Mem. Inst. Butantan,
28: 229-236. 1957/8.

ABSORPTION OF FLUID BY RED BLOOD CELLS AND HEMOLYSIS IN SHOCK INDUCED BY TOURNIQUET*

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Coonse et al (1) observed hemolysis in dogs submitted to traumatic shock but he failed to observe it in those dogs submitted to hemorrhagic shock. Rosenfeld (2) observed that shock induced by histamine, trypsin, peptone, bradykinin and bothropic venom provoked in dogs an increase in mean corpuscular volume accompanied by an increase of the fragility of erythrocytes and hemolysis. These red blood cell alterations were not due to the substances injected because, with exception of bothropic venom, none of them modified the red blood cells "in vitro", even in concentrations higher than those injected. Rosenfeld concluded that the liquid absorption by the erythrocytes with the consequent alteration were not due to the substances injected but to the shock conditions induced by them. By this mechanism the red blood cells are responsible for the disappearance of a great part of the plasmatic liquid loss peculiar to shock.

According to these facts, shock induced by other means would also provoke these red blood cells alterations. In the present paper the modifications that occur in shock induced by tourniquet are studied.

MATERIAL AND METHODS

To dogs anesthetized with Nembutal by intraperitoneal route (35 mg per kg of body weight), a wire torniquet was fastened tight on a hind leg and mantained for six hours or more.

The first blood sample was collected from the femoral vein of the other hind leg while under anesthesia, before the tourniquet fitting, immediatly before torniquet removal, and 5, 15, 30 and 60 minutes thereafter. 5 ml of blood, with 0.1 mg of heparin per ml were collected at each time.

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* This study was supported by the Anastacio Paschoal and M. Pedro Fellowship, and by the Conselho Nacional de Pesquisas. Part. of this paper has been presented at the IV International Congress of the International Society of Hematology, 1952.

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For erythrocyte counting the blood sample was first shaken mechanically for 2 minutes by means of the shaker usually employed for Kahn reaction and then diluted. In order to obtain better results, the pipette containing the sample and diluting fluid was shaken mechanically for 1 minute by means of a standard shaker. All cells present in an area of 0.2 mm^2 of the hematimeter were counted.

For hematocrit determinations, the blood samples were centrifuged in a Wintrobe tube, for 15 minutes at 4.000 rotations per minute.

Hemolysis was qualitatively estimated in the supernatant plasma of the hematocrit. In one case the absolute hemoglobin quantity was determined in a spectrophotometer. Other hematologic data were studied and will be published elsewhere. Blood pressure from the carotid artery was recorded by the usual method.

RESULTS

Data from seven dogs are presented in Table 1. With exception of 1 R all showed hemoconcentration. In dog 1R which showed hemodilution, the tourniquet was maintained for 18 hours, while in the others it was maintained for 6 hours.

All but one dog showed hematocrit increase in a proportion larger than the increase of red blood cells thus indicating a mean corpuscular volume increase.

Hemolysis occurred in 6 dogs. In all cases it appeared only after tourniquet removal and remained till the end of the experiment. Free plasma hemoglobin of dog 2-52 Cg reached 0,528 g/100 ml, amounting to 2,9% of total hemoglobin.

Graph 1, prepared with the mean values presented in table 1, indicates that hemoconcnetration appeared after tourniquet fitting and that the hema-

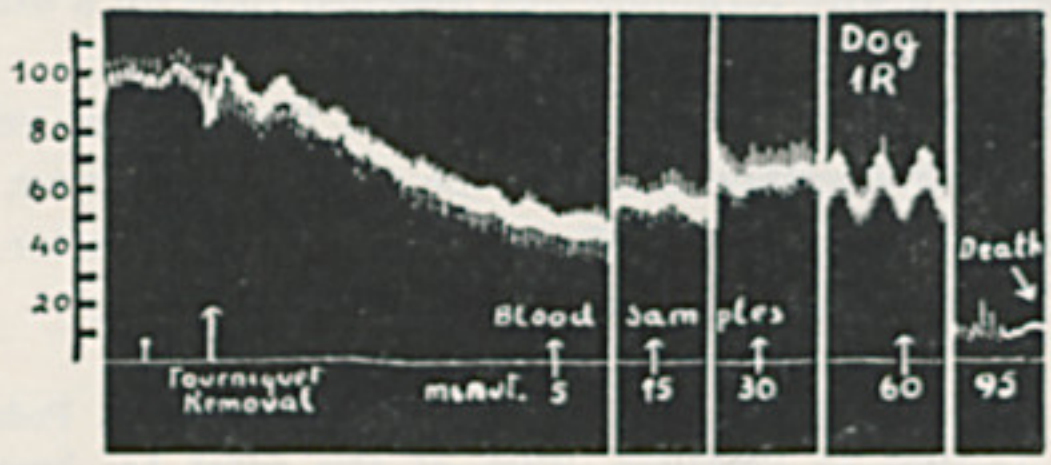


FIG. 2 — Arterial blood pressure recording of dog 1 R. exposed to tourniquet for 13 hours.

