either was entirely free from fatty acids, which SCHULMAN (private communication) believes to be responsible for lytic phenomena in general. The nucleic acid used was kindly given to me by Dr A. E. MIRSKY.

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DESCRIPTION OF THE VISCOSITY CHANGES

pH dependence of viscosity changes. Fig. 1 shows the way in which the larger viscosity changes depend on pH, the values given here being those for systems containing equal volumes of suspensions of $\varphi = 0.005$ and 5 g/l sodium taurocholate. At pH's below 6.5 the mixtures show little viscosity change with time, but at a pH of 7.0 η_{sp} rises to about 2.0 within 15 minutes after mixing. At higher pH's the effect becomes smaller.

As SHATTUCK observes, the viscosity first increases and then decreases with time, passing through a maximum about 10 minutes after mixing. The viscosity changes found 30 minutes after mixing the suspension with the bile salt are accordingly smaller (dotted line), but the form of the pH dependence is essentially the same.

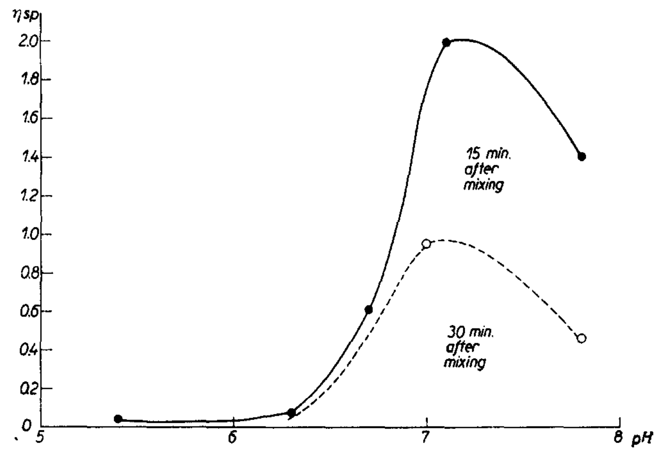

Fig. 1. Dependence of specific viscosity, η_{sp}, on pH for a system containing equal volumes of a suspension of chicken red cell nuclei of $\varphi = 0.005$ and 5 g/l sodium taurocholate. The viscosity effects in this system are usually the largest observed. Comparison of the two curves show that the absolute values of η_{sp} become smaller after having passed through maximum values about 15 minutes after mixing the suspensions of nuclei with the bile salt, but that the form of the pH dependence remains essentially the same.

Dependence of viscosity changes on concentration. When the concentration of suspension φ and of the taurocholate (or any other lysin) c are both varied at a pH which gives the maximum viscosity increases (pH 7.0), the observations being made at a time after mixing at which the effects are greatest (10 minutes), one obtains the set of relations between η_{sp}, φ, and c shown in Fig. 2.

For any value of the concentration of lysin c, the value of η_{sp} passes through a maximum as φ increases. As c increases, the value of φ which corresponds to the maximum in the η_{sp} relation increases. The position of the successive maxima depends on c, the value of φ which corresponds to a maximum increasing with c in such a way that φ/c is substantially constant. Further, the value of η_{sp} at each successive maximum also depends on c, this maximum value of η_{sp} being itself a function of φ (inset in Fig. 2) which passes through a maximum greater than any other ($\eta_{sp} = 2.5, \varphi = 0.005, c = 5$ g/l).

Similar results can be obtained with mixtures of suspensions of chicken red cell nuclei and of sodium glycocholate or of sodium oleate; the maxima, however, are smaller. In systems containing sodium glycocholate (5 g/l), a pH optimum for the effects

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has been found at pH 7.0; systems containing sodium oleate are very difficult to work with because of the hydrolysis of the soap at pH 7 to 8, and satisfactory sets of curves have not been obtained.

