Acta Veterinaria(Beograd), Vol. 46, No.2-3, 87–94, 1996.

UDK 619:612.11:636.7

CHANGES IN THE NUMBER OF CELL ELEMENTS IN CANINE BLOOD DURING PRESERVATION

VERA SAVIĆ-STEVANOVIĆ

Faculty of Veterinary Medicine, Belgrade, Yugoslavia

(Received, 11. January 1996.)

The aim of the investigation was to establish the degree of alteration in the quantitative relationship of formed elements of preserved blood in relation to the duration of storage.

In samples of preserved canine blood the erythrocyte count drops negligibly in relation to the time of refrigerated storage. A significant drop in erythrocyte count was established only on days 20 and 21 ($p<0.001$).

Data on the average leucocyte count in the investigated samples of preserved blood show that this parameter remains stable without any significant change for a long period of time during storage. At the beginning of the investigation the average leucocyte count in preserved blood was $9.960 \pm 1.359 \times 10^9$/l, and no significant changes in the leucocyte count occurred until day 20 of preservation when the value was $8.515 \pm 1.084 \times 10^9$/l, which was significantly lower than the initial value ($p<0.05$). A highly significant drop in the number of leukocytes in preserved blood occurred on day 21 when the average number was $8.194 \pm 0.937 \times 10^9$/l ($p<0.01$).

The number of thrombocytes dropped continuously during storage. The drop in thrombocyte count became significant on day 3 when it was $132.364 \pm 10.628 \times 10^9$/l ($p<0.001$). From day 3 to day 11 the thrombocyte count was significantly below the value at the beginning of the investigation. Namely, there was decrease of 30-40%. The most prononced difference in thrombocyte count occurred on days 20 and 21 when there were 53.14% of the number established at the beginning of the investigation.

Key words: canine, preserved blood, ACD stabilizer, erythrocytes, leukocytes, thrombocytes

INTRODUCTION

Due to its complex biochemical composition and the cell elements it contains, during each moment of storage, the biological value of blood changes,

and the effect is not the same if a patient affected by some disease is given blood on its first or fifteenth day of preservation. During preservation blood undergoes alterations and aging. The limited shelf life of blood is a consequence of these changes, the intensity and speed of which directly depend on the composition of the stabilizer and the conditions of storage.

The aim of preservation is to reduce these changes or to postpone them for as long as possible. For this reason, and in order to maintain the biological value of blood for as long as possible, the most widely used stabilizers are acid-citrate-dextrose (ACD) and citrate - phosphate - dextrose (CPD) which contain glucose as the main source of energy for formed blood elements, especially erythrocytes. In order to slow down changes the temperature of storage is very important, from the moment when blood is drawn until the moment of transfusion. The temperature during preservation must be constant and numerous authors such as Penny. (1953), Komazak and Sova (1963), Combrisson et al., (1964), Grünbaum (1968) and Eisenbrandt and Smith (1973) recommend a temperature of $+4^0$C during this period.

For preserved blood it is of the utmost importance that no significant changes occur among its individual cell elements, especially erythrocytes which are the most numerous, and at the same time the most constant cell element. Their vitality determines the rate of transport of oxygen to tissues, i. e. tissue oxygenation.

MATERIALS AND METHODS

Research was carried out on 11 German Shepherd dogs. The dogs were approximately the same age (2 to 2.5 years), and had approximately the same weight (30 to 35 kg).

Blood samples were drawn into glass bottles into which 18.5 ml of sterile, non-pyrogenic stabilizer (ACD) for 125 ml of blood had been previously placed. Using a sterile needle and a blood flow system, blood was drawn from the jugular vein into the glass bottle and stored at + 4^0C.

The following cell elements were determined in preserved blood: erythrocyte count, leucocyte count and thrombocyte count, using a "Hi-Technicon" hematological analyzer.

RESULTS AND DISCUSSION

The data on mean values of erythrocyte counts in samples of preserved blood, stored for 21 days at + 4^0C and parameters showing the deviatioin from mean values are presented in Table 1.

Table 1. Erythrocyte counts in preserved canine blood (x 10^{12}/l)

Day	n = 11					
	x	SD	SE	CV%	t	p
00	5.807	0.280	0.089	4.829	—	—
01	5.690	0.334	0.106	5.870	0.845	>0.05
02	5.734	0.346	0.109	6.034	0.519	>0.05
03	5.734	0.315	0.100	5.502	0.545	>0..05
04	5.683	0.316	0.100	5.556	0.926	>0.05
05	5.761	0.303	0.096	5.261	0.351	>0.05
06	5.719	0.282	0.089	4.930	0.699	>0.05
07	5.780	0.296	0.094	5.121	0.209	>0.05
08	5.735	0.283	0.089	4.929	0.572	>0.05
09	5.772	0.290	0.092	5.023	0.273	>0.05
10	5.689	0.288	0.091	5.068	0.927	>0.05
11	5.655	0.295	0.093	5.218	1.181	>0.05
12	5.706	0.335	0.106	5.874	0.730	>0.05
13	5.705	0.352	0.111	6.164	0.717	>0.05
14	5.649	0.379	0.120	6.717	1.058	>0.05
15	5.617	0.357	0.113	6.361	1.321	>0.05
16	5.650	0.311	0.098	5.500	1.186	>0.05
17	5.645	0.322	0.102	5.701	1.197	>0.05
18	5.610	0.287	0.091	5.107	1.548	>0.05
19	5.561	0.286	0.090	5.142	1.944	>0.05
20	5.463	0.295	0.093	5.384	2.517	<0.05*
21	5.330	0.212	0.067	3.982	4.282	<0.001***

