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Experimental Study on the Postmortem Changes of Red Blood Cells

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ABSTRACT. In the present study, the author investigated the relationship between osmotic fragility changes measured using coil planet centrifuge (CPC) systems and some other factors involved in membrane resistance.

The animals were killed by stabbing in the chest and blood samples were collected at varing intervals after death. In addition to morphological observation, red cells were assayed for 2,3-diphosphoglyceric acid (2, 3-DPG), potassium, magnesium and zinc.

The disc-echino transformation was observed in the early postmortem period. Morphological changes were correlated well with osmotic fragility changes. The level of 2,3-DPG progressively decreased after death in quite the same manner as osmotic fragility. Among the cations, potassium and magnesium markedly decreased in the early postmortem period and were maintained at almost constant levels. Zinc decreased gradually during the period of the study. The red cells in test tube blood changed more slowly than those in postmortem cells.

From these results, it can be seen that the red blood cells incubated in a carcass rapidly decrease the membrane resistance, leading to hemolysis. It is suggested that the observation of this phenomenon is of great value for estimating the postmortem period.

In legal medicine, the determination of the postmortem time is always a problem. At the present time, it is difficult to assess the time of death accurately. Many authors have reported the postmortem changes of blood components as measures of the postmortem period. Previously, the author reported the postmortem changes of osmotic fragility and red cell shape[1] and that the observation on a course of hemolysis after death was very valuable in estimating the time after death. However, the amount of blood taken from the heart of strangled rats was too small to obtain further information on the osmotic fragility as an indicator of the time after death. In the present study, the rats were killed by stabbing in the breast with a sharp-edged tool and the blood samples were taken from the thorax. With these samples, 2,3-DPG, potassium, magnesium and zinc in red cells were determined and the osmotic fragility and the shape of red cells were examined.

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MATERIALS AND METHODS

Male Wistar-strain rats were used in this study. The animals were slightly anesthetized with ether and killed by stabbing in the breast with a surgical knife. The carcasses were stored at room temperature (26°-29°C). Blood was withdrawn from the thorax at varing intervals after death. Other samples were obtained by decapitating the animals and stocked in the presence of anticoagulants at room temperature (test tube blood).

The measurement of osmotic fragility by CPC systems and the morphological examination by scanning electron microscope were described in detail in the previous report[1].

2,3-DPG in red cells was determined enzymatically with kits (Sigma Chemical Co., Ltd.) and expressed in terms of hemoglobin (μmol/gm). Hemoglobin concentration was determined according to the cyanmethemoglobin method using kits (Wako Pure Chemical Industries, Ltd.).

Potassium, magnesium and zinc in red cells were determined with an atomic absorption spectrophotometer (Perkin-Elmer Co., Ltd.).

Blood was centrifuged at 2,000 rpm for 10 min. After removing the plasma and buffy coat, the precipitated red cells were washed three times with physiological saline and resuspended in saline. Packed cell volume was estimated from the hematocrit value. Potassium was directly determined after dilution of the washed red cell suspension. The samples for magnesium and zinc determinations were prepared by the method of Rosner et al.[2] Red cell suspension was added to 2 volume of 10% trichloroacetic acid, and then centrifuged at 2,500 rpm for 20 min. Zinc and magnesium in the supernatant fluid were determined. The calibration curves were obtained on each analysis. For magnesium determination, lanthanum was added to a final concentration of 1.0%. Potassium, magnesium and zinc were determined at wavelengths of 383 nm, 285 nm and 214 nm, respectively.

RESULTS

The postmortem changes of osmotic fragility as measured by CPC systems are shown in Fig. 1. Within the first one hour, no change in osmotic fragility was observed when the cells were incubated in the thorax of the carcass. A decrease in osmotic fragility was observed at 3 hours postmortem, namely hemolysis starting point (HSP) and hemolysis end point (HEP) significantly shifted to higher osmotic pressure (HSP, $p<0.01$; HEP, $p<0.01$). Thereafter the pattern changed from monophasic one to flat one with the lapse of time after death (Fig. 2). Especially, there was a nearly linear relationship between the shift of HSP and the postmortem time up to 12-18 hours after death ($Y=4.25X+123.52$, $r=0.978$).

