bin addition. The progressive increase in optical density at 350 nm observed during the coagulation of human plasma in presence of 0.4 mg of human fibrinogen (Figure 3, curve a) and in presence of 0.4 mg of the 2, 5, and 30 min ultrasonicates are shown by Figure 3, curves b, c, and d respectively. The lag phase and the slope of the curves suggest the prolongation of plasma thrombin clotting time.

The antithrombin activity of increasing amounts of the 2 min ultrasonicated fibrinogen is evident by the increase in thrombin time shown by Figure 4, curve a. Longer periods of ultrasonication of fibrinogen appears to decrease the antithrombin activity of the ultrasonicate as shown by Figure 4, curves b, c, and d.

Brief period of ultrasonication, at 0–4 °C of purified human fibrinogen resulted in the loss of clottability of the protein, and the appearance of an anticoagulant and anti-thrombin activities. Since the two minutes ultrasonicate of the purified human fibrinogen produced no noticeable changes in the sedimentation rate, electrophoretic mobility and elution from DEAE-cellulose when compared with the purified native fibrinogen, it is suggested that the clottability of human fibrinogen depends on the conformational status of the protein molecule.

Résumé. Placée 2 min sous l'influence de la vibration ultrasonique, la fibrinogène humaine se transforme en une protéine qui ne se fige pas et fonctionne donc comme un anticoagulant. Les propriétés physico-chimiques de la protéine, après action ultrasonique, ressemblent fortement aux propriétés originales de la fibrinogène humaine en voie de sédimentation; mouvement électrophorétique et adsorption sur la cellulose DEAE.

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Posthypertonic Hemolysis in a Sucrose System

The hemolysis which occurs after freezing blood appears to be mainly due to the increase in solute concentration when water separates out as ice during freezing and to the reverse event during thawing[1]. Studies of posthypertonic hemolysis may therefore be of great interest for blood-preservation[2–5].

SÖDERSTRÖM[6] first explained the mechanism of posthypertonic hemolysis in an unfortunately rather neglected paper as being due to salt entering the cell in the hypertonic phase and the cell then reacting to the subsequent isotonic phase in a way analogous to a normal cell reacting to a very hypotonic medium and designated it 'paradoxical hypotonic hemolysis'. By measuring chloride concentrations, he also showed that an uptake of salt occurs in a hypertonic medium. ZADE-OPPEN[3] pointed out that, in view of the rapid passage of water across the cell membrane, a driving force for net solute influx will not occur until a certain concentration in the medium has been exceeded, i.e. that which is just sufficient to shrink the cell to a minimal volume. Further, he calculated that, in order to explain his experimental results on a basis of the SÖDERSTRÖM hypothesis, it was required that the solute permeability should increase with increasing external solute concentration. In an almost simultaneous publication MERYMAN[5] came to very nearly the same conclusions as regards the mechanism of posthypertonic hemolysis and the increase in solute permeability. Both ZADE-OPPEN[2–4] and MERYMAN[5] used electrolyte media and both[2–5] also noted that similar effects were obtained when sucrose was used to produce hypertonicity. Although it has long been known that posthypertonic hemolysis is obtained after exposure to hypertonic non-electrolyte solutions (TAKEI[7] used glucose, VALDIVIESO and HUNTER[8] used sucrose), little is known about the possible difference in the effects of electrolytes and non-electrolytes. This communication describes quantitatively the effect of sucrose under certain conditions and attempts to compare this effect with the effect of NaCl in a similar system, so far as such a comparison is possible.

Human red cells were washed in a buffered isotonic NaCl solution. To an isotonic cell suspension (0.25 ml) first a hypertonic solution (1 ml) was added and then, after a certain time interval, a large volume (20 ml) of isotonic solution. Hemolysis was estimated as the percentage of Hb liberated into the medium. The procedure is given in detail elsewhere[3]. When sucrose was used to vary the tonicity, the electrolyte concentration was kept constant at the isotonic level.

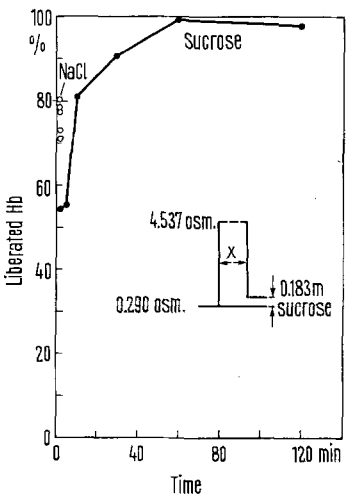

Fig. 1. Posthypertonic hemoglobin liberation after varying times of hypertonicity. Tonicities were varied with sucrose (filled circles) or with sodium chloride (open circles). The inset is intended as a short description of the sucrose experiment. NaCl was used at the same osmolalities.

