THE APPEARANCE OF A PROTEIN IN THE HUMAN ERYTHROCYTE MEMBRANE DURING AGEING

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1. Introduction

W. Ashby [1] showed as early as 1919 that the mean life span of the human erythrocyte was approximately 120 days. This was confirmed by D. Shemin [2] using N^{15}-Glycine which is incorporated into the haemoglobin. More importantly, the latter showed that by far the majority of the erythrocytes had a similar life span. As these cells do not seem to be subject to random removal from the circulation, it follows that an ageing process is involved. Thus, the question to be answered is what is the stimulus for the destruction of the erythrocyte.

Most of the numerous studies so far undertaken have been involved with detecting changes in the enzymic status of the cytoplasm in the hope of showing that senescence is brought about primarily by increasing metabolic incompetence. Unfortunately, no one has yet shown the loss of a critical biochemical function. The somewhat fewer studies on the membrane have revealed the following changes with respect to increasing age: a decrease in surface area [3], total lipid [3–5], cholesterol [3–5], surface-ve charge [6] and deformability [7], and an increase in osmotic fragility [8]. This report is concerned with the proteins of the human red cell membrane as revealed by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE). It is shown that a major protein is not present in the newly formed erythrocyte but gradually appears and becomes more prominent with age.

2. Experimental

Heparinised male blood was used immediately on withdrawal. The cells were sedimented twice at 5000 *g* for 5 min to remove the buffy coat and to increase the haematocrit to approximately 90%. 18.0 ml of this suspension was centrifuged for 1 hr at 1500 *g* at 27–30°C. The resulting red cell column was divided into eight fractions and ghosts were made from each by the method of J. T. Dodge [9], which involved three washes in 10 mM Na phosphate pH 7.4, each followed by centrifugation at 20 000 *g* for 20 min. The resulting pink ghosts were resuspended in 10 mM Tris–Cl pH 7.2 and pelleted at 20 000 *g* for 30 min to remove most of the residual haemoglobin.

SDS PAGE was performed in gels containing 5% acrylamide, 0.05% Bis, 0.2% SDS and 50 mM Tris–Cl pH 7.4. The electrode buffers were 50 mM Tris–Cl pH 7.4 containing 0.2% SDS. A current of 6 mA/gel was employed. The gels were stained for protein with Coomasie Brilliant Blue and scanned using a Chromoscan (Joyce–Loebl) at 560 nm.

3. Results

Fig. 1 compares the protein patterns obtained from all eight fractions using SDS PAGE. The classification of G. Fairbanks [10] has been used to identify the major protein components. It can be seen that protein 4.1. is absent from the youngest membranes (fraction 1), appears in fraction 2 and gradually increases in prominence to become a major component in the oldest membranes. This effect is also visible if the final 10 mM Tris–Cl wash is omitted (results not shown). This is the only consistent difference that has been observed in the non-haemoglobin protein compliment of the membranes. Fig. 2 compares the protein pattern

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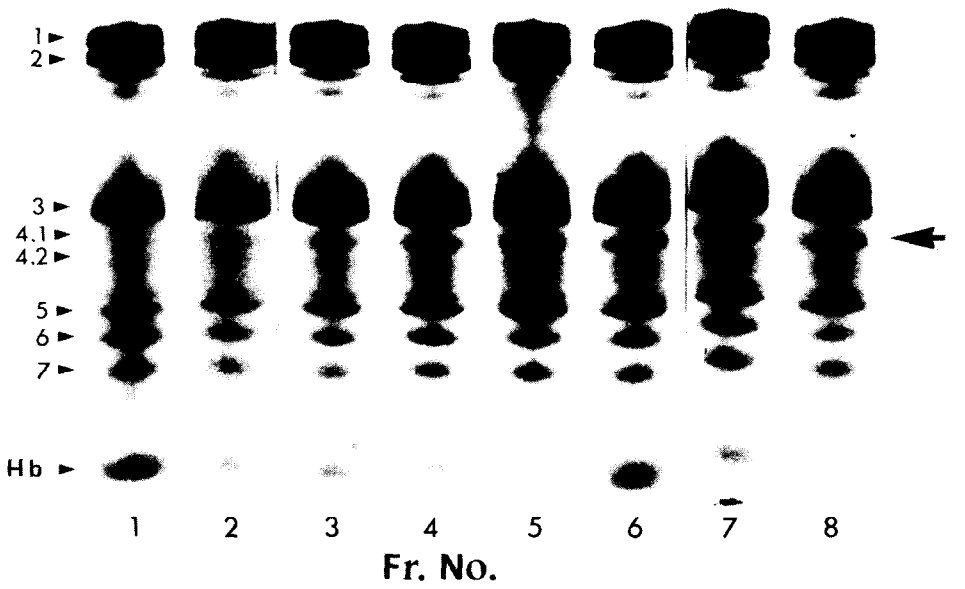

Fig. 1. The proteins of the human erythrocyte membrane as revealed by SDS PAGE, showing the change during the life cycle of the cell. The proteins are numbered according to G. Fairbanks [10].

from the youngest membranes (fraction 1) with those of the oldest membranes (fraction 8) and also includes the corresponding traces obtained by scanning the gels at 560 nm. This shows more clearly the protein change but also shows that the youngest membranes have a much larger haemoglobin component than do the oldest membranes. This has been observed in nearly all cases. The scans show clearly the high background prevailing between bands 3 and 5 (caused by the membrane glycoprotein), which makes quantification of the protein change difficult.