Fig. 3 shows changes in shape of red cells observed by scanning electron microscope. As seen from this figure, the transformation from discocytes to echinocytes was rapid in the early postmortem period.

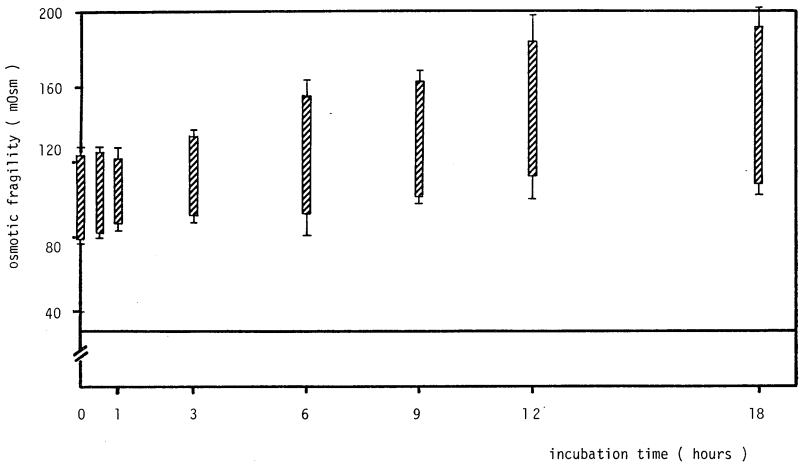

Fig. 1 Postmortem changes in osmotic fragility of erythrocyte membrane. The hemolysis pattern was obtained by CPC systems.

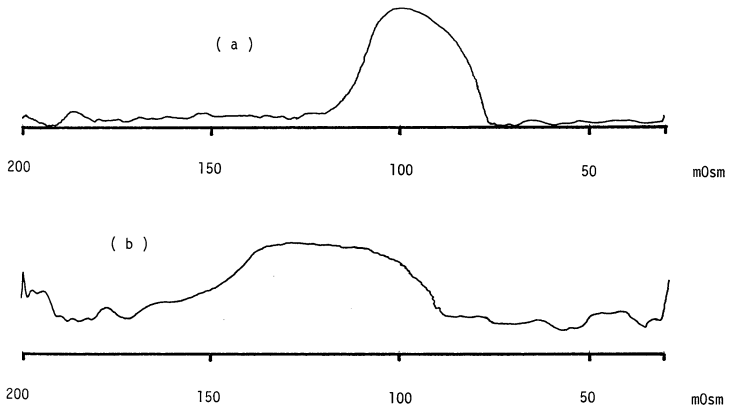

Fig. 2 The hemolysis pattern of postmortem blood by CPC systems. (a) 30 min postmortem (b) 9 hr postmortem

The percentage of discocytes decreased from about 100% to about 50% within one hour postmortem, to 10% within 3 hours and subsequently to 3% within 6 hours. Echinocytes I and II showed the maximum ratios at one hour and three hours postmortem, respectively. As expected, the spicules became smaller, the cells became roundish gradually, and most of the red cells changed to sphero-echinocytes after 6 hours postmortem.

The changes of 2,3-DPG concentration in blood are shown in Fig. 4. The normal value for 2,3-DPG in the present study was 23.4 ± 1.3 μmol/gm Hb. The results were in good agreement with the published data[3]. In the

