the degree of hydrolysis. This supports the supposition of GEORGE et al.[10] that changes in the solvation enthalpy are primarily of significance as regards the change in the free enthalpy on ATP hydrolysis. These changes in the hydrate structure must be connected with a correspondingly large change in the dielectric properties of the systems. The hydrolysis of the ATP hence controls the intermolecular interactions in the system investigated. Since hydrolysing ATP is present in most biological systems, it is clear that the effect observed is of biological significance.

Zusammenfassung. Es wurde die Hydrolyse von ATP in wässrigen Lösungen IR-spektroskopisch untersucht. Innerhalb kleiner Hydrolysegrad-Bereiche treten enorme Veränderungen an den Banden der Wassermoleküle, insbesondere an der Torsionsschwingung auf. Die Bedeutung dieses Effekts wird diskutiert.

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Comparison of Preparations of Erythrocyte Membranes and Membrane Proteins by SDS-Gel Electrophoresis

In the past decade a large number of procedures for preparation of red cell membranes and red cell membrane protein fractions have appeared. The resulting array of protein fractions and properties has recently been described as 'bewildering'[1]. If attention is confined only to protein preparations made by perturbation of ionic strength, there are rarely two preparations of water-soluble protein that are made in exactly the same way whether the starting ghosts are the same or not. These variations make it extremely difficult to compare the results of different laboratories, or even of the same laboratory at different times. Yet it seems probable that many methods should yield preparations with similar properties, since the preparation methods are similar in principle if not identical in detail.

In the course of studies of the principal water-extractable proteins we have compared the protein composition of a number of preparations of ghosts and of water-soluble ghost proteins by gel electrophoresis, to facilitate comparison of our results with the literature.

Materials and methods. Blood was obtained as fresh units unsuitable for transfusion and used within a week. Cells washed 3 times with 0.16 *M* NaCl were used for most ghost preparations; for preparations requiring low ionic strength media, NaCl-washed cells were washed twice in large volumes of 0.32 *M* glucose, which produced clumping.

All chemicals were obtained from commercial sources and used without further purification. Distilled water was deionized and redistilled from glass.

Aliquots of ghost preparations and of preparations of water-soluble protein were analyzed by sodium dodecyl sulfate gel electrophoresis according to the method of FAIRBANKS et al.[2]. The monomer concentration was reduced to 4%, at constant acrylamide: *bis*acrylamide ratio. After staining for protein with Coomassie blue[2], gels were photographed. Protein bands are numbered according to CARRAWAY and SHIN[3].

Results. Ten preparations of ghosts were made, according to each author's directions. SDS-gel electrophoretic patterns of these preparations are shown in Figure 1. The preparations made by low ionic strength hemolysis at neutral to alkaline pH (A–G) are quite similar in composition, with the principal variations being in the low molecular weight bands 8–10 and hemoglobin. Band 10 is not retained by ghosts below 20 imos*M* (note that the preparations in F and G are made at about 10 imos*M*) while band 8 appears to be minimally retained at 20 imos*M*. The presence of EDTA in the lysis mixture does not affect these results, since A and F (prepared with EDTA) are respectively equivalent to C and G (prepared without). The depletion of band 10 below 20 imos*M* correlates with loss of the permeability barrier to ATP[5] and with destabilization of membrane protein conformation[19]. Ghosts prepared at pH 4.6 (H) gave blurred patterns, and show depletion in band 8 and apparent enrichment in bands 1, 9, and 10. The preparations made with Triton X-100 at zero ionic strength (I) are also comparable to the other ghost preparations, with loss of band 10 and possible loss of bands 5 and 6. Preparations in Triton X-100 at non-zero ionic strength are depleted in bands 4, 5, 6, 8, and 10. It should be noted that the apparent variations in bands 1–4 in the gels shown in Figure 1 are primarily due to overloading, as can be seen by comparison of gels with less protein (not shown). Overloading was deliberate, to increase the intensity of the minor bands.

