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POSTMORTEM CHANGES OF OSMOTIC FRAGILITY AND RED CELL SHAPE

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Abstract

The osmotic fragility and morphological changes of red cells in rat corpses were investigated in order to estimate the postmortem interval. The osmotic fragility was measured by a coil planet centrifuge (CPC) system.

Soon after death, hemolysis starting point (HSP) and hemolysis end point (HEP) were shown to shift to higher osmotic pressure and the shift of HSP was observed up to 12 hours postmortem. On the contrary, HEP became fairly constant thereafter and resulted in the change of hemolysis pattern from monophase to flat.

The changes of osmotic fragility especially at HSP well correlated with the morphological changes obtained by the scanning electron microscopy. From these results, it was shown that the shift of HSP together with the analysis of hemolysis pattern obtained by CPC system was very useful to estimate the early postmortem interval.

INTRODUCTION

Estimation of the postmortem interval is an important problem for forensic examiners. Usually it is ascertained that the gross postmortem changes, such as fall in body temperature, degree of cadaveric rigidity, stage of digestion of the gastric contents, are mostly dependent upon the dissector's experience. Recently, Mukai et al. (1977)[1], Suzutani et al. (1979)[2] reported the application of the quantitative method to these postmortem findings.

On the other hand, certain biochemical changes must be also useful in estimating the time of dead, and many authors have tried to correlate the scale of biochemical postmortem changes to the time elapsed since death.[3,4,5] Especially, postmortem changes in many constituents of plasma and cerebrospinal fluid have been examined. However, red cells have not been so well studied because of inherent disadvantages such as poor reproducibility, a time consuming and inaccurate procedures.

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Since the recent advent of a new method for the osmotic fragility test, we carried out the experimental study on postmortem blood taken from rat corpses to investigate the possibility of correlating the osmotic fragility and morphological changes of red cells with the postmortem intervals.