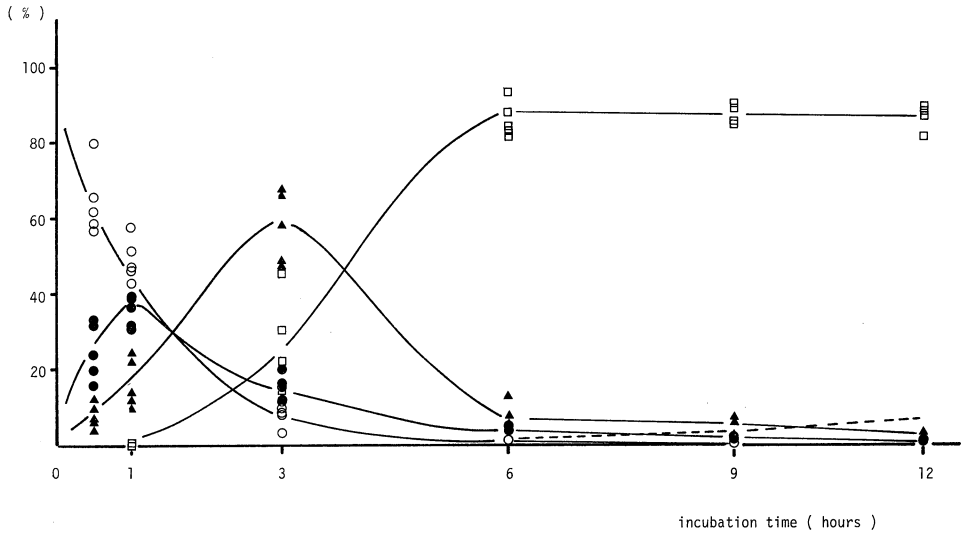

Fig. 3 Postmortem changes in RBC morphology. Observations were made by scanning electron microscope. ○—○ ; Discocyte, ●—● ; Echinocyte I, ▲—▲ ; Echinocyte II, □—□ ; Sphero-echinocyte, ····· ; Spherocyte

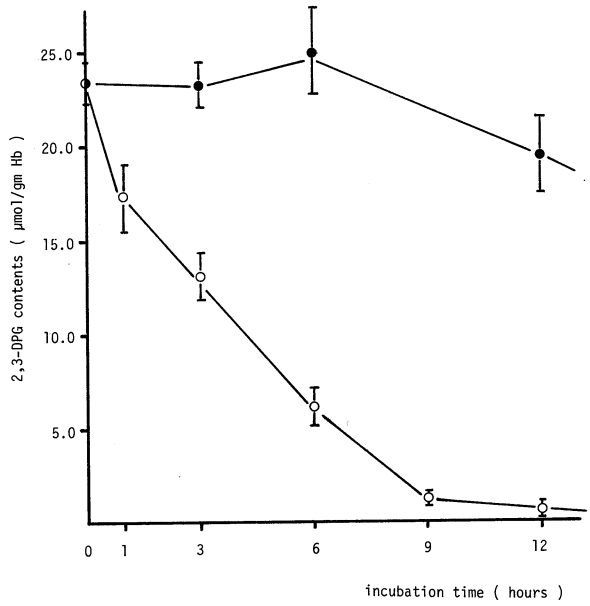

Fig. 4 Time-lapse changes in 2,3-DPG contents.
○—○ ; changes in postmortem blood,
●—● ; changes in test tube blood

presence of anticoagulants without any nutritions, test tube blood maintained its initial 2,3-DPG concentration during the period of this study. On the contrary, during incubation in the rat carcass, the 2,3-DPG concentration in red cells decreased progressively over a period of 9 to 12 hours until it was barely detectable.

The changes in the concentrations of potassium, magnesium and zinc in red cells are summarized in Table 1. As for the postmortem blood, the concentrations of these cations decreased progressively with the passage of time during the early postmortem period. For potassium, the average normal value was 3543.8 μg/ml. However, the potassium value decreased to 2662.2 μg/ml at 3 hours postmortem and ceased to change thereafter. For magnesium, a decrease was noted in the very early postmortem period as seen for potassium. The magnesium value was reduced to half by the time of 6 hours postmortem and remained nearly constant thereafter. On the other hand, zinc in red cells decreased gradually during the study period. With test tube blood, a gradual decrease in each electrolyte was noted and the rate of decrease was lower than that for postmortem blood. Thus, the content of each elctrolyte in red cells taken from the rat carcass decreased in the early postmortem period in quite the same manner as 2,3-DPG concentration.