Nine preparations of water soluble proteins from ghosts made by extraction at low ionic strength are shown in Figure 2. These were also made according to the

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respective author's directions, except that the gel chromatography step in the preparation of 'spectrin' was not performed. The principal protein of these preparations is the high molecular weight doublet, band 1. The other frequently occurring protein is band 7, which, like hemoglobin, is present in the low ionic strength extract if it was originally present in the ghost. Preparations made at slightly higher ionic strength (P1, Figure 2B) tend also to contain bands 5 and 10. The 'tektin' preparation (I) contains an unusually high concentration of band 1, plus a noticeable quantity of band 3. 'Spectrin' (G) and FAIRBANKS et al's EDTA preparation (H) contain only band 1, plus Hb, reflecting in part the depletion of low molecular weight bands in the ghosts from which these preparations were made. 'P2' (C), 'fibrillar protein' (D), and 'torus protein' (E) also contain small quantities of other bands, probably due to nonsedimented membrane fragments or to slight proteolysis. Band 7 may be purified from these preparations by precipitation of most of the band 1 and other protein at pH 5.3, followed by concentration (F). The bands depleted from the ghost preparation shown in Figure 1 (J) are not found in the final supernatant (Figure 2, J) of that ghost preparation.

Several extractions at high ionic strength were made, at 1 *M* NaCl, 1 *M* KSCN, and according to References[2,7,16,17,18]. Extraction was variable, but the predominant extracted proteins were band 1, and, for preparations made below 1 *M* in salt, band 8 and traces of other protein. The FAIRBANKS et al.[2] salt preparation was especially clean, yielding mostly band 8 plus a trace of band 1, as observed by others[3].

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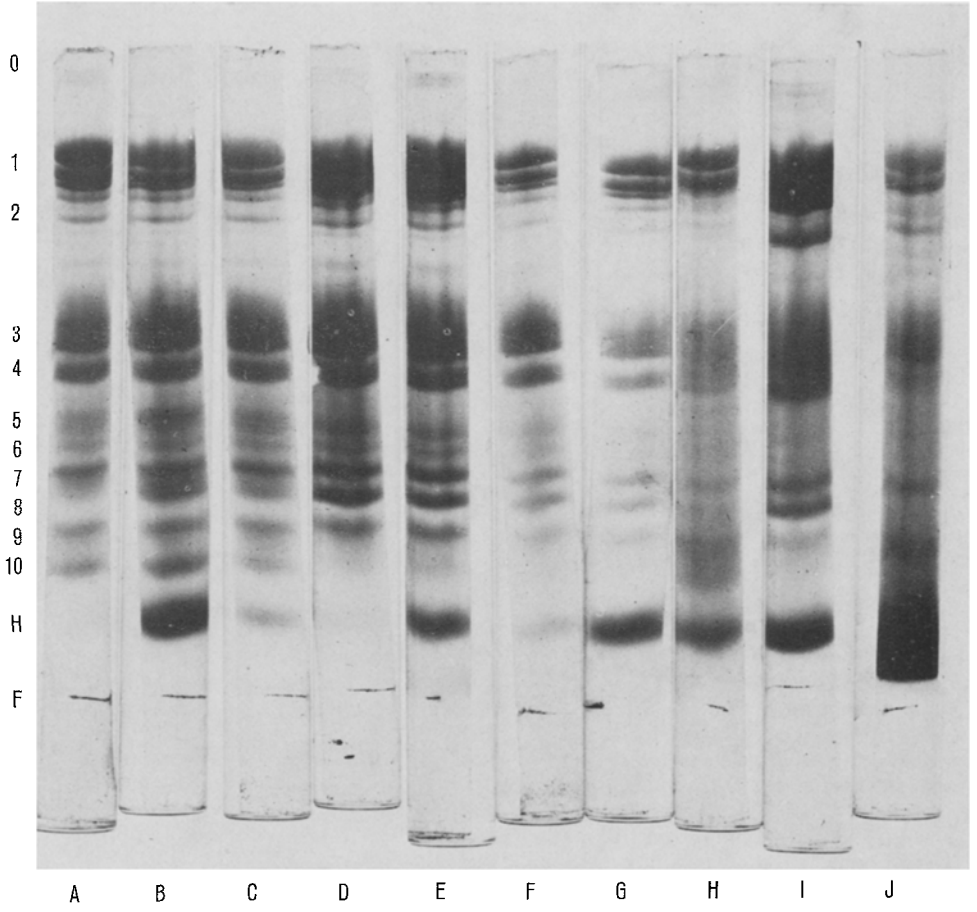

