ON THE TRANSFORMATION OF HEMOGLOBIN IN PROCESSED HUMAN BLOOD DURING STORAGE

G. V. Titova

Dept. of Biochemistry (Chrmn.- Corresponding Member of the Academy of Medical Sciences of the USSR G. E. Vladimirov) of the Leningrad State University

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Hemoglobin can undergo two kinds of change during the action of oxidizing agents. On the one hand, it may be changed into methemoglobin, on the other hand, it may be changed into choleglobin as a result of a profound change in its hemin group.

The formation of choleglobin was discovered during the incubation of a solution of hemoglobin and ascorbic acid [1, 2, 6]. G. E. Vladimirov and A. I. Kolotilova showed that free hydrogen peroxide was formed during this process by using the polarographic method. Hydrogen peroxide played a decisive role since the addition of catalase completely prevented the decomposition of the hemoglobin and the oxidation of the ascorbic acid.

Further study of the conditions and ways of transforming hemoglobin established that the direct agent causing its transformation into choleglobin was not ascorbic acid, but its decomposition product – diketogluonic acid [5]. It was possible to cause the formation of choleglobin from hemoglobin in the presence of diketogluonic acid in the entire system.

The transformation of hemoglobin under these conditions proceeded toward the formation of choleglobin and further bile pigments and did not lead toward the production of free porphyrins.

In connection with the data which were obtained, it was interesting to watch the changes of the hemoglobin in processed human blood during storage.

It is known that, during the analysis of hemoglobin by Sahli's method, no sharp changes are found during the period of processing [5]. However, it was established that a noticeable increase in the iron content of the plasma occurred during the storage of processed blood. It can be deduced that this increase occurs at the expense of the decomposition of hemoglobin which begins during storage. This decomposition, probably developing slowly, cannot be established by such methods as Sahli's hemoglobin determination.

Approaching the analysis of the condition of the hemoglobin in processed blood from another point of view, we set ourselves the problem of watching the changes in the hemoglobin during the usual periods of storage.

EXPERIMENTAL METHOD

Processed blood, prepared with acid sodium citrate and glucose, was used in the experiments.

Changes in the hemoglobin were established photometrically, with the help of Pulfrich's step photometer, using filters S 47, 50, 53, 61, 66 and 72. We gauged the amount of hemoglobin in the blood by the absorption of light at the wavelength of 570 mμ.

Concurrently, we determined the protoporphyrin (by extraction with ethyl acetate and subsequent photometry [5]) and the biliverdin (by repeated extraction with ether and hydrochloric acid and subsequent photometry [6]) content of the blood.

We analyzed the processed blood on the first day of storage and then at varying intervals of time during the course of 20 days. During the experimental period, the processed blood was stored at 0°.

Changes in the Hemoglobin of Processed Blood

No decrease in hemoglobin content could be found by photometric analysis of processed blood for hemoglobin after various periods of storage.

At wavelength 570 mμ, no differences could be observed in light absorption [Fig. 1].

A marked difference in the light absorption of the blood hemoglobin could be observed between the first and 20th days of storage in the zone of 610-660 and 500 mμ. The changes in the light absorption in these zones are a characteristic sign of the appearance of the degradation product of hemoglobin, choleglobin, in the blood [2, 6].

The data obtained permit the conclusion to be drawn that partial degeneration of hemoglobin to form choleglobin occurs in processed blood, although we were unable to find a decrease in the amount of hemoglobin in the blood. Apparently, the latter is to be explained by the impossibility of detecting the small loss of hemoglobin from the blood during storage both by photometric methods of determining the concentrations at one wave length and by the application of Sahli's method for hemoglobin determination by some authors.

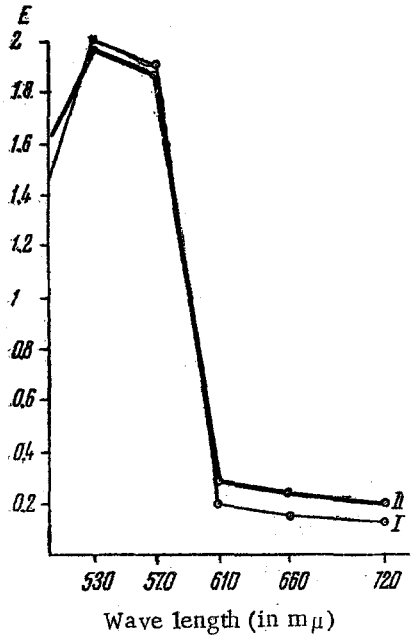

Fig. 1. Light absorption by a solution of the hemoglobin of processed human blood during storage. I) First day of storage; II) 20th day of storage.

