PRELIMINARY NOTES

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The solubilization of human erythrocyte membranes by *n*-pentanol

During the past few years, several studies concerning the isolation and characterization of structural proteins from erythrocytes, have been reported[1-9]. MADDY[1,2] described a method for the solubilization of the proteins of ox erythrocytes by *n*-butanol fractionation. We observed that *n*-pentanol causes a solubilization of erythrocyte ghosts, but in contrast to the butanol procedure, not only the proteins but also the lipids were recovered in the water layer.

Human erythrocyte ghosts were prepared according to the method of DODGE, MITCHELL AND HANAHAN[10]. The haemoglobin-free ghost suspensions were frozen overnight at −25° in hypotonic phosphate buffer (20 mosM, pH 7.4), thawed the next day and washed 3 times with distilled water at 2°. The ghost suspensions in water were mixed with equal volumes of pentanol, butanol or mixtures of both solvents at 0° and 25 min later were centrifuged at 30000 × *g* for 15 min. The upper organic phase was removed and the aqueous phase under the insoluble interfacial film was sucked out with a cold hypodermic syringe and dialysed against water for 20 h at 4°.

With pentanol, 80–85 % of the proteins and over 80 % of the phospholipids (and also cholesterol) of the original ghost were found to be solubilized in the aqueous phase (Fig. 1), whereas only 2–5 % of the phospholipids were recovered in the pentanol phase. Using butanol, we found, in agreement with MADDY[2], that 85–90 % of the proteins and only 3–5 % of the phospholipids were present in the aqueous phase (Fig. 1), while 90–95 % of the phospholipids were detected in the butanol phase. As shown in Fig. 1, the percentage recovery of phospholipid in the water phase is strongly dependent upon the ratio of pentanol to butanol.

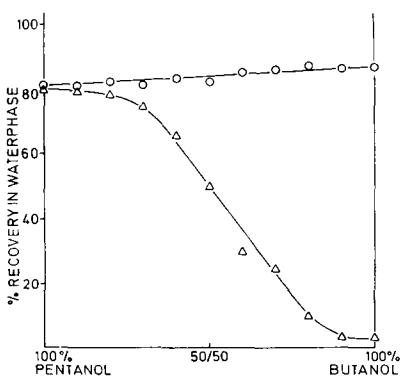

Fig. 1. Protein and phospholipid recovery in the water phase after treatment with pentanol–butanol mixtures of aqueous ghost suspensions. ○—○, protein; △—△, phospholipid. Determinations of protein[11] and phospholipid[12] were carried out by established procedures.

Comparative analyses of the amino acid composition of the proteins from ghost and the water layers after butanol and pentanol extraction, respectively, gave the following values: Asp, 8.3–8.0–7.9; Thr, 5.2–5.0–5.1; Ser, 6.3–6.2–6.1; Glu, 13.9–14.0–13.8; Pro, 5.6–5.8–5.8; Gly, 6.8–7.0–7.0; Ala, 8.1–8.1–8.0; ½ Cys, 0.5–0.5–0.5; Val, 6.6–6.7–6.6; Met, 2.1–2.2–2.2; Ile, 4.8–5.0–5.3; Leu, 11.6–11.6–11.7; Tyr, 2.4–2.3–2.3; Phe, 5.0–4.9–4.9; Lys, 5.0–4.9–5.0; His, 2.7–2.6–2.6; Arg, 5.1–5.2–5.2. (Expressed as mole%; analyses were made with a Beckman amino acid analyser; Thr, and Ser, uncorrected values; Trp, not determined.) These results confirm that the proteins obtained after butanol or pentanol extraction are representative of the original ghost proteins. Disc electrophoresis at pH 3.5 in 6 M urea using 7% polyacrylamide as a separating gel, showed up to 13 protein fractions both after pentanol and butanol solubilization. No significant differences in the electrophoretic pattern between the two preparations were observed.

By density gradient centrifugation in sucrose gradients, a comparative study was carried out between the aqueous solutions obtained after pentanol (Fig. 2A) and butanol (Fig. 2B) extractions. In both cases two major fractions were obtained*. With respect to Fraction I, a difference in density was apparent (pentanol solubilized 1.160; butanol solubilized 1.181) but the densities of Fractions II were about equal (1.090). In another experiment, the water phase obtained after butanol treatment was mixed, prior to the centrifugation procedure, with an aqueous lipid suspension prepared by ultrasonic vibration of total erythrocyte lipids. A separation into three fractions was obtained (Fig. 2C). In a control experiment, it was found that the lipid alone remained floating at the top of the gradient during the centrifugation. The addition of lipid to the proteins solubilized by butanol did not affect the distribution of the proteins over the gradient (compare Figs. 2B and 2C) and the lipid was recovered mainly at the top. However, after centrifugation of the solution obtained after pentanol extraction, no distinct lipid fraction was detectable at the top of the gradient. After extraction of the fractions with chloroform–methanol and examination of the extracts

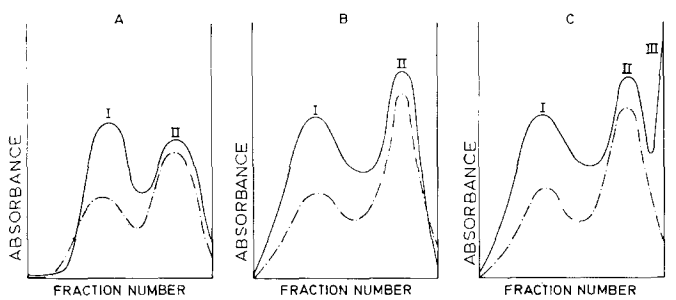

Fig. 2. Density gradient patterns obtained after centrifugation for 3 h in sucrose gradient (13–55%) in a Spinco Model L2-65, SW 39 rotor at 37 500 rev./min. A. Aqueous phase after pentanol extraction of human erythrocyte ghosts. B. Aqueous phase after butanol extraction of human erythrocyte ghosts. C. Protein solution obtained under B, mixed with micellar lipid suspension in water (the ratio of protein to total erythrocyte lipid was 5:4). Samples (0.5 ml) containing 4–5 mg of protein were applied to the gradient and 11 fractions were collected after puncture of the bottom of the tube. ———, absorbance at 280 mμ; ·—·—·, protein content according to the method of LOWRY *et al.*[11] ($A_{750 m\mu}$).

* Disc electrophoresis of Fractions I and II revealed 4 and 9 protein fractions, respectively, which exhibited mobilities corresponding with the 13 fractions detected in the starting material.

by thin-layer chromatography, it appeared that both cholesterol and the various classes of phospholipids were moving downwards into the gradient together with the proteins. Preliminary analyses suggest that differences may exist in the lipid to protein ratio among the fractions. Electron microscopic studies showed that the material in the aqueous layer after pentanol treatment is homogeneous, in contrast to the material in the water phase after butanol extraction mixed with the micellar lipid suspension. These results demonstrate that soluble lipoproteins can be obtained by treatment of aqueous ghost suspensions with pentanol. We hasten to emphasise that it is not known at present whether some of the original lipid–protein associations have been preserved.

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Department of Biochemistry, R. F. A. ZWAAL
Laboratory of Organic Chemistry,* L. L. M. VAN DEENEN
Utrecht (The Netherlands)

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* Postal address: Croesestraat 79, Utrecht, The Netherlands.