MATERIALS AND METHODS

Wistar strain rats were strangled to death and the corpses were stored at

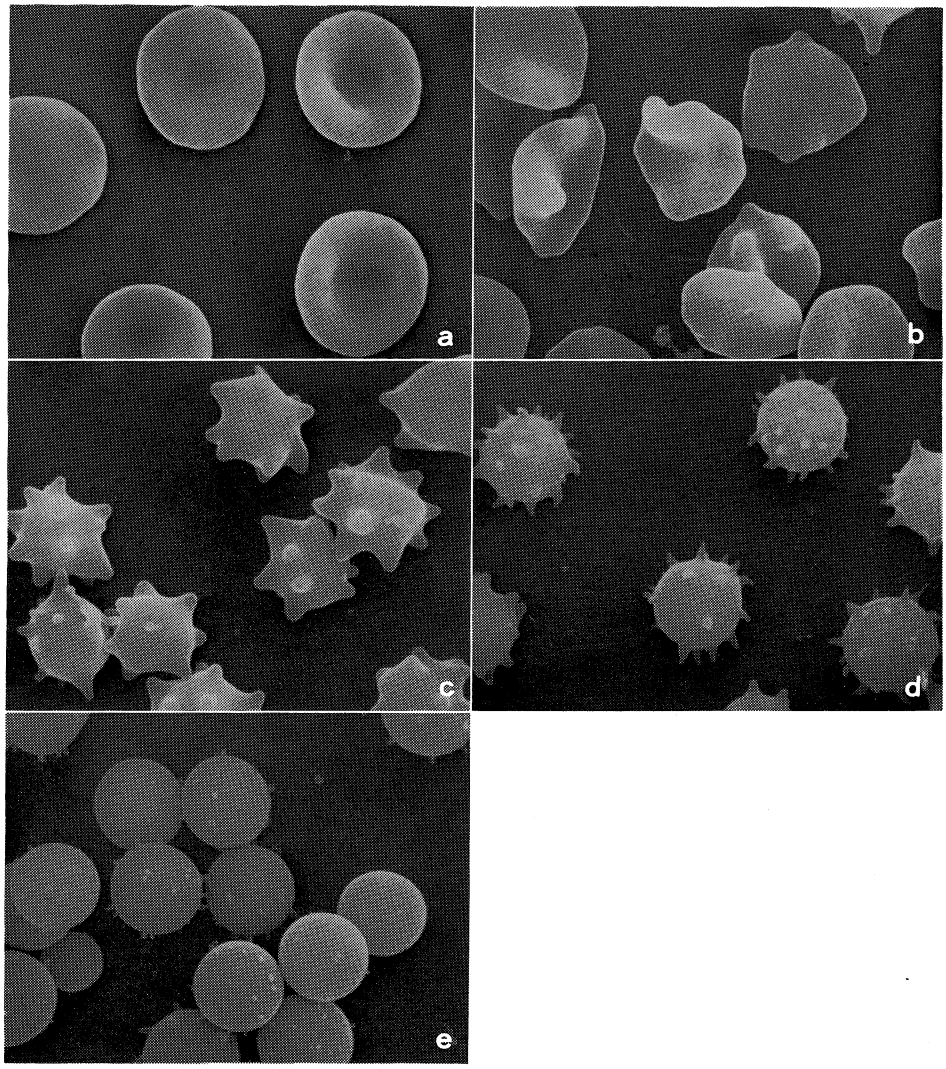

Fig. 1. Cell forms observed by scanning electron microscopy. × 5,000 a : Discocyte, b : Echinocyte I, c : Echinocyte II, d : Sphero-echinocyte, e : Spherocyte

room temperature (26°C–29°C). Postmortem blood was withdrawn from the heart of the sacrificed animals at varying times after death. Other blood samples (test tube blood) were prepared in heparinized test tubes under the same conditions as the postmortem ones.

The osmotic fragility of red cells was measured by the coil planet centrifuge (CPC) system. The red cells were put into a sample coil and forced to pass through the saline solution having a linear osmotic gradient from 200 mOsm to 30 mOsm. Then the hemoglobin distribution pattern thus obtained in the sample coil was recorded with the spectrophotometer specially designed for this purpose.

For morphological examinations, the blood samples were fixed with 1% glutaraldehyde for 40–60 min, then with 1% osmium tetroxide for one hour. They were dehydrated in a graded series of ethanol and isoamyl acetate, dried by the critical point drying method, then coated with a thin layer of gold-palladium using the ion spattering method and examined under the scanning electron microscope (Hitachi, HHS-2R) using 20-kv accelerating potential. Whenever quantitative measurements of the cells seemed necessary, the observation was done with at least 500 cells under 3000–3500 magnifications and divided them into five types according to their shapes as follows : discocyte, echinocyte I, echinocyte II, sphero-echinocyte and spherocyte (Fig. 1).

RESULTS

The osmotic fragility changes of red cell membranes were shown in Fig. 2.

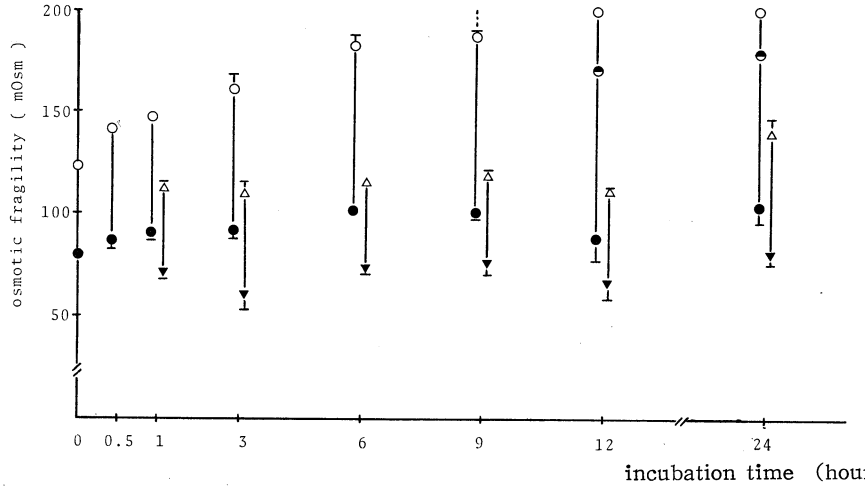

Fig. 2. Time-lapse changes in osmotic fragility of erythrocyte membrane. HSP (open symbols) and HEP (solid symbols) are plotted against incubation time. ○, ● : changes in corpse, △, ▼ : changes in test-tube.

