Placental Blood*

CHANGES OCCURRING ON STORAGE WITH A REVIEW OF THE LITERATURE

(Preliminary report)

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BLOOD preservation is by no means a new practice. It was in 1918 that Dr. Oswald H. Robertson[1] devised the method of preserving blood with a mixture of 5.4 per cent glucose with 3.8 per cent sodium citrate. Rous, Peyton and Turner[2] performed a series of enlightening experiments in rabbits which consisted in bleeding the experimental animals and then administering preserved blood. Their experiments proved that preserved blood was fit for transfusion, at least, in laboratory animals.

In 1934, Ascoli and Vercesi[3] submitted a report on the use of placental blood for transfusions, and prophesied the success that was to be obtained.

In 1936, Norvikova and Farberova,[4] from the Institute of Blood Transfusions in Leningrad, wrote a comprehensive treatise on the use of placental blood. The technique described has been used by them since 1933. The authors observed that placental blood had an average of from 90 to 100 per cent of hemoglobin and contained from 5 to 6 millions of erythrocytes per c.mm.; it also possessed relatively large amounts of bilirubin and a low cholesterin and albumin fraction. The globulin was increased and the potassium, calcium and copper contents were very high.

In 1937, Stavskaya[5] advised the use of placental and retroplacental blood for transfusion in cases of "need of stimulation therapy." He advised its use in cases of anemia because of the large contents of red blood cells, high hemoglobin and bilirubin. The bone marrow is thus stimulated and plastic material is furnished for the regeneration of red blood cells. It was also indicated in cases of hemorrhagic diasthesis, because of the increased amount of coagulating substances in the placental blood. Finally, it is indicated in cases of shock.

It was not until 1938 that Goodall, Anderson, Altimas and MacPhail[6] presented their very comprehensive and complete work on the advantages of placental blood transfusions. The authors, all members of the staff of St. Mary's Hospital in Montreal, have been using placental blood for transfusions since 1936, and three hundred blood transfusions had been given previous to the publication of their article. In their extensive experience the authors outlined two physical reactions resulting from transfusion of placental blood: "One was a mild urticaria"; the second was "a severe accession of fever," both of short duration and without untoward sequelae. One other case had a severe chill, but this reaction was purely nervous, without fever or any other physical changes.

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The physiologic effects, according to Goodall et al[6] are similar to those of ordinary blood transfusion, with very slight difference as to degree. A blood bank of placental blood has been established and the results obtained have been highly encouraging. The advantages of preserved placental blood are outlined as: First, the blood serves to activate preoperatively all the reactive systems of the body and, secondly, it is of real benefit in cases of toxic hemorrhages, no matter what kind.

Goodall et al[6] were attempting to disprove that a placenta that was rigid with blood would detach more readily than when the placenta was empty. The cord was allowed to fall down over the vulvar towel and the cord clamp was released. It was noticed that the blood spurted three feet, due to its pressure and gravity, thus continuing to bleed for a while. An idea struck the observers as to the benefit of saving that blood for transfusions.

The authors of the cited work consider that the best preservative is that of the Moscow Institute of Hematology, which consists of:

Sodium chloride	7.0	grams
Sodium citrate	5.0	grams
Potassium chloride	.2	gram
Magnesium sulphate	.0049	gm.
Bi-distilled water	1000	c.c.

The preservative is supplied in 25 cc. ampules, at such strength that when 100 cc. of distilled water is added, the dilution will be equal to the formula outlined above. The blood is kept in a refrigerator equipped with a clock thermometer, the best suited temperature being between 34–38° F.

The method of collection of placental blood is quite simple, but demands experience on the part of the operator. It is collected through a funnel into an Erlenmeyer's flask after the cord has been thoroughly cleaned and protected from contamination. A Wasser-

mann reaction and blood typing are done from blood collected in separate test tubes during the procedure. The authors do not advise pooling the blood. Nevertheless, numerous samples can be given to the recipient provided they are of the same group. The blood should be warmed up to 105° F. and filtered through two layers of gauze.

Suppose, however, the recipient's blood is type II and there is shortage of this group, group IV can be given, provided the blood of group II has been given previously.