Protoporphyrin and Biliverdin Content of Processed Blood During Storage

Concurrently with the photometric analysis for hemoglobin, we determined the protoporphyrin and biliverdin in the processed blood; the biliverdin was determined in order to confirm the appearance of choleglobin in the blood during storage, as was found by us through photometric analysis. The data obtained previously [5], showed that choleglobin is an intermediary product in the formation of biliverdin both in vitro and in the system.

In addition, determination of the protoporphyrin and biliverdin content of blood should show whether the hemoglobin is destroyed only in order to form choleblobin or whether, simultaneously, protoporphyrin, a substance poisonous to the system, is freed.

It was found that the protoporphyrin content of processed blood did not change in the course of 20 days' storage at 0° [see Table].

The biliverdin content of processed blood changes considerably during storage. From the first to fifth day, we found 0.3 mg of biliverdin per 100 ml of erythrocytes.

A considerable increase in biliverdin content was observed on the tenth day of storage (3 mg per 100 ml of erythrocytes on the average). A still greater increase occurred on the twelfth day the blood was stored (5 mg per 100 ml of erythrocytes); on subsequent days, the biliverdin content remained almost constant (Fig. 2).

The data on the biliverdin content of preserved blood correspond with the data obtained by photometric analysis of hemoglobin.

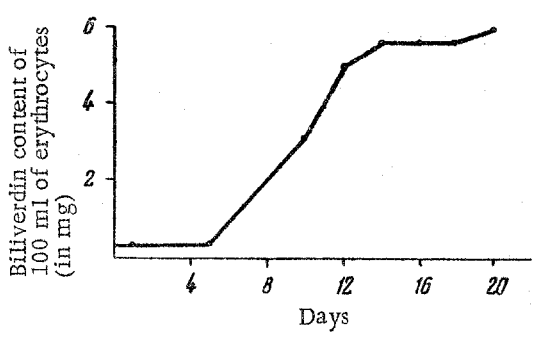

Fig. 2. Biliverdin content of processed human blood during storage.

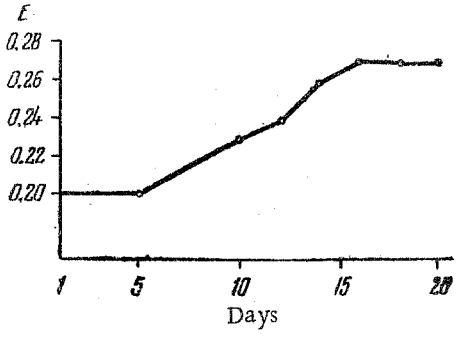

Fig. 3. Light absorption (wave length 610 mμ) of a solution of the hemoglobin of processed human blood during storage.

TABLE

Protoporphyrin Content of Processed Blood After Various Storage Periods (in mg per 100 ml of erythrocytes)

No. of experiment	Days of storage							
	First	Fifth	Tenth	Twelfth	Fourteenth	Sixteenth	Eighteenth	Twentieth
1	0,45	0,45	0,45	0,41	0,45	0,48	0,45	0,45
2	0,45	0,41	0,45	0,45	0,45	0,45	0,45	0,45
3	0,64	0,58	0,66	—	—	0,58	—	0,66

On Fig. 3 is shown the light absorption at wave length of 610 mμ of the hemoglobin of processed blood after various storage periods. At this region of the spectrum a sharper change in light absorption, characteristic of choleglobin, was noted.

As shown in Fig. 3, the appearance of choleglobin in the blood was observed on the tenth day of storage, which corresponds with the time when the biliverdin content rose.

CONCLUSIONS

The storage of processed blood at 0° is accompanied by the gradual decomposition of hemoglobin, which is difficult to show by the usual methods for hemoglobin determination. This change in the hemoglobin occurs as a step in its transformation into choleglobin with subsequent biliverdin formation. The formation of free porphyrins is not observed during this process.

It should be noted that, along with the decomposition of hemoglobin found in our experiments, decomposition of catalase was observed in processed blood. Z. N Katsnelson's investigations [3] showed that the catalase number of preserved blood stored for 5 days does not change at all; it decreases by 26.6% on the 10th day of storage; while by the 15th day it decreases by 42%.

G. E. Vladimirov and A. I. Kolotilova [1] showed that catalase is a powerful factor in protecting hemoglobin from decomposition. Apparently, the decomposition of hemoglobin depends, to a certain extent, on the loss of catalase activity by processed blood during storage.

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