4. Discussion

The very few studies so far undertaken on the effect of ageing on the protein complement of the human erythrocyte membrane have been mainly concerned with the effect of blood bank storage i.e. in vitro ageing. M. J. Conrad [11] observed that protein 3 in outdated blood was less susceptible to SDS solubilisation and concluded that it had become denatured. G. L. Moore [12] showed overall charge and conformational changes. However, to attempt to draw conclusions from these studies and apply them to the ageing process in vivo is a highly tenuous exercise. P. A. Koch [13] using rabbit blood showed that the reticulocyte to erythrocyte transformation is accompanied by the loss of a single membrane protein, but as this report is concerned with the life cycle of the erythrocyte, this observation is not relevant to the discussion.

This is the first time that the proteins of the human erythrocyte membrane have been investigated with reference to the in vivo senescence of the whole erythrocyte. The increase in a protein component reported here seems parodoxical in that one might expect a decrease in complexity during the life cycle of an enucleate cell. As it is an enucleate cell one can fairly confidently rule out protein synthesis. The effect is known to be present after the last two out of a total of four centrifugation steps in the production of the ghosts, so it is unlikely to be produced by a preferential loss of the protein from the younger membranes during the washing procedure. Analysis of the first two pellets is made impossible by the enormous amounts of haemoglobin present. Proteinase digestion of the protein in the younger membranes is also unlikely as the ghosts are prepared at 5°C, but this cannot be completely ruled out. As no other protein species dis-

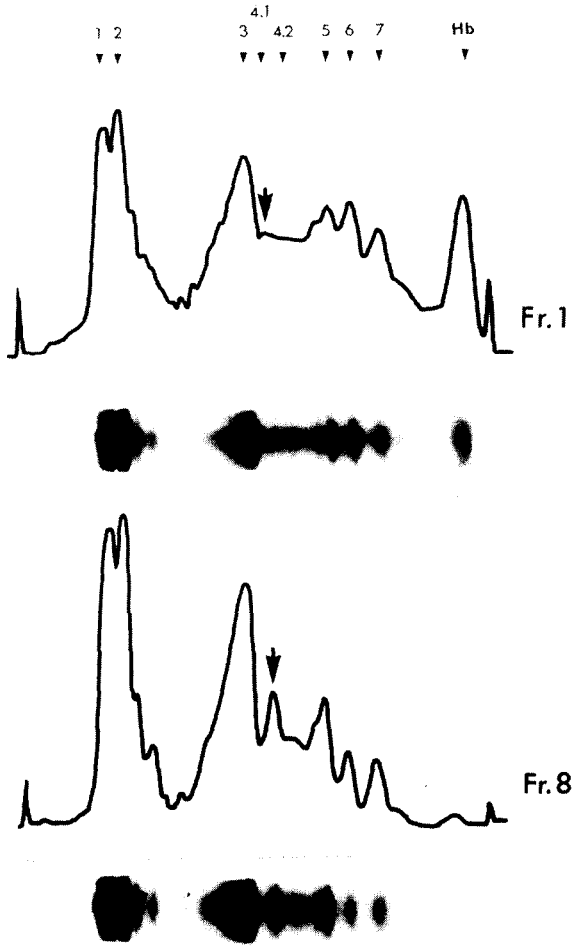

Fig. 2. The proteins of the youngest and oldest membranes as revealed by SDS PAGE and scanning the gels at 560 nm. The proteins are numbered according to G. Fairbanks [10].

appears one can also rule out polymerisation of a low mol. wt. protein, breakdown of a high mol. wt. protein and a change in electrophoretic mobility brought about by some intramolecular alteration.

The most likely explanation would appear to be that this protein is originally an exclusively cytoplasmic one and it gradually becomes adsorbed onto the inner surface of the membrane. Attempts to decide this tissue have been prevented as above by the excess haemoglobin. Preliminary studies show that membranes obtained from outdated blood contain greater amounts of this protein than do membranes obtained from freshly drawn blood. This is what one would expect as this mixed population of cells is 'older', but also suggests that the process is intrinsic to the red blood cell.

Senescence of the human erythrocyte membrane is associated with an increase in a single specific protein and it is suggested that this comes about by the latter adhering to the inner surface of the membrane. This process may be a contributing factor in some of the biophysical changes observed [7,8]. Whether this protein change is a cause of senescence, a result of it or a coincidental accompaniment must await further studies.

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