The results indicate that the erythrocyte count decreases as the period of preservation grows longer. However, a the drop in the number of erythrocytes did not become significant until day 20 and 21 of the investigation ($p<0.001$) which means that most canine erythrocytes endure preservation conditions well until day 20.

Many other authors also found a mild drop in erythrocyte counts in ACD canine blood Mollison, 1975; Muto, 1983; Owen Holmes, 1972).

Muto, (1983) established that, after a relatively rapid drop in erythrocyte counts during the first two days of storage, there is a second phase of slow erythrocyte degeneration, during which around 0.4% of the erythrocytes decompose daily. It is certain that the drop in the erythrocyte count in blood during prolonged storage is a consequence of hemolysis, which first occurs in the least resistant erythrocytes, and this author showed, amounted to 0.4% of the erythrocytes per day of preservation.

In our research the decrease in erythrocyte numbers was significant on day 20 and 21 compared to the initial value. This drop amounted to around 10%. Since erythrocytes are functionally the most important component of preserved blood,

we can indisputably say that this small drop in erythrocyte count should not be a reason to shorten the length of preservation of canine blood, and similarly as for human blood, to heed the suggestion that the maximum period of preservation which is evaluated only from changes in erythrocyte counts should be 21 days.

Our research also covered monitoring the leucocyte count and thrombocyte count in preserved canine blood.

Mean values of leucocyte counts in samples of preserved canine blood and other statistical variation parameters are presented in Table 2.

Table 2. Leucocyte counts in preserved canine blood (x 10^9/l)

Day	n = 11					
	x	SD	SE	CV%	t	p
00	9.960	1.359	0.430	13.649	—	—
01	9.742	1.220	0.386	12.526	0.377	>0.05
02	9.816	1.202	0.380	12.244	0.251	>0.05
03	9.928	1.137	0.360	11.452	0.057	>0..05
04	9.760	1.172	0.371	12.011	0.352	>0.05
05	9.761	1.231	0.389	12.611	0.343	>0.05
06	9.824	1.289	0.407	13.117	0.230	>0.05
07	9.729	1.179	0.373	12.123	0.406	>0.05
08	9.687	1.141	0.361	11.779	0.486	>0.05
09	9.737	1.229	0.389	12.619	0.358	>0.05
10	9.804	1.154	0.365	11.770	0.277	>0.05
11	9.772	1.264	0.400	12.931	0.320	>0.05
12	9.707	1.233	0.390	12.702	0.436	>0.05
13	9.745	1.205	0.381	12.362	0.374	>0.05
14	9.685	1.288	0.407	13.295	0.464	>0.05
15	9.581	1.250	0.395	13.044	0.649	>0.05
16	9.460	1.180	0.373	12.473	0.878	>0.05
17	9.345	1.117	0.353	11.984	1.105	>0.05
18	9.218	1.204	0.381	13.063	1.292	>0.05
19	8.937	1.144	0.362	12.801	1.820	>0.05
20	8.515	1.084	0.343	12.733	2.627	<0.05*
21	8.194	0.937	0.296	11.439	3.383	<0.01**

The results show that the leucocyte count in the investigated samples of preserved blood did not significantly change until day 20, when the count was 8.515 with a standard deviation of 1.084 x 10^9/l, which is significantly lower than the initial value ($p<0.05$).

Beksedić et al., (1963) established that the most pronounced drop in the leucocyte count of human blood stored in various preservatives (ACD, CPD) occurred during the first week of storage, while the subsequent drop was significantly less pronounced. This indicates that canine leukocytes endure

preservation conditions considerably better than human leukocytes, and that their number is not significantly altered until day 20 of preservation.

In the investigated blood samples, apart from determining the leucocyte count, we did not monitor other parameters which would enable us to judge possible alterations of the functional activity of leukocytes.

The data on thrombocyte counts in the investigated samples of preserved canine blood are presented in Table 3.