TABELA 1

RED BLOOD CELL COUNTS, HEMATOCRIT AND HEMOLYSIS DURING THE TOURNIQUET SHOCK IN DOGS

The values recorded under the heading "before tourniquet removal" were obtained from samples taken just before releasing the tourniquet. Hemolysis was assessed rougly by the color of the supernatant plasma obtained during the hematocrit determinations
In case of dog 2-52-Cg the concentration of plasma hemoglobin was determined photometrically
RBC = Red blood cell count' Ht = Hematocrit; MCV = meango corpuscular volume

DOG	Time of exposure to tourniquet	Before the tourniquet application				Before the tourniquet removal				TIME IN MINUTES AFTER THE REMOVAL OF THE TOURNIQUET													
										5				15				30				60			
		RBC X 10^6	Ht %	MCV μ^3	Hemo-lysis	RBC X 10^6	Ht %	MCV μ^3	Hemo-lysis	RBC X 10^6	Ht %	MCV μ^3	Hemo-lysis	RBC X 10^6	Ht %	MCV μ^3	Hemo-lysis	RBC X 10^6	Ht %	MCV μ^3	Hemo-lysis	RBC X 10^6	Ht %	MCV μ^3	Hemo lysis
2 C	5 hours Killed	5.7	45.2	78.3	0	6.3	46.8	73.3	0	6.1	48	78.7	0	6.1	48.5	80	+	6.0	49.5	82.5	+	6.0	51	85	+
3 C	5 hours Killed	6.1	44	72.1	0	7.1	54.2	76	0	7.3	54	74	0	6.4	54	84.3	0	6.7	55.3	82.5	+	6.6	54.4	82.4	+
1 C	6 hours Killed	5.8	41.6	71.3	0	6.6	51.5	82	0	7.8	54.4	69.4	0	6.9	50.2	72.7	++	6.7	52	77.6	++	6.5	48.4	74.4	++
4 C	4.40 hours Killed	4.8	36	75	0	5.4	45	83	0	6	49.9	83.1	+++	5.9	50	83	+++	6.3	50.5	80	++++	6.3	50	79.2	++++
													0.184				0.332				0.304				0.526
2-52-Cg	6 hours Killed	6.6	49.3	74.6	0	7.5	56.9	74.8	0	8.7	61.4	70.5	++	8.5	61.7	71.7	+++	8.6	63.2	72.5	+++	8.1	3.2	78	++++
5 C	5 hours Killed	4.8	40.4	84	0	5.6	42.6	76	+	5.4	44.5	82	++	4.8	40	82	++	5.0	40	80	+++	5.2	41.9	81	+++
1 R	13 hours Died	6.8	48.5	71.5	—	6.0	47	77.3	—	6.0	46	75.7	—	—	—	—	—	6.3	47.5	75.5	—	6.0	46.5	77	
AVERAGES		5.80	43.5	75.2		6.35	49.1	77.5		6.76	51.1	76.2		6.43	50.7	78.9		6.51	51	78.6		6.40	50.8	79.6	

tocrit increase was greater than that of the erythrocyte countings, thus showing an increase in the mean corpuscular volume.