Characteristics of fetal blood, according to Goodall et al[6] are: The cellular contents average 7,000,000 R.B.C. It contains 25–30 per cent more thrombotic powers; it is rich in humoral extracts; it contains many immature nucleated red blood cells having a high degree of fragility.

Cultures in their series were done repeatedly, but have always been negative. They think that if some contamination takes place the organisms will not be able to thrive at the temperature that the blood is kept, and the infecting bacteria will be so attenuated and so diluted that they will be rendered inocuous.

Advantages cited by these authors are as follow: Placental blood is richer in thrombin, thus, of advantage in cases of hemorrhage; being young blood, it will activate the reticulo-endothelial tissue and will thus help in prevention and combat infection; it is of greatest advantage in preoperative medication; for blood obtained from a single placenta is enough to immunize a patient preoperatively, provided that there is no anemia.

Placental blood should not be obtained when membranes have ruptured 48 hours previous to delivery, nor from mothers who have communicable diseases. It should always be collected from full-term or nearly full-term cases. Eclampsia or pre-eclampsia are not contraindications. The authors reach the conclusion that "placental blood preservation has proved to be safe, constant, efficient and time saving source of supply for transfusion."

In the same year, Goodall et al[7] published another enlightening article. They asserted that progressive hemolysis occurs in stored placental blood, but that this is not a contraindication for transfusion. The hemolysis in placental blood is found in too small quantities to produce deleterious effects in the recipient. Hemolysis occurs at the expense of the nucleated red blood cells encountered in placental blood. These cells possess a greater fragility than adult R.B.C.

Grodberg[8] made a study of seventy-five transfusions of placental blood at the Boston City Hospital. His method of preserving blood

departs somewhat from the practice outlined above; he used 15 cc. of 2.6 per cent sodium citrate as a preservative. Citroseroid was used in some cases as anticoagulant. In 23 samples tested and kept in an electric refrigerator for from 1 to 36 days, there was no bacterial growth in any of them. In 235 samples collected, the average yield was 105 cc. and there was a variation of from 35 to 215 cc. The yield depends upon the size of the baby, length and size of the cord and speed of technique. The cytological studies in preserved placental blood demonstrated anisocytosis, poikylocytosis with a tendency to macrocytosis as the days elapsed. The possible disadvantages of anesthetics and analgesics which may pass through the placenta are stressed. Three patients experienced chills in this series of cases. Chills were followed by hyperpyrexia—in two cases the temperature was 101° F., and in the third case, 102° F. In still another case the temperature went up to 104° F., disappearing in 24 hours. The incidence of reactions was 5 per cent lower than the incidence reported by Price[9] who used fresh adult blood (11%); Karavanov[10] who used preserved blood (67%) and Shamov[11] reporting on cadaver blood (14%).

Guynn and Alsever[12] studied the steady drop in sugar concentration during the period of storage of placental blood. The hemolysis that occurs so extensively in some samples is due to the decreasing amounts of glucose. Addition of two grams of glucose per 100 cc. of the preserving fluid will obviate the extensive destruction of R.B.C. It was not until after the 8th week of storage that hemolysis became marked in the samples thus treated. Hemolysis is very much increased by frequent shaking. In regards to both red and white cells, they remain quite normal in appearance after 3 months of storage. Nucleated red cells tend to decrease during the first ten days. In their experience 55 samples were found to be sterile in 5 to 90 days after storage.

Halbrecht[13] used placental blood in 116 blood transfusions using 3.8 per cent solution of sodium citrate. He used blood which had been stored up to the 14th day, and reported results equal to those obtained from ordinary blood.

Page, Seager and Ward[14] advise pooling of placental blood samples provided they are of the same group. It is considered unnecessary and possibly harmful to heat the blood. The authors of this article consider placental blood better than adult's blood because of the absence of food allergies; the clotting power is greater, thus, its advantages in the case of hemorrhages. The best anticoagulant, ac-

cording to these workers, is citrated saline solution, which obviates marked hemolysis in prolonged standing. "Contamination can be avoided by rigid vigilance."