Fig. 1. Comparison of ghost preparations. SDS-gel electrophoretic pattern of ghosts prepared according to the following references with lysis medium indicated: A) HOGEVEEN, JULIANO, COLEMAN and ROTHSTEIN[4], final step 20 mos*M*[a], NaCl-$NaPO_4$, PH 7.2. B) BRAMLEY, COLEMAN and FINEAN[5], 40 imos*M* $NaHCO_3$, PH 7.2. C) DODGE, MITCHELL and HANAHAN[6], 20 imos*M* $NaPO_4$. D) BRAMLEY et al.[5], 10 imos*M* $NaHCO_3$. E) DODGE et al.[6], 5 imos*M*. F) FAIRBANKS, STECK and WALLACH[2], 5m*M* (approx. 13 imos*M*) $NaPO_4$, pH8.0.G)MARCHESI, STEERS, MARCHESI and TILLACK[7], 5 m*M* (10 imos*M*) *Tris* HCl + 1m*M* EDTA, pH 7.5. H) BALLANTINE[8] 0.1 m*M* acetic acid, pH approx. 3. I) MAZIA and RUBY[9], lysed in 'zero' ionic strength 0.1% Triton X-100, washed in 20 m*M* (40 imos*M*) ammonium acetate, pH 6.5. J) FURTHMAYR and TIMPL[10], as I) but nonzero ionic strength at lysis. Gels prepared and run as in text; 'channelling' and overloading of major bands result from applications of protein sufficient to produce photographable minor bands. Bands numbered according to CARRAWAY and SHIN H = hemoglobin; F = dye front. [a] Abbreviation: (i)mos*M*, (ideal) milliosmolar.