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Figure 1 shows the relation between hemolysis when the time of hypertonicity was varied. The shape of the curve is very similar to that obtained in a NaCl-system[3]. The degree of hemolysis was, however, slightly less than that obtained when the high osmolality was produced by NaCl instead of sucrose, as can be seen in Figure 1 with a hypertonic exposure time (HET) of 2 min.

In another experiment the HET was kept constant at 2 min while the degree of hypertonicity was varied. The conditions were chosen so that the increases and decreases in tonicity were made equal on an osmolal basis. Osmolalities were calculated with the osmotic coefficients given by HARNED and OWEN[9]. Interactions between different solutes were neglected. Figure 2, a shows that, using an osmolality scale, the effects of NaCl and sucrose are very similar and differ only at the highest concentrations used. activities were calculated with use of the activity[11] and mean ionic activity[9] coefficients, respectively. The result is shown in Figure 2, b. The previous similarity now disappears, sucrose giving less hemolysis than NaCl at the same concentration. It must, however, be pointed out that the decreases in osmolality after the hypertonic phases are larger for NaCl than for sucrose of equal hypertonic activity in Figure 2, b. A correction due to this difference would therefore again decrease the differences in results on the activity scale. With regard to the points 2 and 4 above it appears, however, incorrect to make a comparison either on the osmolal or the activity scale. The similarity in results on the osmolal scale and the possible similarity on the activity scale appear very interesting, but may be only coincidental. That posthypertonic hemolysis is so easily obtained with a non-

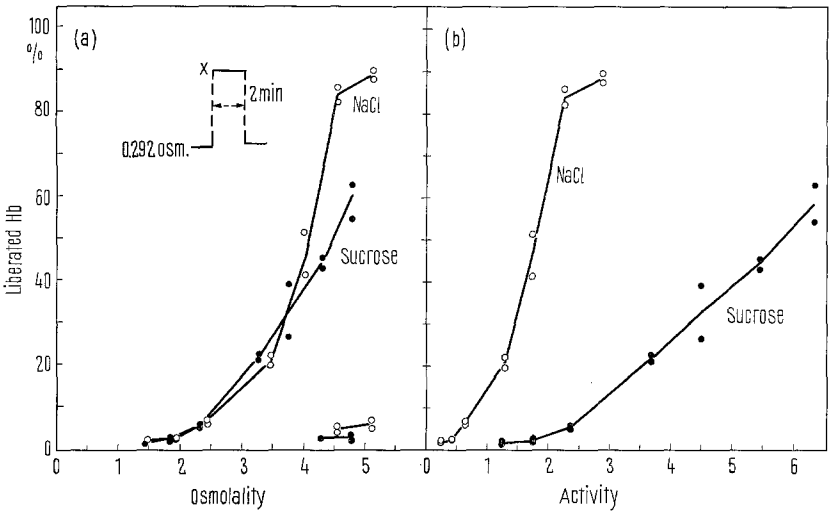

Fig. 2. Posthypertonic hemoglobin liberation after varying hypertonic concentrations. The hemolysis, which occurs during 2 min at the highest (>4 Osm) hypertonic concentrations (less than 7%) is also shown in a). In a) hypertonic concentrations are given in osmolalities; at any concentration, increase and decrease of hypertonicity are similar for NaCl and sucrose. In b) hypertonic concentrations are given in activity. The actual decrease in osmolality after HET at any particular hypertonic concentration is larger for NaCl than for sucrose at the same activity.

Experimentally the degree of hemolysis in one system varies with a) the degree of hypertonicity, as seen in Figure 2 (see also references [2] and [3]), b) the HET, as seen in Figure 1 (see also references [2] and [3]) and c) with the degree of reduction of tonicity after the hypertonic phase[2,4,10]. On the basis of our present concept of the mechanism of posthypertonic hemolysis the degree of hemolysis will vary with the following factors: 1. The time during which solute may diffuse into the cell when this time is less than necessary to reach a (partial) equilibrium. 2. The difference in solute activity between cell exterior and interior. 3. The degree of increase in solute permeability of the cell wall in the hypertonic phase. (Whether the permeability increase is irreversible, as assumed by MERYMAN[5], or reverses to normal for the cells which do not hemolyze after return to isotonicity, remains to be shown.) 4. The decrease in external osmolality after the hypertonic phase. Only point 4 above states that the basis for comparison should be made on an osmolal scale. There is, as yet, no information about point 3 which is likely also to vary with osmolality. However, the solute influx must be related rather to the activity of the solute than to the osmolality of the solution. The experiment shown in Figure 2, a was therefore recalculated on the activity scale; sucrose and salt electrolyte, however, is an interesting observation which is quite in accord with the present concept of the mechanism of posthypertonic hemolysis and will help to rule out any specific salt effects as being of fundamental importance, contrary to LOVELOCK's[1] statement that this type of hemolysis is due to the 'lyotropic properties of salt solutions'.

Zusammenfassung. Die osmotische Hämolyse ist nach Inkubation der Erythrozyten in hyperosmolaren Lösungen von Saccharose und NaCl nahezu identisch.

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