Howkins and Brewer[15] report a 22 per cent contamination in samples of placental blood stored at low temperatures. The organisms more commonly found were *B. subtilis*, *B. coli*, *Staph. albus* and *B. pyocyanus*. These investigators decided the procedure to be uneconomical and unsafe, and recommended it to be used by trained operators.

MATERIALS AND METHODS

Blood was obtained from the umbilical cord by the method devised by Goodall et al.[6] The blood was collected in a flask marked at 65 cc. with enough sodium citrate solution to make a total concentration of 2.5 to 3 per cent solution of the preservative in the blood. The flask was shaken gently in order to mix the blood with the preservative as thoroughly as possible, and was then covered with sterile gauze as soon as it was collected. Then the blood was transferred to 16 different 5 cc. sterile bottles (4 cc. in each bottle) which were then stoppered tightly and transferred to the refrigerator. The temperature was kept at between 4° to 6° C. White cell counts, differential counts, red blood cell counts, fragility test, platelet counts were done, and prothrombin time estimated, in each of the separate samples; each one corresponding to a separate day and the sample discarded as soon as the calculations had been made. A sample was used to make the estimations outlined above not more than two hours after the collection of the blood, and then was considered a normal, fresh sample. For 15 consecutive days a daily study of the stored blood was made. When the calculations were to be made, the sample bottle was gently shaken in order to mix the contents and prevent marked hemolysis. Three sets of specimens were obtained from three different normal placentas. They were studied for fifteen consecutive days, having first been found to be free of communicable diseases. In each case the baby had weighed more than 7 lbs., and the bag of water was intact until immediately previous to delivery. The hemoglobin calculations of the mothers by the Hellige Wintrobe method ranged between 72 per cent and 86 per cent. The initial calculations of hemoglobin in the three sets of samples were as follows:

Set No. I	116%	16.8 gms.
Set No. II	108%	15.6 gms.
Set No. III	112%	16.2 gms.

Red and white blood cell counts were performed in pipettes for red and white cell counts certified by the Bureau of Standards. The diluting fluids used were: Hayem's solution and .1 per cent Hcl. Smears for differential counts and for cytological studies were stained with Wright's stain. Howell's method was used in calculation of the Prothrombin Time.* The fragility test was made in a set of solutions ranging from .6 per cent NaCl to .2 per cent NaCl, with difference in concentration of .025 per cent NaCl. Twenty solutions were used in each test, and small test-tubes were used for that procedure. Results were read at the end of 2 and 24 hours. Platelet counts were done by the direct method.

OBSERVATIONS

1. Changes Occurring in the Erythrocytes

Gross appearance of samples showed evidence of marked hemolysis after the eighth day, when the supernatant fluid or plasma in the bottles became deeply pink. This finding was not in accord with the erythrocyte counts, because, although there was a decrease in the number of red blood cells, yet it could not account for the apparent high degree of hemolysis. (Chart I). Cytological studies in smears stained with Wright's stain revealed a tendency towards anisocytosis and poikilocytosis. Macrocytosis was noticed after the 4th or 5th day. During the last days of the experiment the cells varied tremendously in size and shape. Abundant nucleated red blood cells were seen during the first few days, but very few could be seen after the 8th day.

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* Prothrombin Time (*Howell's Method*):

1) Obtain about 2 cc. of blood from a vein, using a syringe which has been rinsed out with physiologic salt solution.
2) At once place the blood in a centrifuge tube which contains .25 cc. of 1% sodium oxalate in physiologic salt solution.
3) Mix by inverting and centrifugalize thoroughly.
4) Place 5 drops of the clear plasma in each of four test tubes.
5) To these tubes add .5% solution of calcium chloride in increasing quantities: 2 drops in tube 1; 3 drops in tube 2; 4 drops in tube 3; and 4 drops in tube 4. Mix gently.
6) Coagulation will probably occur in all tubes, but not at the same rate. Its occurence is recognized by inverting the tube. The coagulation time of the tube which clots earlier is the "prothrombin time."

Note: In our work we used stored placental blood and the procedure outlined above was followed with some modification.