Inhibition of the effect. If 2 mg of saponin in NaCl-buffer at pH 7.0 are added to each 5 ml of the suspension of chicken red cell nuclei, the subsequent addition of sodium taurocholate is not followed by viscosity increases, and the phenomena described above can no longer be obtained. Apparently the saponin prevents the linking of the bile salt to the nuclear component, and so prevents the formation of the new viscous material (see below). It probably does so by itself combining with the nuclear component. There is some indirect evidence that such a combination takes place, for both the suspension of chicken red cell nuclei and nucleic acid itself act as inhibitors of saponin hemolysis. A suspension of $\varphi = 0.05$ gives an R-value of 1.5 at 37° C, while 0.1% nucleic acid

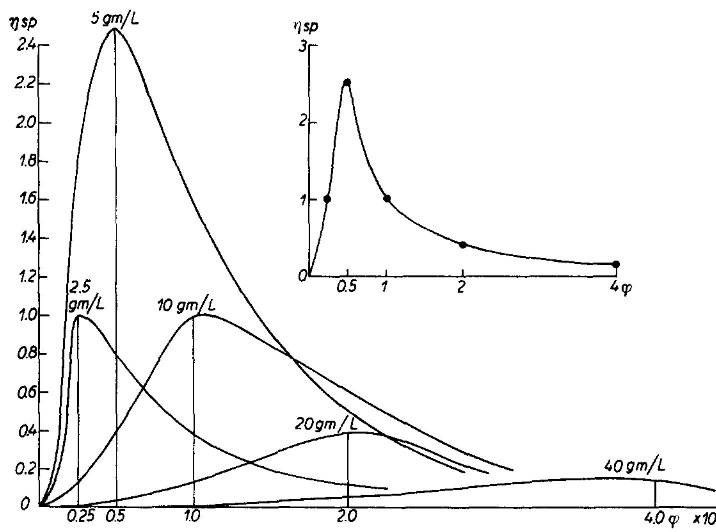

Fig. 2. Dependence of η_{sp} on the concentration S of the suspension of chicken nuclei, shown for different amounts of added bile salt. The concentration *c* of the added bile salt is shown opposite the maximum of each curve. Inset: the value of η_{sp} observed *at the maxima* of the set of curves shown in the Figure, plotted as a function of φ. The maximum value of η_{sp}, occurring at $\varphi = 0.005$, is the greatest of all the maxima. The text should be consulted for an explanation of these relations.

gives an R-value of 1.15. The inhibition produced by the suspension is accordingly some five times greater than that which would be expected on the basis of the inhibitory power of the nucleic acid together with its concentration in the suspension of nuclei; this suggests that the component involved in the viscosity effect is not nucleic acid alone. Further, although solutions of nucleic acid are viscous, mixtures of nucleic acid (0.1 to 0.2%) and sodium taurocholate do not give the elaborate series of viscosity maxima shown in Fig. 2.

Similarly, if formol is added to the suspension of chicken red cell nuclei in an amount which gives a final concentration of formol of 1% or more, the viscosity changes which would otherwise follow the addition of sodium taurocholate can no longer be obtained. Presumably the formol reacts with nuclear components (conceivably nucleoproteins) in such a way as to prevent the linkage of the bile salt with them, and so prevents the formation of the new viscous material.

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It is generally assumed that interaction between lysins and proteins take place, but there are very few instances in which there is independent evidence of these reactions occurring. The viscosity changes described above are of interest in this connection, not only because the viscosity increases themselves seem to be due to reactions between a lysin and a component of the red cell nucleus, but because they are abolished by the previous addition of a lysin of another type.

DESCRIPTION OF A SYSTEM WHICH WOULD SHOW THE FOREGOING PROPERTIES

Bile salt-Hb mixtures do not become viscous, but dilute solutions of nucleic acid are very viscous (the η_{sp} of a 0.1% solution of nucleic acid at pH 7.0 is 2.2). This suggests that the viscosity changes are the result of an interaction between the bile salt and nuclear material, with the result that nucleic acid derivatives are set free. This point of view is consistent with the fact that the viscosity changes cannot be obtained with suspensions of non-nucleated red cells, *e.g.*, mammalian red cells. What appears to be necessary is that the system should contain two components, which exist in concentration φ and c; φ is the concentration of a derivative of nucleic acid, and, were it not for some subsidiary considerations (see above) might be thought of as nucleic acid itself, while c is the concentration of the bile salt or soap. Both components are present in such low concentrations that their individual specific viscosities are very little greater than zero, the changes in viscosity occurring only when they are mixed.

