Adenosine Triphosphate and Shape of Erythrocytes

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The mechanism with which erythrocytes maintain their biconcave shape has remained to be clarified for a long time. An antisphering factor which Furchgott and Ponder (1) once proposed was doubted by Ponder himself (2) later. Recently, Prankerdt (3) suggested that red blood cell envelopes might possess a certain contractile property, since the incorporation of P32 is low in the esters of the stroma obtained from those species with spheroidal red cells or from patients with hereditary spherocytosis.

In the course of the study on metabolism in preserved erythrocytes, following informations were obtained by the authors (4, 5):

1) Adenosine triphosphate (ATP) in erythrocytes, which had been almost entirely eliminated after long-term storage and could not be regenerated with the addition of inosine only, increased rapidly and occasionally rose above the normal level after incubation with adenine and a purine nucleoside at 37°C.

2) Erythrocytes preserved for a long time are smooth spheres and, when they were incubated with adenine and nucleoside, they became crenated at first and changed to cap forms or became discoidal with the increase of ATP.

The present report is concerned with an experimental proof of the existence of an intimate relationship between the shape of erythrocytes and ATP level in them, using this technique of ATP regeneration in erythrocytes. A preliminary experiment along this line has already been published elsewhere (6) and some of the present results were summarized briefly in the 8th Congress of International Congress of Haematology (7).

MATERIALS AND METHOD

Human red blood cells obtained by venipuncture were washed three times with physiological saline and suspensions of these cells in physiological saline with a definite amount of 0.154 M NaF and in the same amount of physiological saline as a control were prepared.

Microscopical observation was carried out using hanging-drop-method.

After 10 ml. portions of about 30% cell suspension were incubated at 37°C under different conditions, each was centrifuged, the supernate discarded and 3% perchloric acid was added to the precipitate to the final volume of 12 ml. Five ml. of the supernate was neutralized with 5 ml of a dilute KOH solution, and KClO4 precipitate was centrifuged off. Then, 5 ml. of the solution was analyzed by the Cohn and Carter's method using chromatography of Dowex 1 (Cl) (8).

RESULTS AND DISCUSSION

During incubation with a final concentration of $2 \times 10^{-4} M$ of sodium or potassium fluoride, the discoidal shape of the cells changed to a crenated disk at about two hours, a crenated sphere at about four hours, and after six hours almost all the cells were transformed to smooth spheres (Fig. 1A). On the other hand, when the cells were incubated without addition of fluoride, their shape remained entirely unchanged even after six hours (Fig. 1B). When the cells were incubated without addition of either glucose or fluoride, the change of the shape to spheres was retarded (Fig. 1C).

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Furthermore, smooth sphere cells obtained after fluoride-treatment for 6-7 hours were washed four times with cold saline in order to wash out fluoride thoroughly and incubated further in a medium with $5 \times 10^{-3} M$ glucose, $1 \times 10^{-2} M$ inosine and $2 \times 10^{-5} M$ adenine to restore the ATP content in the cells as in the previous experiments. Within one hour the shape of the incubated cells turned to a crenated and then after 2 or 3 hours became a biconcave or a shallow cup form again (Fig. 2). The addition of inorganic phosphate and magnesium ion was occasionally effective in accelerating ATP synthesis and transformation of the shape, on account of the compensation of the loss of these ions which flowed out.
from inside the cells during preincubation and washing (Fig. 3). The disk-sphere transformation and vice versa could be repeated several times. The phenomenon was also observed in red cells obtained from preserved human blood or from rat or pig blood as well.

In parallel with the morphological observation described above, the content of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in the cells were estimated according to Cohn and Carter (8).

The data are shown in Fig. 4 and 5. The abscissa indicates the concentration of...
FIG. 3. Washed erythrocytes were incubated with NaF at 37°C for 6 hours and after washing out fluoride, reincubation was carried out with addition of inosine, adenine and glucose (a), and with addition of adenine, inosine and inorganic phosphate (b).

ATP or ADP and the ordinate, the percentage of disk, crenated and smooth spheres respectively throughout the whole population of the cells. As seen in Fig. 4, when ATP level was above a half of normal content, or about 50 μmole per 100 ml. of cells, erythrocytes were discoidal as control erythrocytes; when ATP level was below one tenth of the original level, about 15 μmole per 100 ml. of cells, they became smooth spheres; and when the amount of ATP was between these two levels, they were crenated. However, as Fig. 5 indicates there was no correlation between ADP level and the shape of erythrocytes.

Although the amount of total adenine nucleotide was emphasized in the preliminary report (6), the amount of ATP exactly estimated seems to have a more direct relation-

Fig. 4. Adenosine triphosphate level in erythrocytes and their shape. Abscissa: Percentage of the cells with various shapes. Ordinate: The content of ATP in erythrocytes.

Fig. 5. Adenosine diphosphate level in erythrocytes and their shape. Abscissa: Percentage of the cells with various shapes. Ordinate: The content of ADP in erythrocytes.
ship to the shape.

A similar transformation was also observed during the preservation of blood in Acid-Citrate-Dextrose-Medium at 4°C: the biconcave disks changed to crenated disks at first after several days and then to crenated spheres and finally to smooth spheres after more than several weeks. Calculated from the results which were obtained from gradient elution technique of ion exchange column chromatography on Dowex 1, adenosine triphosphate contents of the cells and photographs of the preserved erythrocytes are shown in Fig. 6. Each cell shape type was quite the same whether it was observed at 2°, 20° or 37°C.

The observations presented here lead us to the following conclusions:

1. If ATP content in red cells is over a certain level, that is about 50 per cent of the original level, the normal shape of the cells is maintained, and if it is below this level the shape becomes crenated, and when it decreases to below about 10 per cent, the shape of the cells changes to a perfect sphere.

2. The presence of plasma protein is not necessary for the red cells to maintain their discoidal shape. Therefore, the presence of antispHERing factor in plasma was decisively denied.

3. Other workers have reported that change of pH and ionic strength causes a similar transformation (10, 11). In this experiment, however, ATP level gives rise to the disk-sphere and also spherical shape-disk

![Fig. 6. Erythrocytes preserved in Acid-Citrate-Dextrose-Medium for 12, 28, 56 and 130 days.](image-url)
transformation of red cells, while pH, cation concentration and ionic strength remained unchanged.

4. The maintenance of the shape of the red cells may depend not on the turnover of ATP, but on the level of ATP, because the cell shape remained quite unaffected by a temperature change alone.

The fact that ghost obtained from long-stored red cells changed to a goblet-like shape with the addition of ATP in the final concentration of 0.03–0.04 M or higher, differing in this respect from intact erythrocytes, has been previously reported by the authors (12).

The observations provide experimental evidence for the assumption that the shape of the erythrocytes is dependent upon ATP level in the cells under normal conditions. The observed phenomena might also recall the relation between the plasticity of muscle and its relatively high ATP content. It might be a general rule that ATP is necessary for the manifestation of the normal shape in various types of the cells.

The extraction of ghost protein is now in progress in our laboratory and will be reported elsewhere.

REFERENCES

(2) Ponder, E., Blood., 9, 227 (1954)