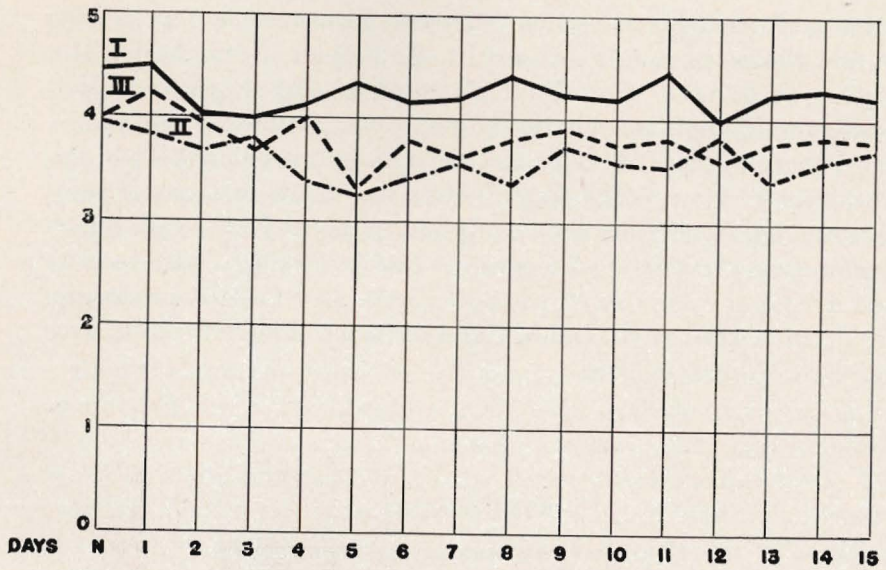

CHART I. Red blood cell counts in millions per cu. mm.

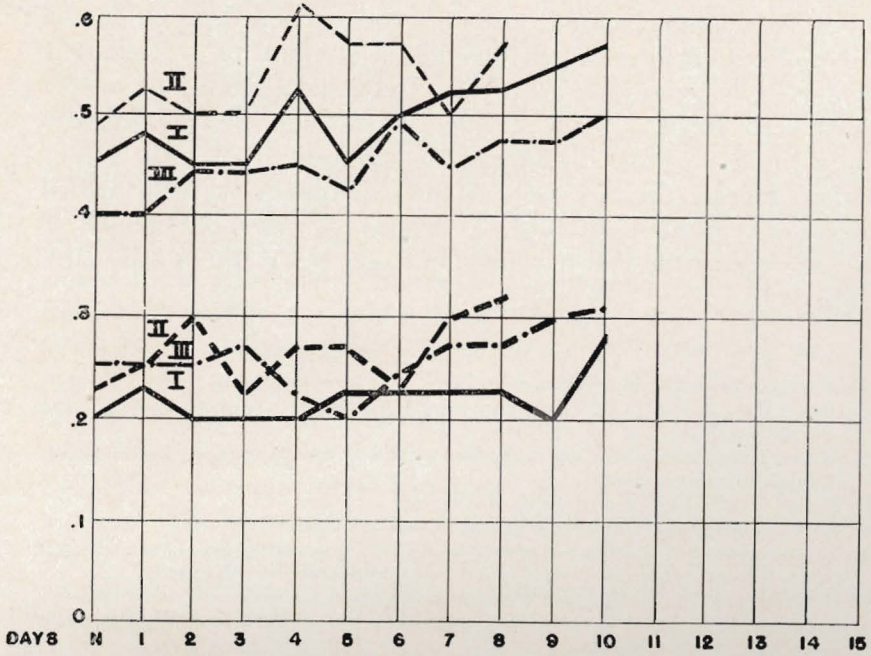

CHART II. Fragility of the erythrocytes calculated in tenths per cent of sodium chloride. Curves above indicate where hemolysis started. Curves below indicate where hemolysis was completed.

2. Fragility of the Erythrocytes

The fragility of the red blood cells markedly increased as the days elapsed, and after the 8th to 10th day, there was hemolysis at all concentrations demonstrating a low degree of resistance of the red blood cells. (Chart II).