As shown in Fig. 2, the osmotic fragility of test tube blood was stable for periods up to 12 hours. On the contrary, postmortem blood demonstrated a significant decrease of the osmotic fragility at 30 min after death : hemolysis starting point (HSP) $p<0.01$, hemolysis end point (HEP) $p<0.05$. Thereafter HSP was shown to shift to a higher osmotic pressure with the lapse of time after death and up to 12 hours postmortem, indicating a linear relationship between the shift of HSP and postmortem interval. However, HEP became fairly constant with little shift, and thereafter the hemolysis pattern changed from monophasic to flat one.

The morphological changes of red cells were shown in Fig. 3 and Fig. 4. In the postmortem blood taken from rat corpses, there was a progressive decrease in the proportion of disc erythrocytes ; about 70% at 30 min postmortem and then about 5% at one hour postmortem. And the disco-echinocyte transformation and then the echino-spherocyte transformation could be observed with the lapse of time after death (Fig. 3). As shown in Fig. 3, firstly echinocytes II had reached its maximum number at one hour after death and then began to decrease rapidly. Secondly, in the case of echinocytes II, an increase in the proportion of sphero-echinocytes was observed gradually and echinocytes in the postmortem blood were nearly all (ca 85%) of crenated spheres at 6 hour-12 hour after death. With lapse of time, an increase in the spherocytes, that are red cells with shorter or negligible needles, were observed.

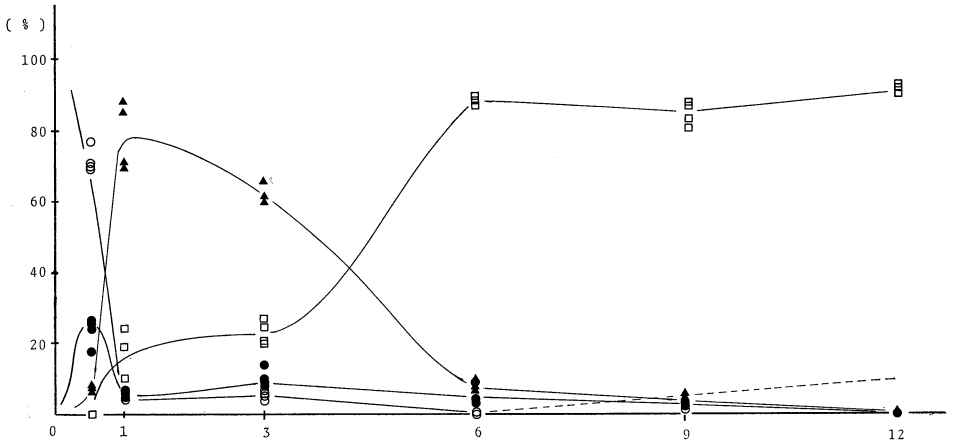

Fig. 3. Time-lapse changes in RBC morphology of heart blood taken from rat corpses. The rats were strangled to death and stored at room temperature. Observations were made by scanning electron microscope (Fig. 1). ○-○ : Discocyte, ●-● : Echinocyte I, ▲-▲ : Echinocyte II, □-□ : Sphero-echinocyte, ····· : Spherocyte.