Suppose that a unit of a new substance, which gives rise to an increase in viscosity, is formed by the rapid linking of one unit of φ to one unit of c*. This linkage is not stable over long times, because the viscosity decreases after 15–30 minutes. In the case of a mixture of φ and of c, the smaller of these two quantities limits the amount of the new substance formed, and the maximum amount of the new substance will be formed, with no material left over, when $\varphi = c$. Thus to obtain the maximum effect on viscosity, the φ/c ratio must be kept constant. If φ and c are unequal, $(\varphi - c)$ or $(c - \varphi)$ units will not enter into the formation of the new substance, and this interferes with the viscosity increases.

An assumption of the following type will give a general explanation of the experimental results. Suppose that c and φ are numerically small and that a network of the new viscous material is formed by their linkage. It is not necessary to think of the network as being regular, and when φ and c are small, it is probably not continuous. Under these circumstances, one would expect the increase in viscosity observed to be smaller than in a system containing more units of φ and more units of c, although in the same φ/c ratio. As the value of φ and c increase, in the optimum ratio for the production of viscosity maxima, the network of viscous material produced by their linkage would become more complete, the threads of the network being related to each other in some special manner which is associated with the viscosity maximum which is greater than any other. Any increase in φ and c beyond this special value would tend to result in a collapse of the network with the best arrangement, and so would lead to the system's having a smaller viscosity than at the maximum just referred to.

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* The proportion of taurocholate to nucleic acid at the largest viscosity maximum corresponds to about 10^5 taurocholate molecules per nucleic acid molecule. Only a small fraction of the taurocholate, of course, may enter into combination with the nuclear material.

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SUMMARY

The increase in viscosity observed in systems containing bile salts or soaps and chicken red cells arises from an interaction of the bile salt or soap with nuclear material, a new viscous substance, probably related to nucleic acid, being formed as a result of a linkage of the two components. The viscosity-concentration relations, which are described in detail, suggest that the new material forms a network throughout the system, this network having some of the regular features associated with lattice-like arrangements. The interaction of the bile salt and the nuclear material is so greatly reduced by the addition of either saponin or formol to the latter that, after such an addition, the viscosity increases can no longer be obtained.

RÉSUMÉ

L'accroissement de la viscosité observé dans des systèmes contenant des sels ou savons biliaires et des globules rouges de poule est dû à une réaction entre le sel ou savon biliaire et la matière nucléaire. Une nouvelle substance visqueuse probablement reliée à l'acide nucléique, est formée par la combinaison des deux composantes. Les relations existant entre la viscosité et la concentration, relations qui sont décrites en détail dans le présent mémoire, permettent de conclure que la nouvelle substance forme une réseau traversant tout le système, ce réseau ayant certaines des propriétés qui caractérisent régulièrement une disposition réticulaire. La réaction entre le sel biliaire et la matière nucléaire est inhibée par l'addition de saponine ou de formol à cette dernière: en effet, après une telle adjonction, l'on n'observe plus d'accroissement de la viscosité.

ZUSAMMENFASSUNG

Das Anwachsen der Viskosität, das in Systemen die Gallensalze oder -seifen und rote Blutkörperchen von Hühnern enthalten beobachtet wird, rührt von einer Reaktion des Gallensalzes oder der -seife mit Kernmaterial her. Als Ergebnis der Bindung der beiden Komponenten wird eine neue viskose Substanz gebildet, die wahrscheinlich mit der Nukleinsäure verwandt ist. Die Beziehungen zwischen Viskosität und Konzentration, die im einzelnen beschrieben sind, lassen vermuten, dass der neue Stoff im gesamten System ein Netzwerk bildet, das einige der charakteristischen Merkmale zeigt, die eine gitterähnliche Anordnung begleiten. Die Reaktion zwischen dem Gallensalz und dem Kernmaterial wird so sehr durch die Zugabe von Saponin oder Formol zu den letzteren herabgesetzt, dass nach einer derartigen Zugabe kein Ansteigen der Viskosität mehr beobachtet werden kann.

REFERENCE

E. G. SHATTUCK, *J. Gen. Physiol.*, 12 (1928) 17.

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