3. The Leukocytes

Total Counts. There was a steady drop in the leukocyte count after the third day. In set of samples No. 1, whose initial count was 15,600, the destruction was marked and by the end of the 15th day it had decreased to 5,500. In set of samples No. II, the initial count was 4,500 and the final calculation was 400. In set of samples III the count decreased from 5,150 to 3,200. (Chart III).

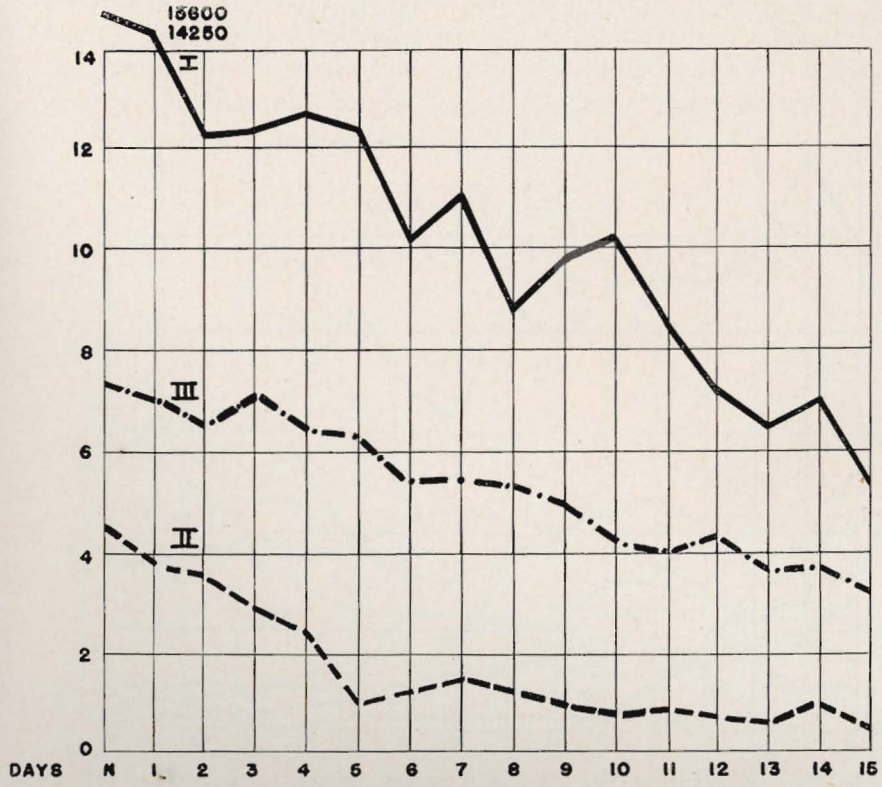

CHART III. Total leukocyte counts in thousands per cu. mm.

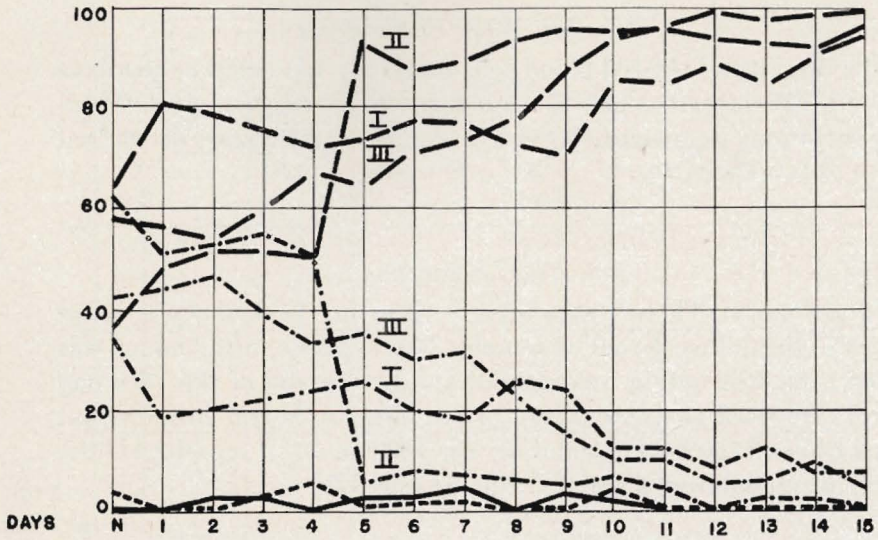

CHART IV. Relative differential leukocyte counts in percentages.
Lymphocytes — — — — — Monocytes ————
Eosinophiles - - - - - - - - Polys. — · — — · —