Table 3. Thrombocyte counts in preserved canine blood (x 10^9/l)

Day	n = 11					
	x	SD	SE	CV%	t	P
00	215.364	38.303	12.113	17.785	—	—
01	205.909	39.851	12.602	19.354	0.541	>0.05
02	182.636	36.307	11.481	19.880	1.961	>0.05
03	132.364	10.628	3.361	8.030	6.603	<0.001***
04	134.000	4.621	1.459	3.442	6.669	<0.001***
05	134.727	3.193	1.010	2.370	6.634	<0.001***
06	136.545	6.128	1.937	4.486	6.425	<0.001***
07	135.727	8.760	2.770	6.454	6.409	<0.001***
08	135.273	3.863	1.222	2.856	6.579	<0.001***
09	129.091	13.392	4.235	10.374	6.723	<0.001***
10	127.455	10.121	3.200	7.941	7.017	<0.001***
11	126.000	10.905	3.448	8.654	7.096	<0.001***
12	121.364	7.413	2.344	6.108	7.619	<0.001***
13	121.818	10.373	3.280	8.515	7.454	<0.001***
14	119.182	7.284	2.303	6.112	7.801	<0.001***
15	121.000	8.246	2.608	6.815	7.616	<0.001***
16	115.182	7.359	2.327	6.389	8.122	<0.001***
17	113.091	5.334	1.687	4.716	8.363	<0.001***
18	114.273	3.645	1.153	3.190	8.308	<0.001***
19	111.727	5.011	1.585	4.485	8.484	<0.001***
20	106.455	4.314	1.364	4.053	8.953	<0.001***
21	100.909	2.968	0.939	2.941	9.421	<0.001***

Unlike the erythrocyte and leucocyte count, the thrombocyte count in ACD blood dropped significantly early during preservation. The drop was first significant on day 3. From day 3 to day 11 the number of thrombocytes became significantly lower by 30% to 40% as compared to the value at the beginning of the investigation. The most pronounced drop in thrombocyte count, (53.14% compared to the count on day 0) was established on days 20 and 21. Beksedić. (1963) and Jakubec, (1965) while investigating the thrombocyte count in preserved human blood, noted that the drop in the number of thrombocytes was most pronounced during the first three days of storage (up to 60%), after which

the number of thrombocytes gradually fell until day 7. These authors state that even after 21 days of storage the loss of thrombocytes was not complete (around 20% remain).

We did not evaluate the functional activity of thrombocytes in the investigated blood samples. The point which is evident in our investigation is the decrease in the thrombocyte count by over 50% up to days 20 and 21 of preservation. We can presume, with a high level of security, that the thrombocytes which are left in the blood are not as functionally active as they were during the first few days of preservation.

CONCLUSION

Based on the results of our research, the following conclusions are possible:

— in samples of canine blood preserved in ACD solution, stored 21 days at + 4^{0}C, the erythrocyte count is not significantly altered until day 20 of preservation,

— conditions of preservation in ACD solution do not result in significant changes in the leucocyte count until day 20, while the thrombocyte count drops significantly by contuining on day 21 of preservation the thrombocyte count is 53.14% lower than the count established on day 0 of the investigation.

REFERENCES

1. *Beksedić J., Jeremić S., Kostić M.* 1963. *Bilten transfuzije,* 11-16.
2. *Combrison-Le Bolloc H. A., Jourdanneau, J. C., Marcenac, P. et Houset, E.* 1964. Etude de volume danguin et de da duree de vie des hematites de chien. *Prese Med., 72,* 2775-2776.
3. *Eisenbrandt, D. L. and Smith, J.* 1973. Use of biochemical measures to estimate cells in canine blood stored in acid citrate dextrose solution, with and without added ascorbic acid. *J. A. V. M. A., No. 8,* 984-987.
4. *Grünbaum, E. G.* 1968. Möglichkeiten und Grenzen der Bluttransfusion beim Hund. *Vet. Med. Habilschr., Leipzig.*
5. *Jakubec Z.* 1965. IV Čuvanje konzervisane krvi i promene u njoj u toku konzervisanja i čuvanja. *Transfuzija krvi.*
6. *Komazek, J. and Sova, Zd.* 1963. Veranderungen an Blutkonzerven von Rindern, Pferden und Hunder während der Lagerung. *Arch. Exper. Vet. Med., 17,* 819-825.
7. *Mollison, P. L.* 1975. Blood transfusion in clinical medicine. *IV Ed. Blackwell Scientific Publication, Oxford and Edinburgh.*
8. *Muto, B.* 1983. Untersuchungen über das Verhalten von erythrozytenzah., Hämatokritwert, Kaliumgehalt in Blutkonzerven von Hund. *Inaugural Disertation, Universität Giesen.*
9. *Owen, R. R. and Holmes P. H.* 1972. An assessment of the viability of canine blood stored under normal veterinary hospital condition. *Vet. rec. 90.* 231-235.
10. *Penny, S. G.*1953. Blood substitutes or plasma extenders for small animal practice. *Vet. Med., 48,* 151-156.

PROMENA BROJA ĆELIJSKIH ELEMENATA KRVI PASA U TOKU KONZERVISANJA

VERA SAVIĆ-STEVANOVIĆ

SADRŽAJ

U krvi pasa čuvane 21 dan u ACD rastvoru na +4^{0}C ispitivan je broj eritrocita, leukocita i trombocita.

Na osnovu rezultata postignutih u radu zaključak je da se u uzorcima konzervisane krvi pasa broj eritrocita značajno ne menja do 20. dana konzervisanja. Zatim, uslovi konzervisanja krvi u ACD rastvoru ne dovode do značajne promene broja leukocita, dok broj trombocita značajno opada od 3. dana konzervisanja i 21. dana broj trombocita je manji za 53, 14% u odnosu na broj utvrđen nultnog dana ogleda.