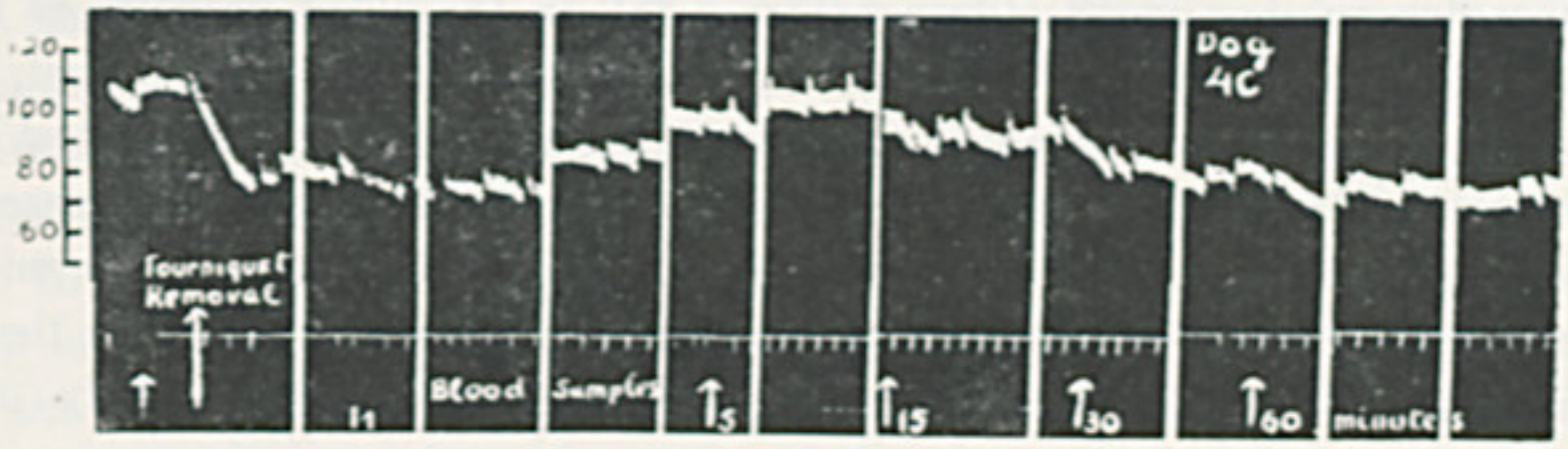
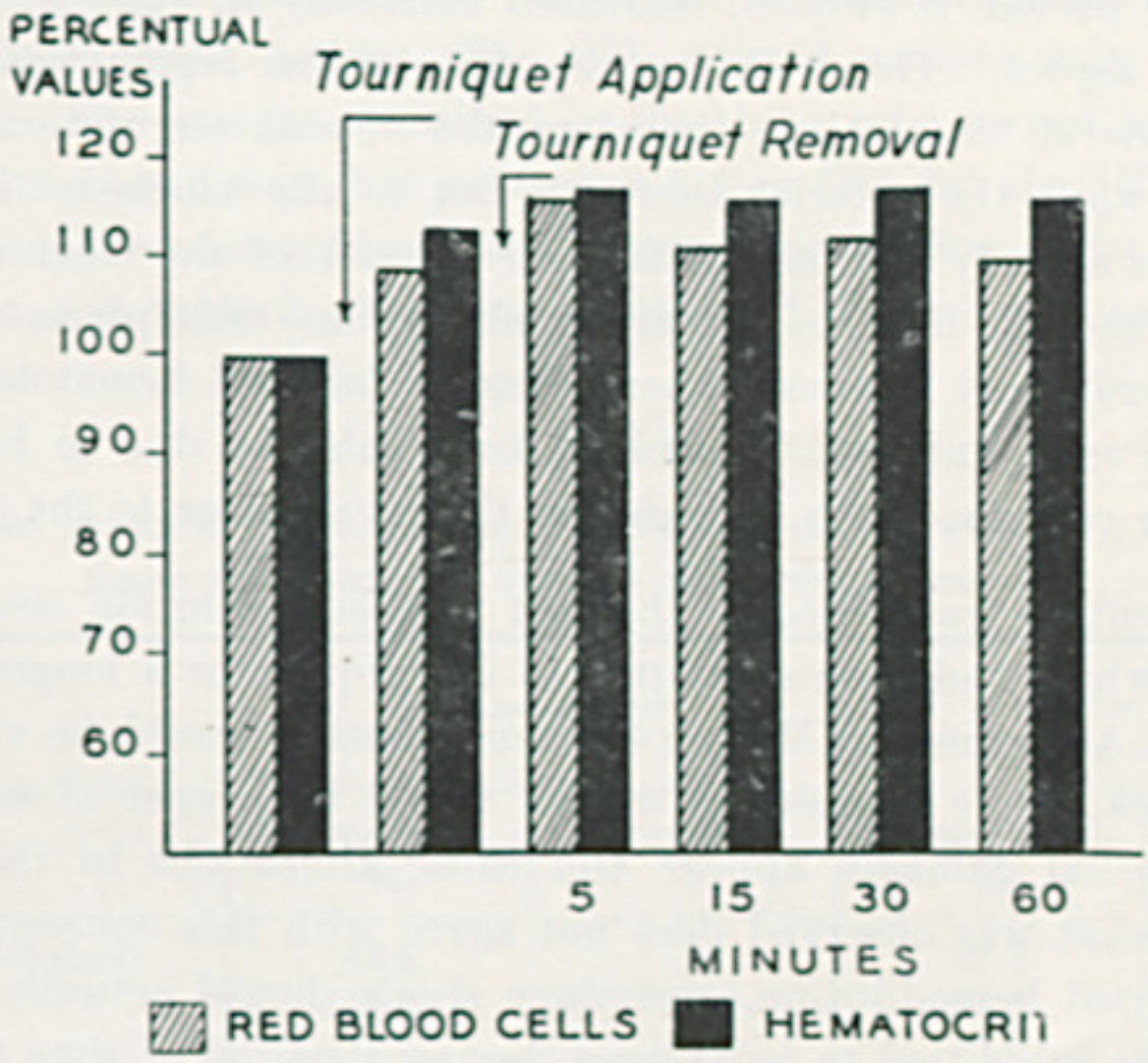