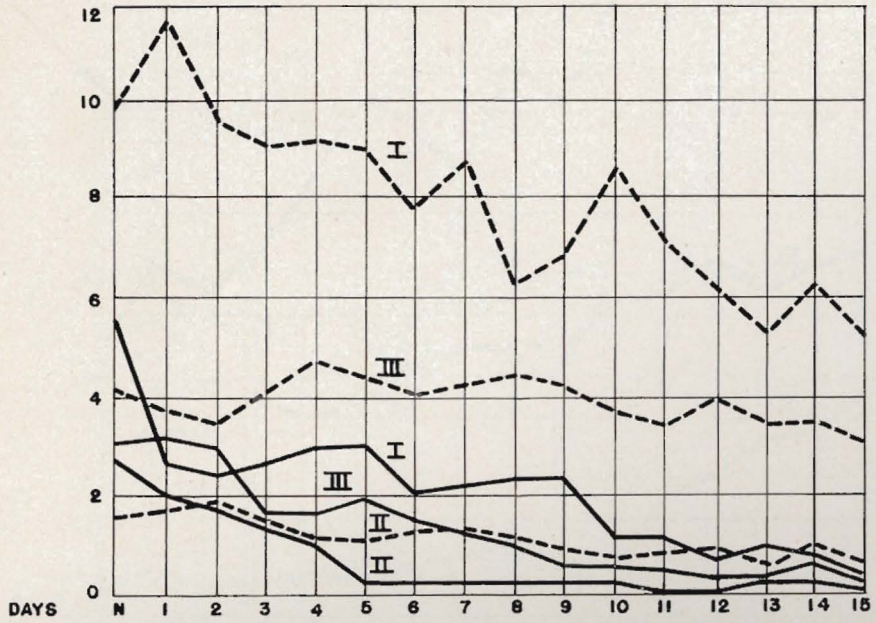

CHART V. Actual leukocyte counts in thousands per cu. mm.
Lymphocytes ———— Polymorphs. - - - - - - - - -

Differential Counts (Relative). There is a marked decrease in the percentage of polymorphonuclear leukocytes with a corresponding increase in the percentage of lymphocytes. Therefore, the marked increase in percentage of lymphocytes as the days passed was at the expense of the polymorphonuclears, but this is a *relative* and not an absolute or actual happening. (Chart IV).

Actual Leukocyte Counts. There is a decrease in number of both the lymphocytes and the polymorphonuclears. The polymorphonuclears, nevertheless, showed actually more evidence of destruction, but not to such a marked degree as would be thought by the curves obtained with differential calculations. (Chart V).

4. Cytological Studies

The lymphocytes maintain their integrity quite well. During the last few days, it was observed that these cells became smaller and more deeply basic on staining. The plymorphonuclears were destroyed with much more ease. The cell suffered clumping of the nuclear segments. The nuclei showed a "moth eaten" appearance, and became quite small. The nuclei was destroyed, and on disappearing degenerated, non-nucleated segments could be seen. Undoubtedly, a process of necrobiosis took place.

5. Platelet Counts

A steady decrease in the number of platelets took place. (Chart VI).