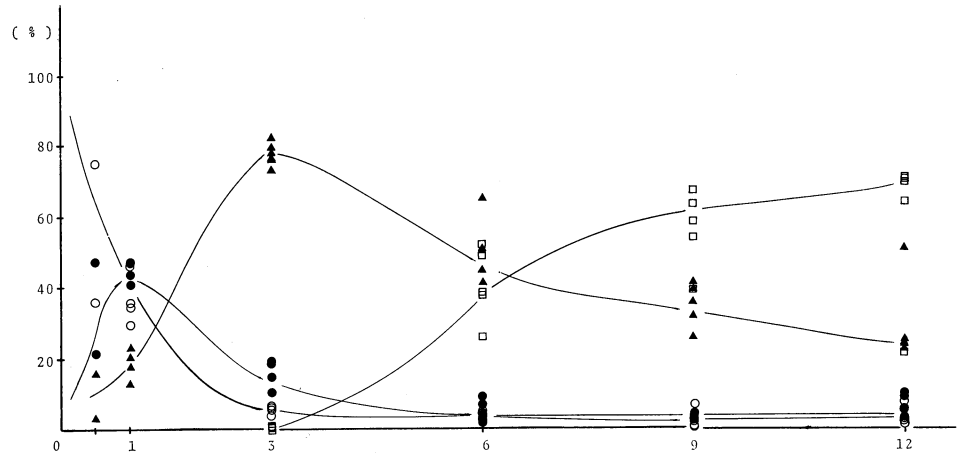

Fig. 4. Time-lapse changes in RBC morphology of blood left standing in the test tube. Observations were made by scanning electron microscope (Fig. 1). ○-○ : Discocyte, ●-● : Echinocyte I, ▲-▲ : Echinocyte II, □-□ : Sphero-echinocyte.

On the other hand, in comparison with postmortem blood, erythrocytes in test tube blood showed a gradual change from the disk cells to spheroid cells. As shown in Fig. 4, during the storage at room temperature, the discoidal shape of the cells changed to a crenated sphere and after 3 hours almost all the cells were transformed to echinocytes II. And then the transformation from echinocytes II to sphero-echinocytes was gradually observed ; the proportion of sphero-echinocytes was about 40% after 6 hours, about 60 % after 9 hours and about 65% after 12 hours.

DISCUSSION

With cessation of life, dissolution begins and many chemical changes follow.[6,7,8,9,10] Determination of such postmortem chemical changes is thought to be useful for estimating the time of death. Various properties or capacities of the whole blood and plasma have been tested for a relationship to the duration of death. With regard to the red cells in corpses, many attempts have been reported as to hematocrit values, sedimentation rate, viscosity, increase of plasma hemoglobin and morphological changes[5,6,12,13] and it has been generally considered that the hemolysis phenomenon of red cells is the main process that is observed with blood in corpses. De Bernardi (1958)[11] described an ever-decreasing avidity for the dye and crenation developing in the first 24-hr period, increasing deformation, clumping and fragmentation in the second

24-hr period, and the predominance of red cell ghosts in the third 24-hr period. Momose et al. (1958)[14] estimated the lapse of time after death by the hemolytic rate of blood in corpses and the rate after one minute's passage of time represented hemolysis of 0.0037%. While, in general, all these efforts are not necessarily to dwell on precise details, partly due to no rigid experimental conditions, the possibility of artifacts, and an inadequate method. Then, to answer the question when and how the hemolysis would come out, we measured the osmotic fragility of red cells by the coil planet centrifuge method that was recently developed in our country as a precise and highly reproducible method.[15,16,17,18] As shown in Fig. 3, there could be observed a great difference in the osmotic fragility changes between postmortem blood and test tube blood. Soon after death, at 30 min after death, HSP and HEP of blood taken from rat corpses showed a significant shift to a higher osmotic pressure and thereafter the shift of HSP was observed up to 12 hours postmortem. In contrast, HEP showed little shift and the hemolytic pattern became wider with a plateau during the lapse of time after death. Whereas the osmotic fragility of test tube blood remained stable for a fairly long period of time without any addition of nutrious. From these results, it was demonstrated that the osmotic fragility of red cells in corpses decreased rapidly with wide hemolysis distribution after death. The morphological changes observed at postmortem red cells had a good correlation with the results obtained by CPC method. The red cells in rat corpses transformed from discocytes to echinocytes and then to spherocytes more rapidly than those in test tubes (Fig. 3 & 4). In spite of the phenomenon of hemolysis band mentioned above, erythrocytes in postmortem blood were homogeneous and were nearly all of crenated spheres at 6 hour-12 hour after death. These differences remain to be investigated in future.

These results clearly indicate that the great difference between postmortem red cells and test tube ones with lapse of time might be attributed to the disparity of environmental conditions to which the cells are subjected. Thus the shift of HSP together with the analysis of hemolytic patterns was thought to be useful for the estimation of the early postmortem interval, particularly of the period within 12 hours after death.

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