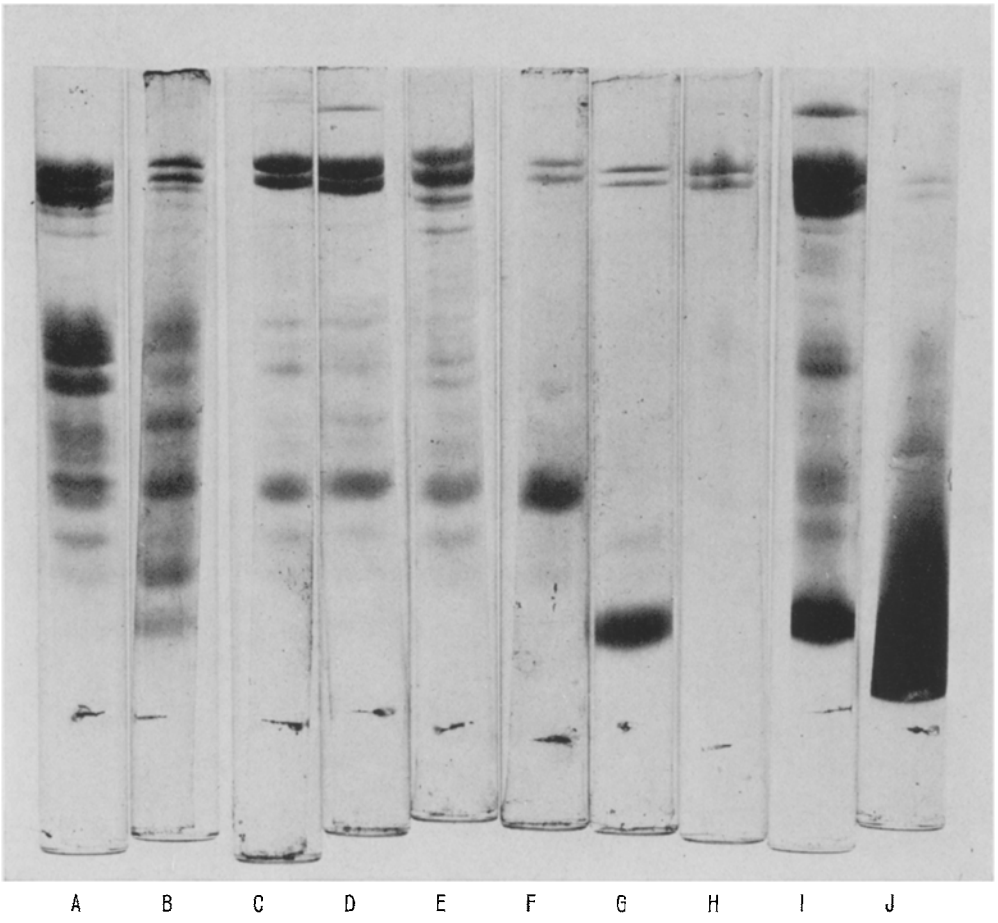

Fig. 2. Comparison of low ionic strength protein extracts. A) Ghosts (same as Figure 1A). B–F) Extracts were made from ghosts of Figure 1A: B) 'P1', JULIANO, ROTHSTEIN and LAVOY[11], overnight extract at 25 °C in 20 mos*M* NaCl-$NaPO_4$, pH 7.2, centrifuged at 35,000 × *g* for 1 h. C) 'P2', JULIANO et al.[11], pellet from P1 dialysed overnight vs. 1 m*M* EDTA, pH 8.5, centrifuged 140,000 × *g* 1 h. D) 'fibrillar protein', ROSENTHAL, KREGENOW and MOSES[12], dialysis of ghosts vs. 0.5 m*M* EDTA, pH 7.4, centrifuged at 140,000 × *g*, 1 h. E) 'torus protein' HARRIS[13], also HAGGIS[14], dialysis vs. distilled water, adjusted to pH 7.5, frozen and thawed, centrifuged 140,000 × g, 1 h. F) Supernatant of E) after precipitation of protein at pH 5.5, centrifugation, and concentration. G) 'spectrin', MARCHESI et al.[7], dialysis of their ghosts (Figure 1G) vs. 5 m*M* EDTA, 2 m*M* mercaptoethanol, pH 7.5; 35,000 × *g* pellet re-extracted with distilled water, 15 min at 22 °C, pooled supernatant centrifuged 140,000 × *g*, 1 h. H) 'EDTA extract', FAIRBANKS et al.[2], extracted from their ghosts (Figure 1F) by stirring ghosts 15 min at 37 °C with 0.1 m*M* EDTA, pH 8, and centrifuged 140,000 × *g*, 1 h. I) 'tektin', MAZIA and RUBY[9] or CLARKE[15] from MAZIA and RUBY ghosts (Figure 1I) dialysed overnight vs pH 9.5 distilled water, centrifuged 140,000 × *g*. J) Last supernatant of preparation of FURTHMAYR and TIMPL ghosts, Figure 1J, showing absence of 'missing' protein bands.

The high-molecular weight doublet, band 1, has been the subject of considerable interest and speculation[12, 7, 9, 11, 3, 15]. Because of variation in reported molecular weight for the high molecular weight doublet[2, 3, 4, 7, 9, 11, 15], it has not previously been known if the various water-soluble protein preparations contained the same components. It is clear from our results that the water-soluble red cell protein extractions in the literature yield a small number of components, and that the exact extraction procedure is not especially critical. The most homogenous preparations of band 1 are obtained from ghosts depleted in low molecular weight proteins. Although such ghosts are permeable to ATP, and thus not 'native'[5], they are clearly the preferred material for extraction of the high molecular weight doublet.

Résumé. Quelques préparations de membranes érythrocytaires et de leurs protéines extraites en phase aqueuse ont été comparées par électrophorèse sur gel de SDS-polyacrylamide. De faibles différences seulement ont été notées d'une préparation à l'autre. Les extraits obtenus à force ionique basse ou élevée contiennent tous la même protéine de poids moléculaire élevé ('spectrine') mais different quant à leur contenu en peptides de faible poids moléculaire. Les préparations obtenues à l'intérieur de chaque groupe s'avèrent fondamentalement semblables.

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