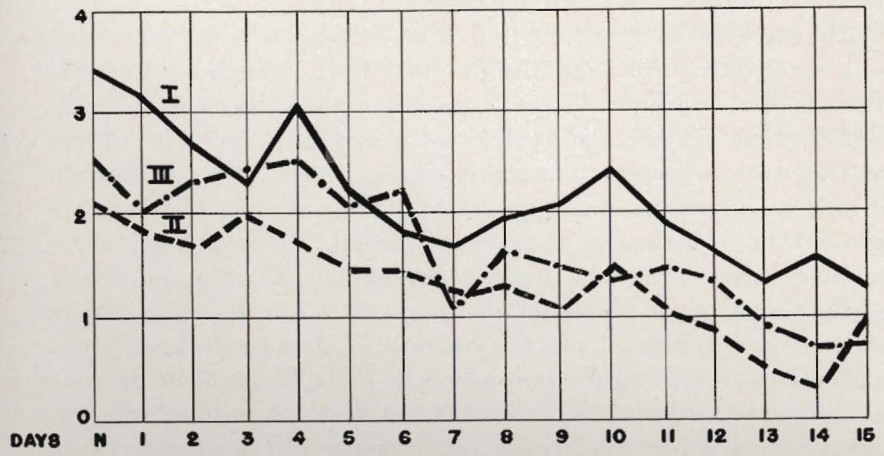

CHART VI. Platelet counts in thousands per cu. mm.

6. Prothrombin Time

Howell's calculations,[16] place 10 minutes or less as normal. In placental blood, even during as many as 15 days of storage, the prothrombin time remains quite low, demonstrating that the thrombotic powers possessed are quite marked. In the chart below, the reader will find all variations occurring in the three separate sets of samples during the 15 days of storage.

(*Time in minutes and seconds*)

	SAMPLE I	SAMPLE II	SAMPLE III
Day	2: 45 (Normal)	2 (Normal)	2:5 (Normal)
1	2	2:15	2:25
2	2:15	2:15	2:0
3	2:5	2:5	2:75
4	3:5	3:5	3:25
5	3:0	3:0	3:50
6	3:25	3:5	3:25
7	3:15	3:15	3:30
8	3:45	3:55	3:50
9	3:0	3:45	4:00
10	4:50	4:15	4:30
11	5:0	4:5	5:00
12	5:0	5:15	4:45
13	5:0	5:30	5:50
14	5:30	6:00	5:25
15	6:0	5:30	5:50

DISCUSSION

With the idea in mind to find how storage affected placental blood, three different sets of samples have been studied for 15 days. Although there is a great deal of literature on the subject of placental blood, there is yet much to be learned about its properties. Therefore, this work is presented as a preliminary report. Up to this time the medical literature has been mainly interested in the benefits derived from transfusion of placental blood. Possibly such overwhelming interest may be slightly dangerous! There is no doubt that the properties of placental blood are efficacious in many abnormal conditions, but much attention should be paid to the techniques of collection, storage, and administration of the blood. The procedure of administration, as many other emergency procedures, may on some occasions have fatal results. An analysis of the literature has been made, and the high points been stressed. Although the general opinion of experts is overwhelmingly in favor of the practice of placental blood transfusion, yet there are some authorities who consider the practice dangerous, and at the same time, useless.

SUMMARY

1. The literature has been reviewed on methods of collection, storage and preservation of placental blood for the object of immediate and delayed transfusions. The incidence of successful results from this practice has been emphasized. The dangers of contamination and bacterial growth have been considered.

2. A study of placental blood in storage at 4 to 6° C. has been made for a period of 15 days.

3. Although, grossly, there is evidence of marked hemolysis, this finding is not in accord with the erythrocyte counts for 15 days. The cellular preservation would indicate its use for transfusion, if the blood could be kept sterile.

4. After the 8th to 10th day, the red blood cells become fragile, as evidenced by hemolysis occurring in all concentrations.

5. The red blood cells show tendency to poikilocytosis, anisocytosis and macrocytosis, as the time of storage is prolonged.

6. The leucocytes decrease steadily in numbers as the days elapse.

7. The polymorphonuclear leukocyte is less resistant to storage than the lymphocyte.

8. A marked and steady decrease in the number of platelets occurred during storage.

9. The prothrombin time calculated by Howell's method, demonstrates beyond any doubt that the thrombotic powers of placental blood are extremely high even during prolonged storage.

10. After 15 days of storage there are enough cellular contents and enough thrombotic powers to make placental blood fit for transfusion.

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