FIG. 1 — Arterial blood pressure recording of dog 4 C, exposed to tourniquet for 4.4 hours.



GRAPH 1 — Percent changes of red blood cell count and hematocrit of dogs exposed to tourniquet. The graph was prepared by averaging the results included in table 1.

In all cases the blood pressure fell soon after removal of the tourniquet, but remained normal while the latter was on. This can be seen from figures 1 and 2; it must be mentioned that the former is a record of blood pressure of dog 4 C, which was exposed to tourniquet for 4.4 hours, while the latter belongs to dog 1 R, exposed to tourniquet for 13 hours.

DISCUSSION

Tourniquet fitting is sufficient to produce hemoconcentration and mean corpuscular volume increase even before a fall in the blood pressure as it can be seen from the Fig 1 and 2 and table 1. On removing the tourniquet, a fall in the blood pressure was noted and 5 minutes thereafter the described modifications were found to be more intenses, the corpuscular volume increased and hemolysis appeared. The alterations remained during the next 60 minutes, period of time in which observations where done. It would seem that the tourniquet induces hematological alterations peculiar to shock probably due to blood stasis in the neighborhood of the place where the tourniquet was fitted.

As reported in this paper hemoconcentration appeared even with the tourniquet on, a phenomenon which cannot be explained by elimination of the circulation through the leg to which the tourniquet was fitted, since during this experimental period, it swelled, increased markedly in volume, and acquired a hemorrhagic aspect. This indicates that the swollen region was drawing blood from the general circulation; therefore the venous circulation was probably interrupted while the arterial flow was not totally blocked. Besides, at this stage the was already a trend towards an increase of the mean corpuscular volume which showed that more marked alterations took place. Hemolysis appeared on toruniquet removal, when pressure fall and hematological modifications become more intense. Hemolysis could also be due to hemoglobin liberation by the red blood cells demaged in the region near to the tourniquet.

Our attention was called to the fact that hemodilution occurred specially in dog 1 R, which had been submitted to tourniquet for a longer time than the other dogs. According to Moon (4), hemodilution would be characteristic of primary shock, while hemoconcentration would be proper of secondary shock, when endothelial damages appear and cause alterations in the liquid equilibrium, but what was observed does not agree with this concept. In dog 1 R, which presented hemodilution, secondary shock should actually have occurred because it was submitted to tourniquet during a period of time three times longer than others and it was the only dog that died spontaneously. Besides, the noticeable increase of the mean corpuscular volume showed that an alteration of red blood cell permeability had occurred, probably by the same mechanism that affects the endothelium of the blood vessels.

These results showed that in shock induced by tourniquet there is an increase in red blood cell volume by fluid absorption and consequent hemolysis. These alterations occur even before the arterial pressure falls, and are probably due to stasis or maceration of the tissues adjacent to the place where the tourniquet was placed.

In order to see if the increase of the red blood cells volume could be due to an increase of the CO_2 concentration with consequent a fluid absorption, the following experiments were done:

A sample of venous blood of dog taken with Ethylenediaminetetraacetic acid, disodium salt, as a anticoagulant (1 mg/ml of blood), under the technical condutions used in the laboratory (3). 5 ml of blood was transfered to each of three tubes of 20 ml capacity. Close to the surface of the blood contained in the first two tubes a stream of oxygen or carbon dioxyde was injected for several minutes in order to substitute the atmospheric air and to saturate the cells; the stream was regulated so as to provoke agitation of the blood without foaming.