TABLE 1. Time-lapse changes in potassium, magnesium and zinc levels in red blood cells.

incubation time	postmortem blood			test tube blood		
	K	Mg	Zn	K	Mg	Zn
0	3543.8±109.7	73.8±5.8	12.3±0.6	3610.7±43.9	64.6±3.1	10.7±0.9
3hr	2662.2± 79.7	50.6±6.5	9.2±0.4	—	—	—
6hr	2535.7± 45.5	31.6±0.8	8.1±0.7	3265.7±61.7	50.5±2.4	9.5±0.3
12hr	2809.4± 91.1	32.7±1.2	9.7±1.2	3317.6±19.5	41.1±3.4	8.6±0.5
18hr	2615.9± 56.2	35.4±1.6	7.2±0.3	3033.9±27.4	30.9±0.9	8.7±0.4

μg/ml

DISCUSSION

Various biochemical changes have been noted in the body fluid obtained from human and animal bodies soon after death. Recently, Laiho et al. have reported that the osmotic resistance is correlated poorly with the postmortem time.[4] However, the author previously found that the osmotic fragility change measured by CPC systems, which is very simple and accurate method developed in our country, was very useful in estimating the time after death so far as the early postmortem period was concerned[1]. Especially, HSP had a good correlation with the postmortem period. Penttilä et al. investigated the morphology of rat postmortem blood cells[5]. They found that the red blood cells were transformed in a characteristic way in the blood of a rat carcass. They also found that the progress of this change was related to the postmortem period, though as for red cells of a human cadaver this change seemed to be

of minor use for the estimation of the time of death[6]. The present observation on the rat red cells, as reported previously[1], indicated that a series of transformation of red cells occurred after death by the autolytic process. The transformation was clearly related to the osmotic fragility change which seemed to be a good parameter for estimating the time of death. Then the author investigated the relationship between the osmotic fragility and some other factors involved in membrane resistance. One of these factors seems to be 2,3-DPG, or an organic phosphate which is present most abundantly and that almost exclusively in red cells of man and many other mammals. It is known that anemia and some hereditary hemolytic disorders[7,8] change the 2,3-DPG level of blood. It has also been reported that the concentration of 2,3-DPG in stored blood decreased exponentially with time[9,10,11]. Albala et al. reported the physiologic features of hemolysis associated with the changes in cation and 2,3-DPG contents[12]. The present study showed that 2,3-DPG level in the rat carcass decreased quickly during the first few hours in a manner very similar to osmotic fragility changes measured by the CPC method. 2,3-DPG level of blood from the rat carcass decreased to about 25% of the normal value within 6 hours and was not detectable at and after 12 hours postmortem. On the contrary, test tube blood maintained approximately 100% of 2,3-DPG during the study period (Fig. 4).

It is also well known that cations play an important role in cellular membranes. Sumuvuori et al.[13] studied red blood cells in vitro and in human cadaver materials. They observed a decrease in cellular potassium and magnesium contents and in osmotic resistance of red cells during the early postmortem period at room temperature. In this study, the red cells showed marked losses of potassium and magnesium during the early postmortem observation period. The losses of these cations are known to be related with the death of cells. These phenomena seem to occur as a visible expression of cell autolysis, while in others Walia et al. described that heart-serum potassium was raised at the time of death, even if there is a five minutes' delay in the collection of the blood[14]. The issue of this cation from red cells has been described to precede hemolysis[15]. Zinc is also an important constituent of red blood cells, being approximately 10 times the amount of zinc in serum. Bettger et at.[16], Chvapil et al.[17] and Montgomery et al.[18] demonstrated that erythrocyte membranes are stabilized by zinc. There are several possible explanations for the stabilizing effect of zinc. Chvapil et al. assumed that the effect of zinc was confined to the surface of the membrane[19]. They have clarified thereafter that zinc is part of erythrocyte ghost, linked to protein as well as to lipid phases[20]. Zinc in red cells of postmortem blood decreased gradually with time after death (Table 1). It was suggested that zinc was related to the change of the osmotic resistance. The more rapid accumulation of toxic products in postmortem blood than in test tube blood may be an obvious reason for more rapid degradation of red cells in postmortm blood.

As mentioned above, the time course of changes in osmotic fragility

measured by CPC systems seemed to be in connection with some other factors. The present study showed that these parameters were valuable in estimating the time after death.

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