After a gentle rotation of the tube, two portions of the O_2 saturated blood were transfered to two Wintrobe tubes for hematocrit determination. The O_2 layer contained in the tube was carefully replaced by air, the sample was mechanically shaken for 2 minutes, as for hematimetric determinations, and hematocrit was determined again. The CO_2-satured sample was treated in a similar manner. The sample contained in the third tube served as control and was treated in the same way, but replacing O_2 or CO_2 by air. The whole experiment was repeated using blood taken from a second dog. The results obtained are presented in table 2 where the averages of the two replicates are regis-

TABLE 2

Hematocrit changes produced by saturation of the blood sample with $C0_2$, 0_2 or air. Each figure represents the average of 2 determinations

Experimental conditions	Gas saturated		Gas saturated and shaken afterwards in atmospheric air	
	Hematocrit %	Variation %	Hematocrit %	Variation %
Atmospheric air (control)...	43,6	—	43,2	− 0,9
Oxygen.....	42,4	− 2,8	42,8	− 1,8
Carbon dioxyde.....	46,8	+ 7,3	44,6	+ 2,3

tred. The results indicate that, as expected, the volume of the red blood cells decreases in presence of O_2 (-2,8%3 and increases in presence of CO_2 (+7,3%); but their size change again towards the normal values when shaken mechanically in presence of air under the conditions of shaking as for hematimetric determinations. In fact the final hematocrit variation was -1,8% and + 2,3% for the portions of blood which ad been saturated with O_2 and CO_2 respectively.

A second experiment was done to see if the cells obtained after a venous stasis, in wich an increased volume was observed, they diminished when shaken

in an atmosphere of O_2. A blood sample was obtained from the vein of the dog leg without tourniquet or stasis; after this, a tourniquet was put to provoke stasis for 5 minutes and another sample was taken. 5 ml of each blood was put in tubes of 20 ml. The tubes were mechanically shaken for 5 minutes and the hematimetric values were determined. Afterwards the air was substituted by O_2 by means of a stream no so strong as to provoke agitation of the blood contained in the tube, and shaken again the hematimetric values were determined once more.

All determinations were made in duplicate and the results of Table 3 are the means obtained.

TABLE 3

INFLUENCE OF AIR OR OXYGEN ON THE HEMATOCRIT VALUE AND MEAN CORPUSCULAR VOLUME OF DOG BLOOD COLLECTED WITH OR WITHOUT STASIS

Blood	Erythrocytes $mm^3 \times 10^6$	Hematocrit %	Mean corpuscular μ^3	Variation %
Without Stasis + atmospheric air	4,43	35,3	79,7	—
Without Stasis + 0^2	4,39	35,0	79,7	0
With Stasis + atmospheric air	4,63	39,4	85,1	+ 6,78
With Stasis + 0^2	4,64	39,3	84,7	+ 6,27

The results obtained in this connexion show that the mechanical shaking, either with air or with O_2, is not sufficient to annul the increase in erythrocyte volume produced by stasis. This finding seems to indicate that this increase in erythrocyte volume is not due to increased saturation with CO_2, since the excess of this gas should have been eliminated by the mechanical shaking as indicated by the results of the first experiment (table 2). This seems to be more so, since, in the second experiment, a 5 minutes mechanical shaking with oxygen was unable to reduce the erythrocyte volume towards the normal figure.

The results presented in this paper show, therefore, that, in tourniquet shock, there is a marked absorption of fluid by the red blood cells. This leads to the conclusion that the loss of plasma volume which accompanies this type of shock is due in part to fluid absorption by the red blood cells and not only to the loss of blood plasma through the membrane of the blood vessels, as usually accepted. Since similar findings have been reported by Rosenfeld (2) in shock produced by histamine, trypsin, bradykinin and bothropic venom, it would seem that this may perhaps apply to all types of shock independently of its cause.

SUMMARY

In the shock induced by tourniquet in dogs there is an increase of the mean corpuscular volume of the red blood cells by absorption of fluid with consequent hemolysis. These alterations appear even before the fall of blood pressure. In this kind of shock a part of the plasmatic fluid that disappears enters in the red blood cells, so that the hemoconcentration seems not to be caused only by escape of plasma from the blood vessels, as is the classic concept.

RESUMO

No choque produzido pelo torniquete em cães há um aumento do volume médio das hemácias pela absorção de líquido e conseqüente hemólise. Essas alterações aparecem mesmo antes de se observar queda da pressão arterial. Contrôles indicam que êste aumento de volume das hemácias não é devido ao eventual aumento de CO_2 conseqüente à deficiência circulatória, pois as técnicas usadas parecem eliminar esta causa de êrro. Nesse choque uma parte do líquido plasmático que desaparece penetra nas hemácias, e portanto a hemocentração não é devida sòmente a uma saída de líquido dos vasos como é conceito clássico.

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